CORTICOSPINAL EXCITABILITY OF THE SOLEUS MUSCLE DURING WATER IMMERSION

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ABSTRACT

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Water-based exercising is a common form of physical activity used in both rehabilitation and athletic training. However, despite being widely used its' effects on neuromuscular control is poorly understood. Therefore, the main aim of this study was to investigate how a brief (15 min) immersion to thermoneutral (34°C) water would affect corticospinal excitability and/or intracortical circuitry when soleus muscle is targeted, compared to dry land measures. The experiments were conducted with the subject either resting (passive experiment) and separately during a low-level submaximal (20% of maximal voluntary contraction) isometric voluntary contraction (active experiment) while seated. The depth of immersion was at sternum level.

In total nine (9) subjects underwent the measurement protocols, results from six (6) of the total were analysed. Corticospinal excitability was assessed with transcranial magnetic stimulation via input-output -curve. Intracortical circuitry was measured with short-interval intracortical inhibition and intracortical facilitation for inhibition and facilitation, respectively. In addition, in both experiments force (Newtons) and electromyography (root mean square) values were measured and analysed. The main findings of the study were that at rest, the corticospinal excitability was significantly higher on dry land at mid (120% of the resting motor threshold, $p = 0{,}001$) and high (140% of the resting motor threshold, $p = 0{,}007$) end of input-output -curve. However, these changes were not reflected at 130% of the resting motor threshold level ($p > 0{,}05$). In the active experiment the input-output -curve had no statistically significant differences ($p > 0{,}05$ for all the measured levels). Intracortical inhibition was unaltered ($p > 0{,}05$) in both experiments, while intracortical facilitation measurement protocol was deemed unsuccessful. Also, in both experiments both the force values and electromyography values during maximal voluntary contraction had no statistically significant differences ($p > 0{,}05$). In the active experiment the level of background electromyography activity was also similar in water than in dry land ($p > 0{,}05$). Furthermore, the motor thresholds (measured as percentage

maximal of stimulator output) and all stimulation intensities were similar in both conditions in both experiments ($p > 0,05$).

Taken together, the results of this study suggest that water immersion alone is not enough to alter corticospinal excitability of the soleus muscle at either rest or during a low-level submaximal isometric contraction. Similar results have been obtained when upper extremity muscles are targeted in either partial or complete water immersion (Sato et al. 2014; Sato et al. 2015). Further, the intracortical circuitry seemed to remain unaltered by the environmental change. While hypothesized, spinal excitability may have an effect in the observed increase in corticospinal excitability in the resting experiment. Further studies are needed to clarify the contribution of spinal and cortical mechanisms in the observed changes during thermoneutral water immersion.

Key words: Motor control; corticospinal excitability; water immersion; intracortical circuitry;

ABBREVIATIONS

αMN	Alpha-motoneuron
AE	Active experiment
aMT	Active motor threshold
BG EMG	Background electromyography activity
CNS	Central nervous system
EMG	Electromyography
GABA	Gamma-aminobutric-acid
H-reflex	Hoffman reflex
ICF	Intracortical facilitation
IO-curve	Input-output curve
ISI	Interstimulus interval
M1	Primary motor cortex
MEP	Motor evoked potential
MSO	Maximal stimulator output
MT	Motor threshold
MTAT	Motor threshold assessment tool
MU	Motor unit
MVC	Maximal voluntary contraction
NMDA	N-methyl-D-aspartate
PE	Passive experiment
RMS	Root mean square
rMT	Resting motor threshold
S1	Primary sensory cortex
SICI	Short-interval intracortical inhibition
SP	Silent period
TA	Tibialis anterior muscle
TES	Transcranial electrical stimulation
TMS	Transcranial magnetic stimulation

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1 INTRODUCTION

Being able to interact purposefully with the environment is one of the main achievements of the whole locomotor system. This purposeful action occurs in different hierarchical levels that act in unison and equally contribute to motor control. These levels of motor control can be roughly divided into cortical, spinal and peripheral level. Cortical mechanisms of motor control include the primary cortices and neuronal commands descending through various modulatory units, through brainstem and to spinal cord. At spinal level the motor command is relayed to muscles through alpha-motoneurons. Input rising from periphery can modulate the motor command at this level through reflex activity. The peripheral control of movement ends in muscle-tendon-mechanics and the lever system created by muscle-tendon units and bones, providing a mechanical framework which is under neuronal control. These peripheral components (muscles, tendons and bones) can all adapt independently from neural input, therefore possibly altering neural control (Kandel et al. 2013). One such challenge could be the change in environment in which the individual operates (Pendergast & Lundgren 2009).

Water based physical activity is one form of cardiovascular exercising that is widely used e.g. in rehabilitation. Training in water has been shown to improve the functional capacity of different patient groups (Park et al. 2014; Stevens et al. 2015). Water provides a unique medium for exercising where gravity is reduced thereby reducing loading of the joints while also providing resistance to functional movements (Pendergast & Lundgren 2009). Despite being widely used as an exercising environment, the effects of water immersion caused on neuromuscular system are largely unknown. In addition, only a handful of studies have explored the before and after effects of water immersion on neural control of movement. The evidence base is further limited when examining the effects of water immersion on neuronal control of movement during immersion.

Based on the hierarchy described above, effects of water immersion have been studied on spinal level via reflex excitability studies. It has been shown that the excitability at spinal level can either increase or decrease (Pöyhönen & Avela, 2002; Cronin et al. 2016; Nakazawa et al.

2004). Cortical mechanisms are even less understood, currently it seems that water immersion does not change either corticospinal excitability or intracortical circuitry of the upper extremities while subjects are at rest (Sato et al. 2014; Sato et al. 2015). However, these studies are limited by not immersing their subjects completely (i.e. only the hand under measurement was immersed) or that the lower extremities were submersed while measurements were done on upper extremity muscles.

To the author's knowledge, there are no studies which would have examined the corticospinal excitability of lower extremity muscles. Further, all the studies that have examined the cortical mechanisms of movement during water immersion have done so with the subject at rest. Therefore, the aim of this study was to examine if water immersion can alter corticospinal excitability of soleus muscle when the subject is at rest or during a submaximal, isometric contraction. The experiment was designed so that all the measurements can be conducted with the subjects seated and immersed to sternum level. Literature review covers the above described hierarchy of the motor control in more detail while also examining how the corticospinal system can be examined and how water immersion is known to influence humans. Results from this study can further increase the evidence base of water-based exercising and provide insight how the corticospinal system adapts into water, therefore aiding the research design in experiments conducted on patient groups.

2 INTERACTING WITH THE ENVIRONMENT

Nervous system allows humans to interact with the environment by both sensing and influencing in it. The nervous system is anatomically divided into central and peripheral nervous system. Central nervous system (CNS) includes the brain (cerebral hemispheres, diencephalon, cerebellum and brainstem) and the spinal cord. The peripheral nervous system consists of sensory neurons that relays information from various sensory receptors to relevant CNS structures. In addition to sensory neurons, the peripheral division has a motor portion linking the brain and the spinal cord to skeletal muscles to enable movement. Also, in the motor portion of the peripheral system are located the visceral motor efferents responsible for the innervation of smooth and cardiac muscle (Purves et al. 2012, 13).

Mainly two types of cells are residing in the nervous system; nerve cells (or neurons) and glial cells (or glia). A neuron is typically divided based on its morphology into the cell body (or soma), dendrites and axon. Functionally, the soma hosts the nucleus and the genetic code of the cell and the endoplasmic reticulum where the cells proteins are synthesized. From the soma onwards several short dendrites and one long tubular axon arises. Dendrites form a tree-like structure and acts as the main site for receiving signals from other neurons. The axon on the other hand is much longer, ranging from 0,1 mm to 2 m, carries the electrical signals to other neurons (Kandel et al. 2013, 22).

The surface of the neuron is a phospholipid bilayer that ions cannot penetrate. However, specialized protein structures, called ion channels, allow the ions to pass through the membrane. When the neuron is at rest the cell membrane is charged to its' resting membrane potential. Nerve cell has an excess of negatively charged ions inside compared to positively charged ions outside, leading to a resting membrane potential of -65 millivolts (mV). Mainly potassium (K^+) is concentrated inside the neuron and sodium (Na^+) and chloride (Cl^-) in the extracellular space. Difference between the two spaces leads to concentration gradient that allow the movement of ions. If the resting membrane potential reaches a threshold level, it leads to an all-or-none response called action potential. It is a short reversal in the potential difference across the membrane that is transmitted rapidly. The cell depolarizes by a sudden influx of Na^+ ions

through the ion channels, briefly overshooting. As the cell is depolarized, voltage-gated K^+ channels are opened, allowing a repolarization. However, this efflux of K^+ ions causes hyperpolarization leading to afterhyperpolarization. This causes a refractory period lasting for a few milliseconds in which the cell is unable to produce another action potential (Figure 1) (Enoka 179, 186). The speed by which action potentials are conducted can be increased with a lipid substance called myelin. The myelin sheath around the membrane is interrupted at nodes of Ranvier where the action potential is regenerated (Kandel et al. 2013, 23).

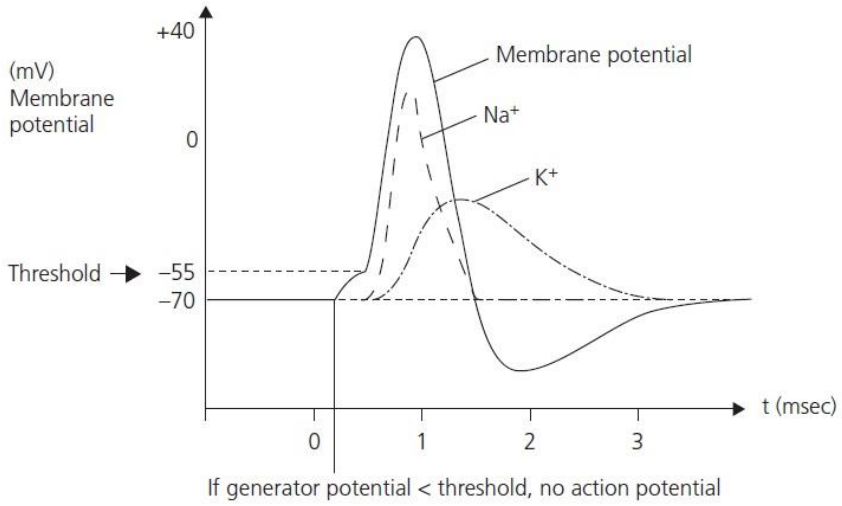

FIGURE 1. Action potential time course and the change in Na^+ and K^+ concentration (From Michell 2013).

Action potential is the signal by which the information is received, analysed and relayed onwards by the brain. An important characteristic of the action potential is that it is created by a variety of events from mechanical touch to odour, so the signal is independent from the incident that caused it. However, the brain does not interpret the signal by its form but rather by the pathway it travels. After analysis and interpretation, the brain creates our various sensations of sight, touch or smell. (Kandel et al. 2013, 23).

3 FROM ACTION POTENTIALS TO PURPOSEFUL MOVEMENT

As stated earlier, purposeful movement with which to interact in the environment is one of the main achievements of the human brain, together with the rest of the locomotor system. This purposeful movement consists solely of action potentials occurring in an estimated 86 billion neurons that reside in the human brain (Herculano-Houzel, 2009). The massive neuronal activity organizes into voluntary movements and reflexes that underlies the purposeful movement, both of which are different in their expression. As the name suggests, voluntary movement is initiated by an internal choice to interact with the environment. Reflexes on the other hand are automatic responses triggered by external stimuli. Moreover, voluntary action involves the choice between actions and the choice to not act at all. Voluntary actions in addition to being internally initiated are internally motivated to achieve a certain goal. Also, as experience increases the voluntary actions often improve in effectiveness to reach a desired goal or complete a movement sequence. In other words, motor learning occurs. The motor system is therefore capable of learning new behavioural responses to familiar and predictable environmental stimuli, or to learn new skill to cope in them. This leads to the conclusion that voluntary actions are more complex than initiating a certain pattern of muscle activity involving sensory, perceptual and cognitive processes (Kandel et al. 2013, 836).

Voluntary control seems to occur in sequential stages. Systems involved in motor control are schematically illustrated in Figure 2. Firstly, the perceptual mechanisms creates a sensory representation of the external world and of the individual in it. Secondly, the cognitive processes decide on the purposeful action based on the internal representation formulated in the first stage. And lastly, an according motor plan is relayed to the action systems (Kandel et al. 2013, 839).

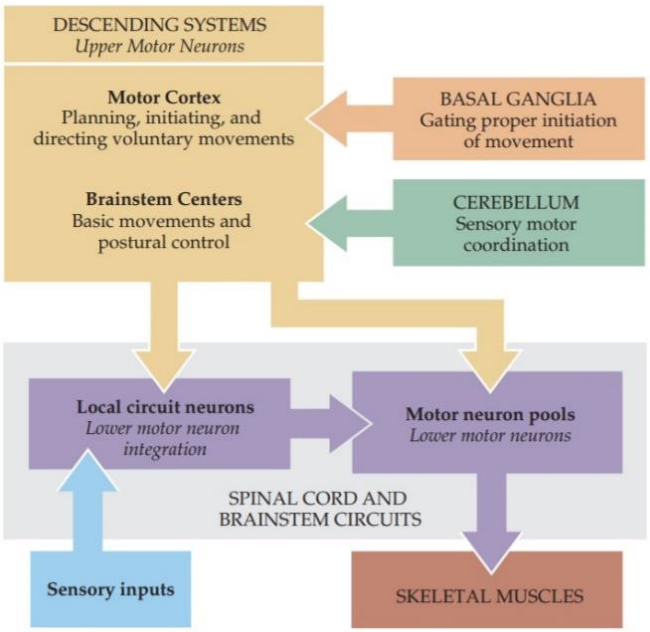

FIGURE 2. Schematic of the human motor system and their relative contributions to movement control (From: Purves et al. 2012).

3.1 Brain structures of motor control

Multiple structures of the central nervous system are underlying voluntary motor control (Figure 3). In a descending order, structures such as the motor cortex and the pyramidal tract, basal ganglia and thalamus, cerebellum and spinal cord are reviewed and their role in the motor control elaborated in the following (Shumway-Cook & Woollacot, 46). It is crucial to understand that the motor control function of the brain is cannot be separated from its sensory function. The unity of the two systems is referred to as a single sensorimotor function. This sensorimotor function relies on information being transmitted from the brain to the spinal cord and from the peripheral receptors to the brain. These relay pathways are named descending and ascending pathways, respectively (Latash 2012, 190).

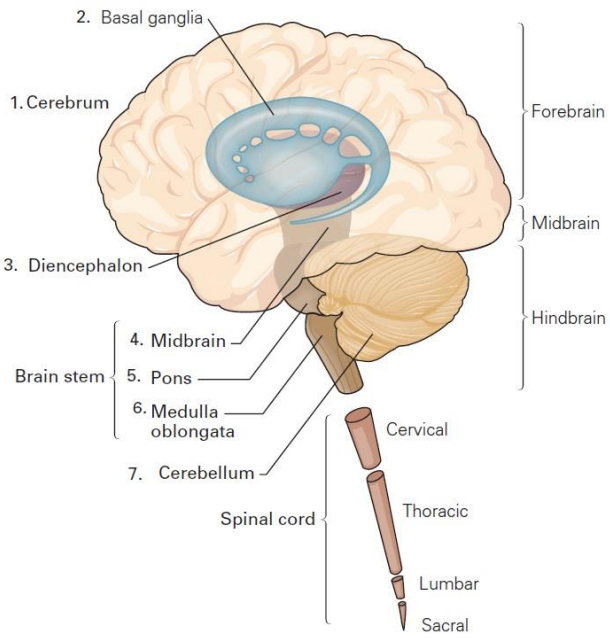

FIGURE 3. Central nervous structures involved in motor control (From Kandel et al. 2013).

Motor cortex. The primary motor cortex (M1) and premotor areas of the frontal lobe are responsible for planning and precisely controlling complex sequences of voluntary movements. Motor areas containing the upper motor neurons responsible for movement can be found in the posterior frontal lobe. Collectively called motor cortex, it can further be divided into primary motor cortex and premotor area. Low threshold in the primary motor cortex suggests a large and direct pathway to the lower motor neurons in the brainstem and spinal cord (Purves et al. 2012, 377). Motor cortex contains a topographical map of motor output to different parts of the body (Kandel et al. 2013, 837) (Figure 4).

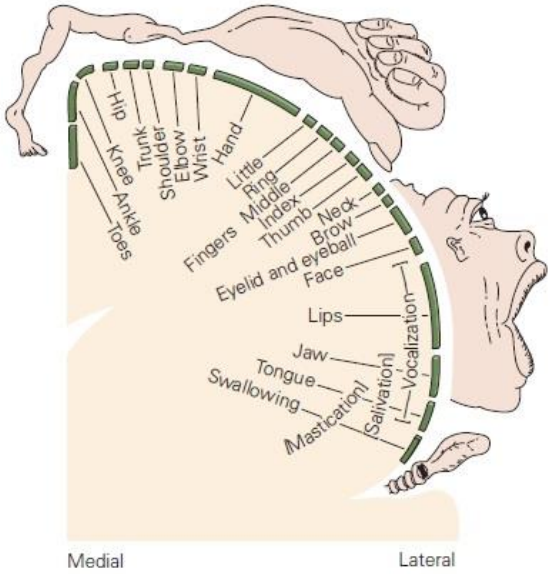

FIGURE 4. Topographical map of motor representations in the motor cortex (From: Kandel et al. 2013).

Neurons are organized in the cortex into a laminar structure with six layers. It consists of two major groups of neurons, stellate cells and the pyramidal cells. Stellate cells are primarily interneurons as their axons do not leave the cortex whereas pyramidal cells leave and project vastly across the structures in the CNS (Latash 2012, 191). Pyramidal cells located in the layer five, represents the main output from brain towards periphery (Avela & Gruber, in Komi 2011, Purves et al. 2012, 377).

Basal ganglia. Basal ganglia influence movement similarly to cerebellum by regulating the activity of upper motor neurons. Basal ganglia consist of large and functionally diverse set of nuclei deep within the cerebral hemispheres. From the motor control perspective, caudate, putamen (collectively referred to as striatum) and globus pallidus are of interest. Closely related are the substantia nigra (in the midbrain in brainstem) and subthalamic nucleus in the ventral thalamus. The combination of these structures creates a loop linking most of the upper motor neurons in the motor and premotor cortices and the brainstem. Functionally, this loop can modulate the activity in the above-mentioned upper motor neurones in anticipation and during the performance of voluntary movements (Purves et al. 2012, 399).

Functional relevance of the basal ganglia is revealed by the role of efferent neurons from both globus pallidus and substantia nigra; they use gamma-aminobutric-acid (or they are GABAergic). Hence, the main output of the basal ganglia is inhibitory. Both output structures are highly active, spontaneously preventing unwanted movement by continuously inhibiting thalamus and superior colliculus. As the medium spiny neurons in the striatum are also inhibitory, the consequence of the excitatory inputs from the cortex to the striatum is the inhibition of the continuously active inhibitory cells of globus pallidus and substantia nigra, resulting in disinhibition. This disinhibition of thalamic neurones allows the relay of information to upper motor neurons and these in turn can send commands to lower motor neurons that initiate movement. Functional connectivity of the basal ganglia is illustrated in Figure 5. (Purves et al. 2012, 404).

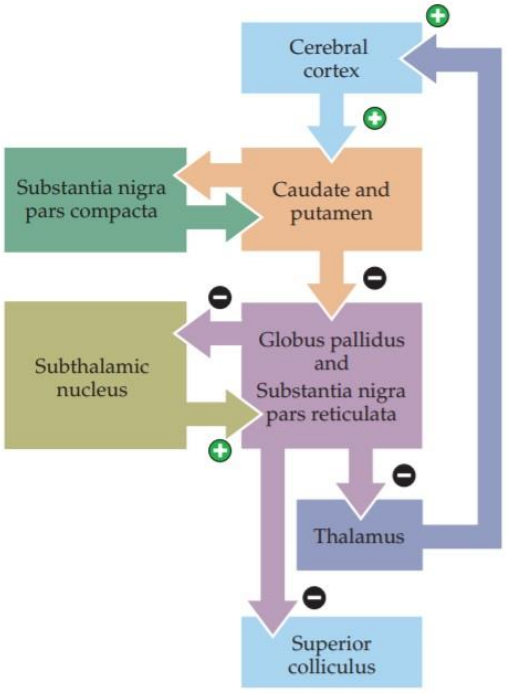

FIGURE 5. Schematic of basal ganglia motor components. “+” refers to excitatory and “-“ to inhibitory connections (From Purves et al. 2012).

Thalamus. Even though the primary role of the thalamus is to convey sensory information to the primary sensory cortices, the role of thalamus in human motor control cannot be brushed aside (Kandel et al. 2013, 360). It was noted earlier that sensory and motor processing are entwined and cannot be separated and therefore it is noteworthy that thalamus is discussed shortly (Latash 2012, 190). Thalamus acts as a gatekeeper that either prevents or enhances the arrival of specific information to the cerebral cortices, depending on the current state of the system. It is made up of numerous different thalamic nuclei and some of which are specialized to a specific sensory information, projecting to a specific site in the cerebral cortex. One major group of thalamic nuclei, the ventral nuclei are important to motor control as they relay information to basal ganglia, cerebellum and M1. Also, posterior nuclei in the ventral group sends somatosensory information to the primary sensory cortex (S1) (Kandel et al. 2013, 362). Thalamocortical connections are vast and reciprocal in nature. As these neurons are myelinated, it appears as white to the naked eye and is therefore referred to as white matter. Thalamocortical system is also changing the large-scale activity of the brain in daily brain states such as waking, sleeping and rapid-eye movement dreams (Baars & Cage, 248).

Cerebellum. Posterior to the brainstem and below the large cerebral hemispheres is the cerebellum. While being only 10% of the total volume of the brain, it contains more than half of the whole neuron population. Cerebellum is organized into highly regular and repeating microcircuit –units. However, projections from different parts of the brain and spinal cord arrive to different parts of the cerebellum and, from cerebellum the output is distributed across the motor systems. As the architecture and physiology remains the same throughout cerebellum it can be theorized that different parts of the cerebellum perform similar processing operations to different inputs (Kandel et al. 2013, 960). Cerebellar hemispheres participate in the movement control of the ipsilateral part of the body, thereby having a major difference to the primary motor areas (Latash 2012, 203). Despite having a large neuronal population, the cerebellum does not have a primal role in either sensory or motor function. This can be elaborated in the case of cerebellar damage; the result would not be a paralysis or loss of sensation, instead it would have a detrimental effect on the ability to perform movements. As it also receives ascending input from almost every sensory system, the input and output connections make the cerebellum an effective error detector being able to regulate motor output (Shumway-Cook & Woollacot, 73).

Brainstem. In a descending order, the brainstem consists of the midbrain, pons and the medulla. From the motor control perspective, the brainstem has several important functions. First, parallel to the motor neurons in the spinal cord mediating sensation and motor functions in the periphery the brainstem is responsible for the motor and sensory control of head, neck and face. Second, brainstem is the entry point for specialized senses such as balance. Third, from the brainstem ascending and descending pathways carry information to other parts of the CNS. Fourth, the core of the brainstem, the reticular formation is a diffuse network of neurons that receives most of the ascending sensory information while also regulating alertness and arousal (Kandel et al. 2013, 341). Lastly, to emphasize the role of brainstem in motor functions it is worth noting that all the descending motor pathways except for the corticospinal tract originate in the brainstem (Shumway-Cook & Woollacot, 48).

Collectively, the main output route from the cortex towards periphery is referred to as the pyramidal tract. Pyramidal tract is one of the best-known output tracts of the cortex and has long axons of cortical cells that project onto various structures involved in movement control. Primarily it consists of neurons that originate from the motor areas but neurons from parietal somatosensory areas are also included. Pyramidal tract can be further divided into corticospinal tract that travels down the spinal cord and into corticobulbar tract that terminates in the brainstem controlling muscles of the face and neck (Latash 2012, 192). Upper motor neurons of the corticospinal tract eventually form either monosynaptic connections with lower motor neurons at spinal level or terminates with interneurons. These descending projections often synapses into lower motor neurons belonging to several motor pools thereby influencing on more than one muscle (Enoka, 289). Immediately below the medulla, majority (90%) of the corticospinal tract cross the midline entering the lateral columns of the spinal cord on the opposite side. The axons that do not cross (10%) forms the ventral corticospinal tract that terminates ipsilaterally or bilaterally (Purves et al. 2012, 381). These uncrossed neurons innervate mostly proximal muscles e.g. those located in the trunk. Therefore, an injury to a cortical area responsible for movement often leads to impairments in the contralateral extremities (Latash 2012, 193).

3.2 From spinal cord to skeletal muscles

Spinal cord houses the motor neuron pools from which the alpha-motoneurons (αMN) originate towards muscles they innervate. Motor neuron pools are located in an orderly manner not only along the length of the spinal cord but also in medial to lateral orientation (Figure 6). Motor neuron pool consists of all the motor neurons innervating muscle fibres of a single muscle. The arm muscles for example: each motor neuron pool is in the cervical enlargement of the cord. In a similar manner all the motor neuron pools of lower limb muscles can be found from the lumbar enlargement. As for the medial to lateral orientation, the motor neuron pools for trunk muscles can be found from the medial spinal cord and the more distal arm muscles can be found from the lateral ventral horn of the spinal cord. This somatotopic organization provides a framework for understanding descending control as well: medial motor neuron pools that are responsible for balance and postural control receives input from long projection systems running medially in the white matter of the spinal cord. The lateral motor neuron pools descend from the cerebral cortex and run laterally in the spinal cord white matter (Purves et al. 2012, 400).

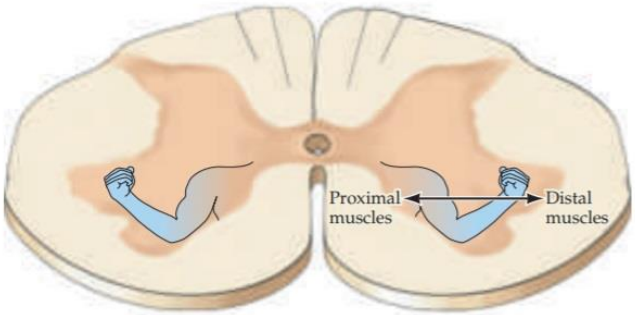

FIGURE 6. Somatotopic arrangement of the spinal cord (From: Purves et al. 2012).

3.2.1 Motor units and force production

Motor neurons are responsible for the innervation of striated muscle fibres required for force generation (Purves et al. 2012, 356). Further, the basic functional unit with which the nervous system controls movement is the motor unit (MU). It comprises of a single αMN and all the

muscle fibres it innervates. The number of muscle fibres can range from few to several thousands (Figure 7). When the αMN is depolarized, the action potential propagates along the axon to its terminal in the muscle. At its' terminal, the neuromuscular junction, the action potential causes a release of neurotransmitter that in turn depolarizes the sarcolemma of the muscle which leads to muscle contraction. Electrical activity on the sarcolemma can be recorded with surface electrodes using electromyography (EMG) (Kandel et al. 2013, 770).

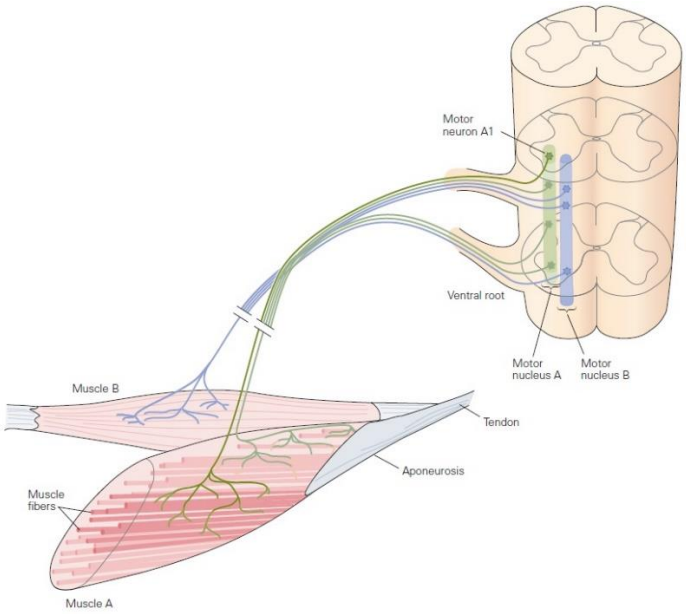

FIGURE 7. Alpha-motor neurons originating from the ventral horn of the spinal cord. Motor units innervating a single muscle can extend over one to four segments within the spinal cord. Motor nucleus A and B represent single motor neuron pool (From Kandel et al. 2013).

Firing of the MUs and their subsequent firing rates occur in highly in unison. A phenomenon that has been observed in upper and lower limb muscles, small and large muscles and in muscle contractions of muscles with or without muscle spindle input as well. As such it suggests that MUs are controlled by the same source. This concept of common drive states that in order to achieve a desired force output, the CNS regulates the net sum of both excitatory and inhibitory inputs to the motor neuron pool. Thus, all the motor neurons in the pool receives the same net drive at any given time (DeLuca & Erim 1994). Motor neurons are brought to recruitment

threshold by synaptic inputs that cause a change in membrane potential that exceeds the voltage threshold of the motor neuron. Recruitment threshold is the muscle force at which the voltage threshold is exceeded. As the MUs convert the neural activation signal into muscle forces it can be said that the discharge behaviour of the motor unit contains information about the descending control signal (Farina et al. 2016).

The discharge rate, or rate coding measured in pulses per second (pps), is dynamic and varies across the spectrum of muscle actions. The importance of rate coding in force production is reflected in force-frequency relation that is a sigmoidal relationship between firing rate and exerted force (Macefield et al. 1996). Gradually increasing force during an isometric contraction leads to increase in both MU recruitment and rate coding. Peak discharge rates can be observed in the later recruited MUs while peak rates for earlier recruited MUs tends to remain constant despite the increase in muscle force and continual increase in net excitatory synaptic input. In contrast to modest increases in rate coding during gradual increases of force levels, rapid ballistic contractions lead to instantaneous high bursts of MU discharges (60-120pps) that declines with successive firing. However, due to changes in synaptic inputs and MU specific properties, rate coding is often modulated by 5-10 pps between tasks like submaximal steady contractions, eccentric and concentric contractions. Even though the raw increases in pps seem small, they occur at the steep rise of the sigmoidal curve of the force-frequency relationship that leads to relatively large increases in MU force (Enoka & Duchateau 2017).

Motor units are not made equal as both the MUs and the αMNs themselves vary in size and in function. Smaller αMNs innervate a relatively small number of muscle fibres creating MUs that produce small forces. Large motor neurons innervate larger and more powerful MUs. Small MUs are also fatigue resistant whereas large units fatigue more easily due to higher power output and smaller number of mitochondria in the innervated muscle fibres. Functionally, small MUs have smaller activation thresholds and are tonically active during sustained effort such as balance. Large MUs, on the other hand, participate in the force production during rapid movements when great force is made such as jumping. An important aspect of MU recruitment is the orderly fashion of motor neuron activation from small to large. This is called the Hennemans' size principle where the synaptic activity in the motor neuron pool first activates

the small MUs and as the input increases, progressively larger units are recruited to generate larger forces (Purves et al. 2012, 358).

3.2.2 Role of sensory receptors

Spinal cord is the lowest level of the perception or action hierarchy. Circuitry of the spinal cord receives and processes somatosensory information from the periphery that allows for reflex and voluntary control of posture and movement via motor neurons (Shumway-Cook & Woollacot, 46). These activations of spinal pathways always produce changes in the excitability of spinal motoneurons and thus have been called 'the final common path' in the motor system. The spinal stretch reflex (or its' electrical equivalent, Hoffman reflex (H-reflex) is the most studied reflex, however either the extent of its involvement or the contribution of monosynaptic Ia connections in its generation are not yet completely understood in normal motor control (Pierrot-Deseilligny & Burke, 1, 63). Coordinated movement patterns of muscle contraction are one of the main functions of spinal reflexes. The stretch reflex has an especially important part in locomotion because of its resistance towards the lengthening of a muscle (Kandel et al. 2013, 792). The simple stretch reflex is composed of a receptor sensing the change of length in a muscle, a receptor called muscle spindle and type Ia axon from this receptor that has a direct connection with homonymous motoneurons that are excitatory. Muscle spindles sensitivity for stretch is controlled by CNS via gamma-motoneuron (γMN). In addition to this direct excitatory synapse, the Ia afferent axon has connections to inhibitory interneurons that inhibit the antagonist motoneurons thereby creating a reflex loop that can prevent possible muscle contractions capable of resisting the movement caused by the stretch reflex (Figure 8). This inhibitory capability results in reciprocal innervation (Kandel et al. 2013, 795).

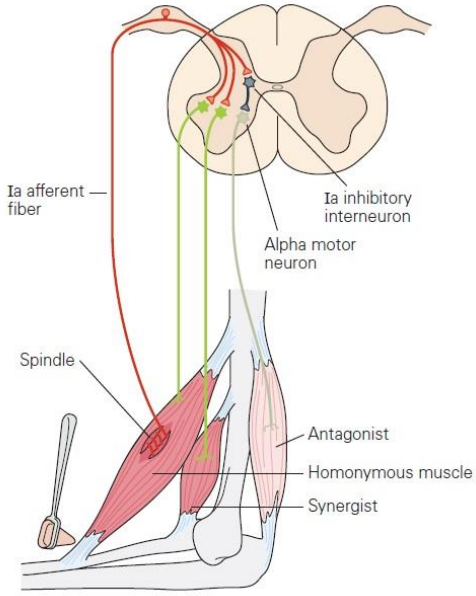

FIGURE 8. Stretch reflex of the biceps muscle. Activation of the Ia afferent excites homonymous αMNs and inhibits through Ia inhibitory interneurons the antagonist muscle (From Kandel et al. 2013).

Spinal interneurons activated by group Ia afferents (through Ia inhibitory interneuron) are responsible for mediating inhibition between antagonist motoneurons (Nielsen 2016). It has been demonstrated that these inhibitory interneurons are activated by descending input, namely corticospinal, rubrospinal and vestibulospinal tracts, together with their corresponding α- and γMNs thereby creating a functional unit to coordinate flexion-extension movements (Nielsen 2016, Knikou 2008). With this organizational feature the control of voluntary movement is simplified: higher centres do not have to send separate commands to the opposing muscles. Reciprocal innervation is a part of stretch reflex, but it is also useful in voluntary movements. As the antagonist muscles relax during movement, it enhances the speed and efficiency because the muscles acting as prime movers are not met with the contraction of opposing muscles (Kandel et al. 2013, 797).

4 PROBING THE CORTICOSPINAL TRACT

In 1985, Barker and colleagues introduced a non-invasive, pain-free method to stimulate the human cortex. They demonstrated that with a single transcranial magnetic stimulation (TMS) pulse applied over the primary motor cortex, a response in the muscles innervated by those cortico-motoneuronal inputs can be recorded with surface electrodes. As these motor responses, termed motor evoked potential (MEP) are easily obtained, TMS has gained a lot of popularity among clinical and basic research concerning pyramidal tract (Barker et al., 1985; Groppa et al. 2012). Based on variable stimulation parameters, TMS can either inhibit or excite the brain or it can be used for functional mapping of the cortical region. Besides clinical neurophysiology and motor control research, TMS is a common method in cognition research and as a therapeutic tool in psychiatry or in neurological rehabilitation (Hallet 2000).

Magnetic stimulation utilizes a powerful and rapidly changing current pulse that is produced in a coil of wire placed over the scalp. The resulting magnetic field penetrates the scalp, fat and bone with little to no attenuation and induces an electric field in the brain depolarizing the neuronal membranes that leads to excitatory or inhibitory postsynaptic potential (Figure 9) (Rossi et al. 2009, Avela & Gruber in Komi, 2011). According to the Faraday's law of electromagnetic induction, the magnitude of the electric field induced by this time-varying magnetic field is proportional to the time rate of change of the magnetic field. In the case of TMS, it is determined by the rate of change of the current in the coil (Rossi et al. 2009). Orientation of the electrical field in the tissue is perpendicular to the magnetic field. The electrical field in the tissue also has an opposite direction relative to electrical current in the stimulation coil (Groppa et al., 2012).

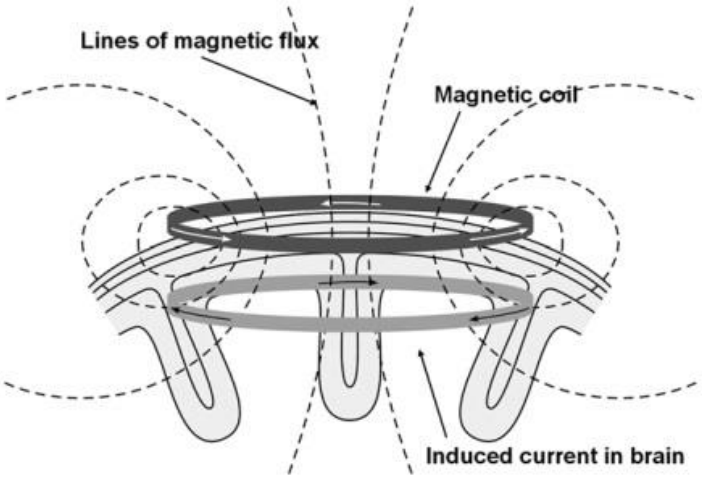

FIGURE 9. Current flows in a magnetic coil and in the brain illustrated. From Hallet 2007.

At its' initial state, TMS was introduced with a large circular coil of wire with a diameter of 10 cm. With this kind of configuration, the most effective site of stimulation is under the coil and weakest at the centre of the ring. As such, with a tangential placement over the scalp it covers a large area of the brain but does not penetrate deep into the brain. To overcome the issue of large stimulation area with a shallow depth, a figure-of-eight coil has been developed. This comprises of two small round coils with oppositely directed currents in them that yields the highest electrical and magnetic amplitude at the intersection of the two, resulting in a more focal and deeper stimulation (Figure 10) (Rossini et al. 2015). An adaptation from the figure-of-eight coil is the double-cone coil. In double-cone coil, the two circular coils are connected to each other at an angle of 90° – 100°, allowing even greater depth penetration making this type of a coil better suited for lower limb stimulation. However, the term focal stimulation might be misleading as the physics of magnetics dictates that the fields diverge after they leave their source. This adds to that the combination of coil geometry and structural organization of the neuronal circuits in the cortex together determines the ultimate site of stimulation (Avela & Gruber, 2013). At rest the lowest threshold for eliciting a MEP in the upper extremity is when the stimulus induces a posterior to anterior current across the central sulcus in the brain (Di Lazzaro 2004). However, it seems that the coil orientation causing a medial to lateral direction

current yields the lowest motor threshold for lower limb muscles when using a figure-of-eight coil (Smith et al. 2017).

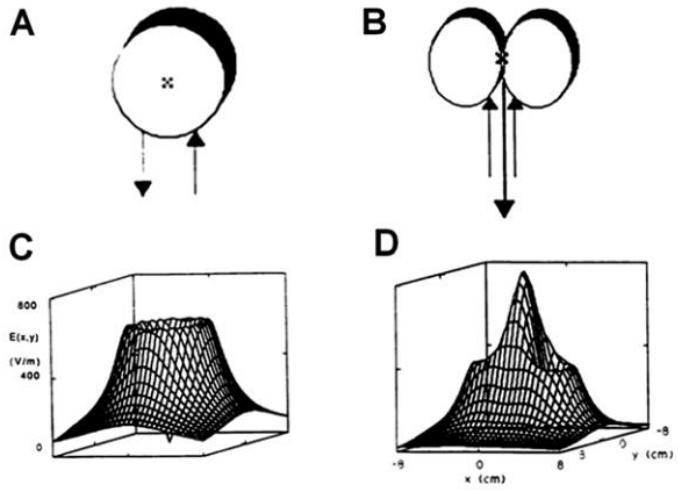

FIGURE 10. Magnetic coils (A and B) and their resultant electric fields (C and D). From Hallet 2007.

In general, there are three methods of TMS application. The first, single-pulse TMS can be used in studying central motor conduction time, examining causal brain-muscle response behaviour and in mapping of the cortical regions. The second method is the paired-pulse TMS that utilizes a pair of stimuli separated by a variable time interval to allow examination of intracortical circuits as well as cortico-cortical interaction. Paired-pulse stimulation can be targeted to a same cortical with one coil or by two coils to different regions of the brain. In addition, a single TMS pulse can be paired with a peripheral stimulus, a method that is called paired associative stimulation (PAS). Lastly, TMS can be applied in a repetitive manner using a train of multiple stimuli (rTMS) (Rossi et al. 2009).

4.1 Physiology – what is activated?

Stimulation of the human motor cortex, whether magnetic or electrical, results in two types of responses. The first response results from a direct activation of the pyramidal axons and is termed the D-wave (direct). The D-wave is followed by other descending volleys with an

interval of approximately 1,5 ms. These responses are thought to occur due to transsynaptic activation of the same pyramidal neurons thus termed the I-waves (indirect). It has been concluded that TMS at lower intensities recruits I-waves and as the stimulation intensity is increased both I- and D-waves are recruited. (Di Lazzaro 2004). The neural activation caused by the stimulus is limited to the cortex or subcortical white matter unless the stimulus intensity is very high. The action potential is transmitted through at least two synaptic connections from the corticospinal tract to spinal motoneuron in the spinal cord and onwards from a peripheral motor axon to the muscle (Rothwell 2011).

4.2 Measures of corticospinal excitability

Motor threshold. In a scientific study the intensity of TMS is individually adjusted to motor threshold (MT). 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). Physiologically, the MT reflects the excitability of individual neurons and their local density. As MT value can be manipulated with drugs that influence sodium and calcium channels it is a measure of membrane excitability (Hallet 2007). Hand and forearm muscles have the lowest thresholds, progressively increasing in truncal, lower limb and pelvic muscles. Intrinsic fluctuations of the cortical and spinal neuron excitability cause variations in the MEP amplitude. As this physiological fluctuation cannot be avoided other technical and physiological variables should be kept constant. These include coil position and orientation, background muscle activity, cognition and environmental noise (Rossini et al. 2015). The MT can be determined with the subject at rest and it is thus called resting motor threshold (rMT). Alternatively, it can be assessed during a slight tonic contraction such as 20% of maximal voluntary contraction resulting in active motor threshold (aMT).

As there is an inevitable variation in the MEP amplitudes, different methods for estimating the MT have been developed. The first is the relative frequency method where MT is defined as the lowest stimulus intensity (% of maximal stimulator output, MSO) required to induce a MEP in 5 out of 10 trials based on visual observation with a 0,05mV amplitude as cut-off value for MEP and no-MEP. Alternatively, to reduce the amount of time needed for relative frequency

method and to take in to account the probabilistic nature of MT an adaptive method has been developed. The idea is to estimate the probability of evoking a MEP at a given intensity. The adaptive method is based on Parameter Estimation by Sequential Testing and Maximum Likelihood regression. At each trial, the model predicts the TMS intensity that would result in MEP with a 50% chance. After each trial the program suggests the next intensity based on previous result (Groppa et al. 2012). With the computer program (Motor Threshold Assessment Tool v. 2.0), as little as 14 to 17 stimuli are needed for a reliable MT estimation (Awiszus, 2011).

Motor evoked potential. MEP provides a variety of different parameters that can be studied. These include the latency from stimulation to response called central motor conduction time and the size of the MEP based on amplitude, duration or area. When compared to compound muscle action potential resulting from peripheral nerve stimulation, MEP is more variable. Where compound muscle action potentials corresponds to the number of activated motor units and is thereby proportional to the number of activated motor axons, the size of the MEP theoretically reflects the number of activated corticospinal motor neurons. However, MEP is influenced by three basic physiological mechanisms: 1) number of recruited motor neurons in the spinal cord, 2) the number of motor neurons discharging more than once to the stimulus and 3) the synchronization of the TMS-induced motor neuron discharges (Figure 11) (Rösler & Magistris 2008). As the TMS creates temporally dispersed volleys in the corticospinal tract, motor neurons are activated in slightly different latencies and these latencies are further increased in the peripheral nerve. This leads to phase cancellation of motor unit potentials and to MEPs that are less synchronized, more prolonged and of smaller amplitude than compound muscle action potentials. This remains true even at higher stimulation intensities (Rossini et al. 2015). Maximal stimulation of corticomotor pathway through high TMS intensities leads to muscle twitches that can match or even exceed the force of muscle twitches that are evoked by peripheral nerve stimulation. This can happen because the same αMNs can discharge multiple times through high TMS intensity, compared to a single discharge with PNS (Groppa et al. 2012).

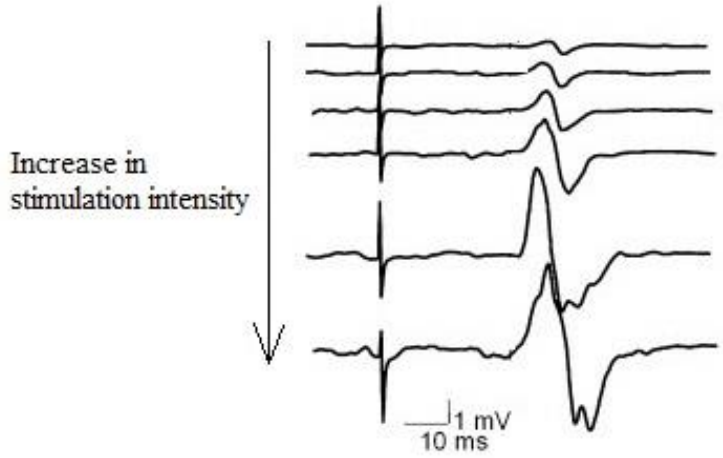

FIGURE 11. Varying sizes of MEPs evoked by increasing stimulus intensity. From Hallet 2007.

As is the case with MT, excitability level of the corticospinal pathway leads to different MEP amplitudes. Voluntary contraction unexpectedly leads to an increase in corticospinal excitability and thus to larger MEPs without an increase in the stimulus intensity (Rossini et al. 2015). Afferent input also effects the MEP sizes somatotopically. Terao et al. (1995) applied air-puffs to the tips of the finger and observed an increased MEP response in the corresponding mainly to the finger it was targeted. The effect was less pronounced in the dorsal side of the finger (Terao et al. 1995). Air-puff induced MEP facilitation was abolished in patients with primary sensorimotor or partial thalamic lesions. They concluded that the sensory inputs leading to the facilitation ascends the dorsal column and first reaches the thalamus from where the input is fed to S1 and onwards to M1. Based on these studies the facilitation route barely involved other cortical regions (Terao et al. 1999). Thereby it can be said that MEPs are subject to dynamic changes that reflect the present physiological state of the motor system. A relaxed or active muscle, movement preparation period, motor imagery or the current flow of afferent information all has an effect to MEP response highlighting the dynamic state of the cortico-motor system (Rossini et al. 2015).

Input-Output -curve. The relationship between stimulus intensity and MEP amplitude modelled by a cumulative Gaussian and described by a sigmoid curve. This relationship in a sigmoid

curve is called the recruitment curve or input-output -curve (IO-curve) (Figure 12). Initial segment is relatively flat and starts to deviate from the zero at the level of MT. The ascending part of the curve reflects a linear increase in MEP amplitude with increasing stimulus intensity (usually from 120% to 140% of the MT). The final part of the IO-curve is a plateau with no further increase in the MEP amplitude (Rossini et al. 2015). The slope of the IO-curve reflects the excitability of the motor cortex with steeper slopes reflecting increased cortical excitability. Increase in the slope of the IO-curve also increases glutamate levels in the cortex. As such it seems that there is a link between glutamatergic neurotransmission and corticospinal excitability that can be examined with IO-curve (Stagg. et al. 2011, Rossini et al. 2015).

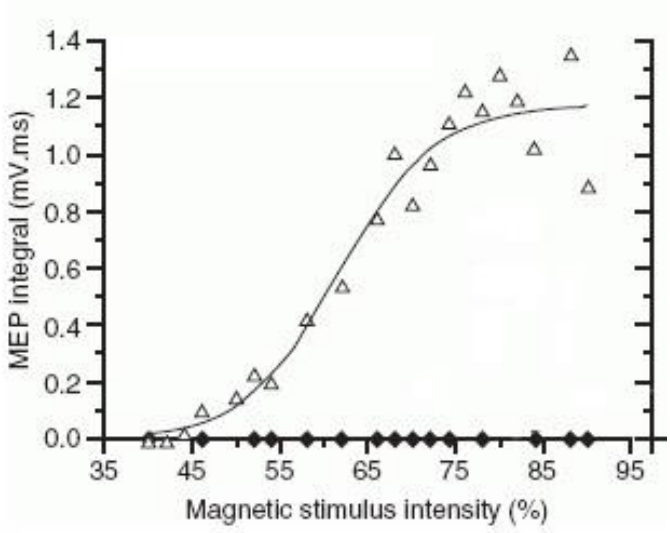

FIGURE 12. An example of an IO-curve. Soleus MEP integral (mV x ms) as a function of stimulator output. From: Rothwell 2008.

Silent period. During an isometric muscle contraction, a single suprathreshold TMS pulse targeted to the corresponding motor area of the M1, a period of silence can be observed in the EMG recording of the muscle activity (Figure 13). The length of this EMG suppression, or silent period (SP) lasts 100-300 ms and consist of both spinal and cortical mechanisms. Currently it is understood that spinal inhibitory mechanisms, such as recurrent inhibition, refractoriness of spinal motor neurons and postsynaptic inhibition through Ia interneurons, contribute to the first 50 ms of SP but the later part of the inhibition is originated from the M1

(Wolters et al. 2008). However, there is evidence suggesting that the spinal contribution of silent period may exceed 50 ms up to 150 ms (Yacyshyn et al. 2016). Still, as the greater part of SP is generated by cortical mechanisms the total duration of SP is usually altered only by the same cortical mechanisms. Mechanisms behind SP are further elaborated by comparing TMS and transcranial electrical stimulation (TES): TMS stimulation produces significantly longer SP than TES. Since TMS preferentially activates excitatory intracortical neurons that in turn activate the pyramidal neurons (compared to the direct activation of pyramidal neurons through TES), a predominant role of intra-cortical inhibitory phenomena is suggested in the formation of SP (Rossini et al. 2015). Further, the duration of SP is compatible with a long-lasting inhibition mediated by $GABA_B$ receptors. It has been shown that the duration of SP is longer when subjects have orally taken a GABA re-uptake inhibitor, suggesting $GABA_B$-ergic circuitry in M1 as a cortical mechanism of SP. Also, motor preparation, motor task, attention to motor function, visual input or hyperventilation can all modulate the SP (Groppa et al. 2012)

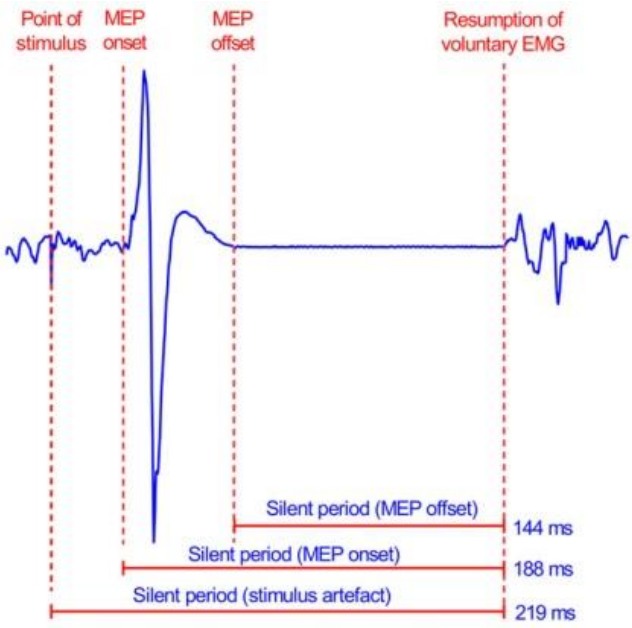

FIGURE 13. Silent periods depending on the definition of the onset. From Škarabot et al. 2019

4.3 Intracortical circuits

Paired-pulse techniques essentially allows the examination of how local circuits or afferent input arriving from other areas of the brain modulate motor cortical excitability. The inputs can have either inhibitory or facilitatory effects and can be applied through connections from within the cortex (intracortical), from the same hemisphere (intrahemispheric) or from the opposing hemisphere (interhemispheric). The method relies on conditioning stimulus that is given somewhere in the brain prior to a test stimulus. Results can then be analysed by comparing the size of the conditioned MEP with a MEP response from a test stimulus alone (Hanajima & Ugawa 2008).

Short-interval intracortical inhibition. Kujirai et al. (1993) observed that when a subthreshold stimulus is applied prior to a suprathreshold from the same stimulating coil, with an interval of 1-6 ms it would lead to a suppression of the MEP (Figure 14) (Kujirai et al. 1993). However, the same conditioning stimulus does not reduce the amplitude of MEPs induced by TES nor does it inhibit the size of H-reflex either. This led to the conclusion that reduction in the MEP size occurs at primary motor cortex and is termed short-interval intracortical inhibition (SICI). (Hanajima & Ugawa 2008). It has been observed that SICI peaks at interstimulus intervals (ISI) of 2,5 ms and likely represents post-synaptic inhibition from $GABA_A$ receptors as drugs that enhance $GABA_A$ neurotransmission also enhances SICI. The intensity of the test-stimulus should be chosen so that it is at the mid-range of the IO-curve as MEPs elicited with this intensity are sensitive to both inhibition and facilitation. Often an intensity set for 110-120% of the MT (Rossini et al. 2015).

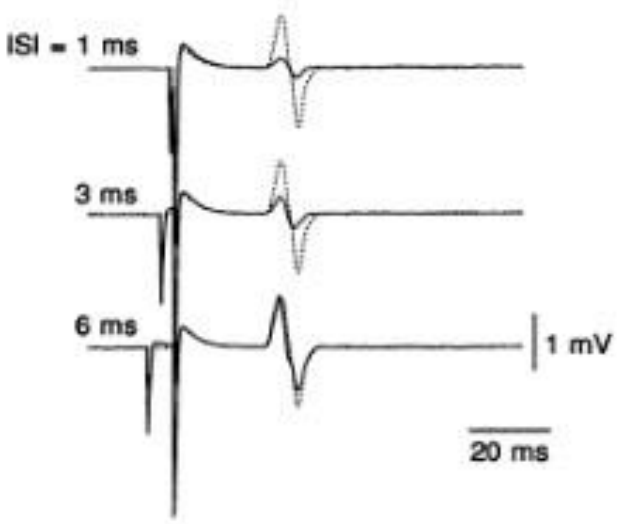

FIGURE 14. Conditioned responses with different ISI. Dashed line represents the unconditioned MEP. From Kujirai et al. 1993.

Voluntary activation decreases SICI in comparison to resting state in intrinsic hand muscles (Roshan et al. 2003; Ortu et al. 2008). Soto and colleagues studied the effects of rest, voluntary activation and postural activity on cortical parameters of soleus muscle control. Firstly, they found out that SICI is similar in the soleus muscle in terms of magnitude and time course as in the intrinsic hand muscles. Secondly, they observed that SICI is reduced when comparing resting state and voluntary activation. Additionally, SICI was equally reduced in the postural task as it was in the steady-state voluntary contraction task. They concluded that motor cortex might control soleus muscle also in the postural tasks (Soto et al. 2006). Hunter et al. studied how fatigue changes the intracortical circuits in biceps brachii contractions. During the contractions, a steady motoneuronal output was maintained via matching the contraction to EMG levels instead of force. In their brief control contractions at 25% of maximal voluntary contraction (MVC) both SICI and intracortical facilitation (ICF, explained below) were observed. As the 10-minute, sustained fatiguing contraction started, both the amount of SICI and ICF were shown to reduce, meaning that the conditioned and unconditioned MEPs were similar. Throughout the sustained fatiguing contraction, the size of the unconditioned MEPs did not change. Interestingly, as the excitability of the intracortical circuits were changed, it was

not directly associated with an increase in the perceived exertion or decrease in maximal voluntary force (Hunter. et al. 2016).

Similar to how MEPs are modulated through afferent input, SICI is also subject to changes due to different afferent modalities. Muscle vibration has been shown to increase MEP amplitudes while at the same time decreasing the SICI response. This result followed the somatotopical arrangement meaning that the responses were only observed in the muscles vibrated. However, SICI was seen to increase in the non-vibrated muscles (Rosenkranz & Rothwell, 2003). Another afferent modality that effects SICI is mechanical stimulation. When the whole hand is stimulated mechanically at 25Hz, SICI decreases. The decrease in SICI was found to last 1 h post-stimulation. At lower frequency (10Hz) these changes were not seen (Christova et al. 2011). Whole-hand electrical stimulation has a similar effect on SICI. Compared to sham-intervention, electrical stimulation of the hand leads to a decrease in SICI and overall increase in corticospinal excitability. The effects of electrical stimulation also are dependent on the stimulation parameters. Most prominent changes in SICI were observed at intensity above sensory perception threshold at 50Hz and motor level at 2Hz. (Golaszewski et al. 2010; 2012).

Intracortical facilitation. In the same study in which Kujirai et al. (1993) described an inhibition of MEP, they found a facilitation of MEP with subthreshold conditioning stimulus at ISIs of 10-15 ms with an identical protocol (Figure 15). H-reflexes also remained unfacilitated, so it was concluded that it occurs at the level of the motor cortex and called intracortical facilitation (ICF). It is thought of as a net facilitation from both higher facilitation and weaker inhibition. The facilitation is thought to be mediated by glutamatergic N-methyl-D-aspartate (NMDA) receptors as an antagonist to these receptors decrease ICF. However, ICF is less extensively studied compared to SICI but it is accepted that it mainly tests the excitability of the NMDA receptor dependent excitatory circuits (Hanajima & Ugawa 2008). Another mechanism involved in ICF might be a recruitment of additional circuits that are not present in the single pulse generated MEP and thereby producing additional descending activity (Di Lazzaro & Rothwell, 2014).

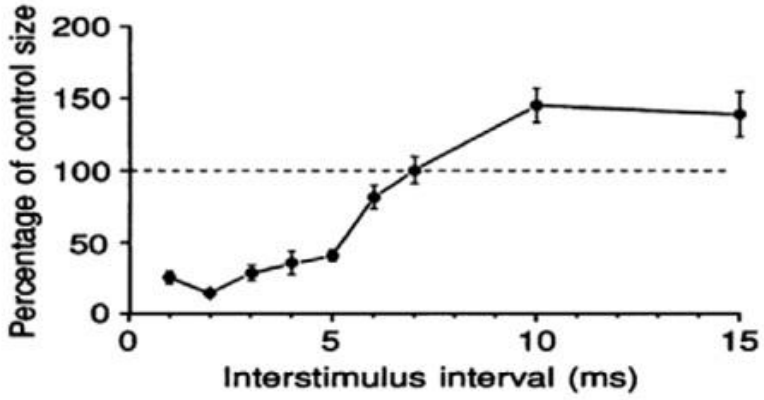

FIGURE 15. Facilitated MEPs in paired pulse-pulse paradigm with ISI of 10 – 15 ms. From: Kujirai et al. 1993

Short-interval intracortical facilitation. When using a pair of magnetic stimuli of 100% MT or suprathreshold conditioning stimulus followed by a subthreshold test stimulus a facilitation of MEP can be observed. This facilitation occurs at ISIs of 1.0-1.5 ms, 2.5-3.5 and at 4,5 ms. Again, as test stimulus elicited with TES does not show any facilitation the likely location for it is the motor cortex and is termed short-interval intracortical facilitation (SICF). Physiologically it is thought that SICF represents a direct (non-synaptic) activation of the interneuron axons that were made hyperexcitable by the excitatory post-synaptic potentials elicited by the conditioning stimulus (Hanajima & Ugawa 2008).

Long-interval intracortical inhibition. When two suprathreshold stimuli are given from the same stimulating coil at long ISIs of 50-200 ms an inhibition of the test MEP can be seen. Of intracortical origin and mediated by $GABA_B$ receptors, the long-interval intracortical inhibition (LICI) resembles the silent period but the two phenomena are not identical. Epidural recordings have revealed that at 50 ms ISI the I-waves are enhanced even though MEPs recorded by surface EMG are inhibited. Hence the conclusion that like cortical silent period, MEP inhibition at 50 ms probably has a spinal mechanism that overrides the supraspinal facilitation (Hanajima & Ugawa 2008; Rossini et al. 2015).

5 WATER – AN ENVIRONMENTAL CHALLENGE FOR THE SYSTEM

Early documents date the use of water for healing and religious purposes as early as 2300BC. Originally from the Far East, the use of water as therapeutic medium arrived in Europe and England in 800BC. Hippocrates (460-375BC) has documented the use of hot and cold-water immersion in treatment of various bodily ailments including muscle aches and joint pain. Recreational bathing culture originated from Greece but was expanded by Romans for both recreational and exercise purposes. In 330AD, Romans used their baths for healing and treatment of rheumatic disease, paralysis and injuries. Spanning centuries, with varying interest towards water, whether for recreational, healing or exercise purposes, few therapeutic modalities has such a long history as the use of water has (Brody & Geigle 2009, 4).

Today benefits of aquatic exercise have been investigated for different clinical populations to establish an evidence base for aquatic therapy as rehabilitation modality. Aquatic exercise has been shown to improve on short term patient-reported pain, disability and quality of life in people with hip and knee osteoarthritis. Also, it is considered as safe and accessible treatment modality (Bartels et al. 2016, Waller et al. 2014, Lu et al. 2015). When expanding the literature to include other musculoskeletal disorders, such as rheumatoid arthritis, fibromyalgia, low back pain or osteoporosis, aquatic exercise seems to reduce pain and improve physical function and quality of life equally to land based activities (Barker et al. 2014, Bidonde et al. 2014). Although, insufficient resistance application might hinder some studies, opposing results have been found with land exercises having superior outcomes when comparing achieved strength levels (Heywood et al. 2017). Patients suffering from cardiovascular diseases, such as heart failure or high blood pressure benefits equally from aquatic exercising when compared to land-based activities again providing an alternative for patients unable to join traditional training programmes (Adsett et al. 2015, Igarashi & Nogami, 2018). Also, in hypertensive patients low-intensity aquatic exercise has been shown to improve anxiety levels, functional autonomy and oxidative dysfunction (Da Silva et al. 2018). Water-based exercise interventions have also been used in neurological rehabilitation. In Parkinson's disease, gait variability has been shown to reduce, or become more stable after a six-week aquatic intervention programme compared to usual care or when added to land-based training programmes (Carroll et al. 2017, Palamara et

al. 2017). Improvements in static and dynamic balance were also seen in patients with hemiplegia (Methajarunon et al. 2016). Children affected by cerebral palsy have improved their gross motor skills after aquatic exercise, although more research is warranted (Roostaei et al. 2017). Women suffering from multiple sclerosis have been shown to improve their functional capacity, balance, gait stability and fatigue perception, one of the key features of MS (Kargarfard et al. 2018, Methajarunon et al. 2016). Spinal cord injury patients have also been shown to improve physical function, aerobic fitness and spasticity levels (Li et al. 2017, Kesiktas et al. 2004). When gait was specifically trained with underwater treadmill training, significant improvements were found in both physical function and walking ability in adults with spinal cord injury (Stevens et al. 2015).

5.1 Physical characteristics of water

Aquatic environment poses unique physiological stresses on humans. Considering that our day to day activities occur on dry land and with oxygen available, aquatic environment can be viewed as hostile towards humans. However, as a failure to maintain homeostasis in underwater environments would lead to injury or even death, our bodies are capable of multiple adaptations towards the physiological stressors occurring in water. Some of the environmental challenges include increased hydrostatic pressure that effects circulation, fluid and electrolyte balance and locomotion. In addition, water temperature, whether cold or warm, challenges our thermal regulation systems. These challenges must be countered immediately even at the surface levels, but they are further increased with increasing depths (Pendergast et al. 2015). Much of the biological effects water immersion causes are related to the principles of hydrodynamics (Becker 2009).

Density. The relative density of an object determines whether an object will float. Relative density is the ratio of the weight of the object to the weight of an equal volume of water. If the value is greater than one the object will sink and if the value is lower than one the object will float. If, however the value is exactly one, the object will float just below the surface (Bates & Hanson 1996, 21). Despite human body being mostly water, the density of the human body is slightly less than the density of water. It averages a relative density of 0.974 with men averaging

higher density than women. Typical density of lean body mass, including bone, muscle, connective tissue and organs is near 1.1. Fat mass, including both essential body fat and excessive fat mass, on the other hand has a density of approximately 0.9. Therefore, fit and muscular individuals tend to have relative densities greater than one whereas unfit and obese individuals might have lower densities (Becker 2009).

Hydrostatic pressure. Fluid has a pressure effect that is the product of its density and depth called hydrostatic pressure (Pöyhönen 2002). The pressure is directly proportional to both values and therefore, the hydrostatic pressure increases with increasing fluid depth and density (Becker 2009; Pöyhönen 2002). While submerged the Pascal law states that the body experiences the pressure equally from all directions and it is distributed equally along the surface at given depth (Pöyhönen 2002) (Figure 16). From atmospheric pressure baseline the hydrostatic pressure increases linearly with increasing depth. Hydrostatic pressure has a significant effect on cardiovascular system. In short, the external pressure against the human body increases the blood volume by up to 60% that is displaced towards the centre of the body away from periphery. Increase in blood volume leads to increase in cardiac volume by up to 30% that in turn leads to increase in stretch of the myocardium and thus to a stronger contraction. Taken together increase in both central blood volume and heart muscle contraction force, stroke volume increases on average by 35% when a human is immersed to neck level (Brody & Geigle 2009, 29).

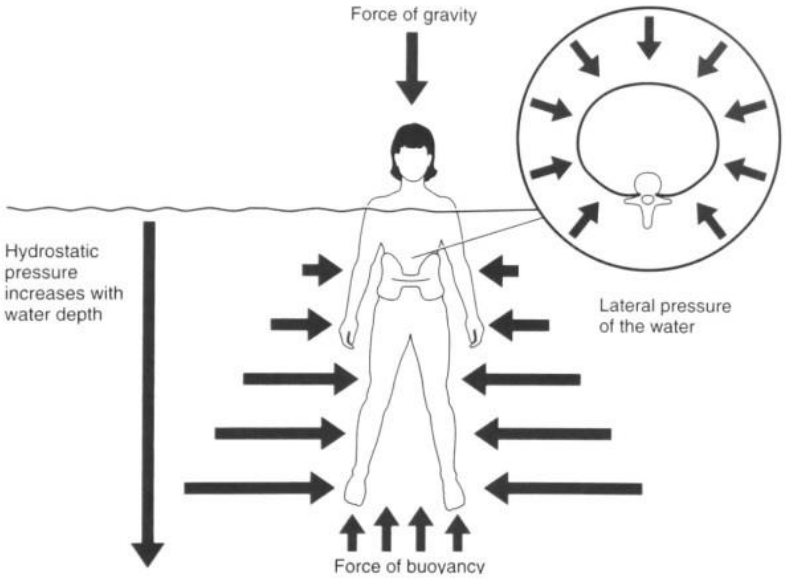

FIGURE 16. Hydrostatic pressure, buoyancy and gravitational forces illustrated on a human during water immersion. From: Bates & Hanson, 1996.

Buoyancy. Archimedes' principle states that when a body is fully or partially submerged in a fluid at rest, it experiences an upward thrust equal to the weight of the fluid displaced (Bates & Hanson 1996, 22). Buoyancy, that is the opposite force against gravity, is related to the density of the immersed object. During gradual body immersion water gets displaced and simultaneously the force of buoyancy starts to affect. A human body that has relative density of 0.97 needs to have 97% of the total body volume submerged in order to float, meaning that through the displacement of water the upward force of buoyancy is equal to the downward force of gravity (Becker 2009, Bates & Hanson 1996, 22). Buoyancy either assists, resists or supports movement in water (Brody & Geigle 2009, 26) (Figure 17). Brito Fontana et al. (2015) compared peak ground reaction forces and cadence between dry land running and varying depths of water running from hip level up to chest depth. Unsurprisingly they found out that peak ground reaction forces were 17 to 58% lower during water running. Also, cadence was observed to be lower in water running. Both parameters were found to decrease as a function of increasing water depth, but cadence did not influence ground reaction forces.

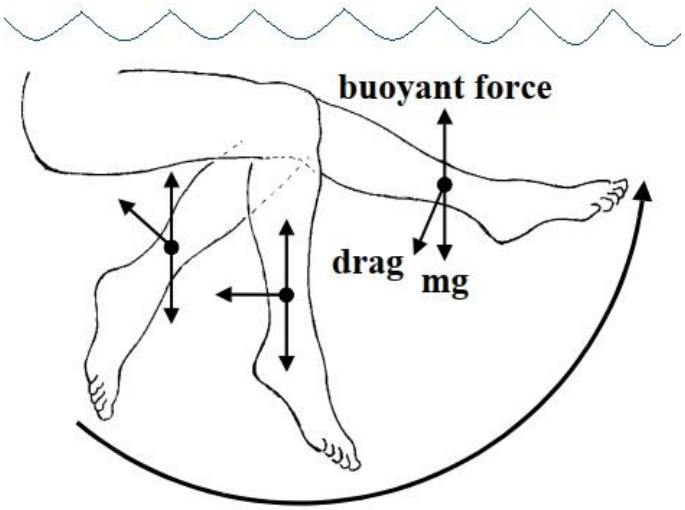

FIGURE 17. Buoyancy together with drag force schematically presented acting on the moving leg during knee extension and flexion during water immersion (mg = mass x gravity). From: Pöyhönen 2002.

Movement slows down quickly in the water. The water resists movement with drag force that is a function of viscosity, frontal shape of the object, size of the object and the relative velocity between the object and the water. Viscosity is the friction between liquid or gas molecules. It results in either adherence to each other (cohesion) or as is the case in water, adherence to submerged body (adhesion). Because water is more viscous than air, water causes more restriction to movement than air. Combination of objects (or extremities) surface area and speed determines the amount of resistance of movement caused by viscosity (drag). During functional movements, drag provides different stimuli to muscles when compared to land-based activities (Aquatic exercise association, 101-102). Castillo-Lozano et al. (2014) compared muscle activity between different movement speeds and movement planes during an arm elevation in and out of water, movements where drag would be the force behind possible changes. Movements were flexion, abduction and scaption (elevation at 45º shoulder abduction and 45º external rotation) from 0-90º at three different speeds, (30º/sec, 45º/sec, and 90º/sec). Overall their findings show that during water immersion there is a trend towards lower muscle activities with lower movement speeds. This is expected when buoyancy assists arm elevation at lower

speeds. However, when transitioning into higher movement speeds the muscle activity was higher underwater, as would be expected because of increase in drag force.

5.2 Cardiovascular system and fluid shifts

Amount of fluids in the human body ranges from 50 to 60%. This fluid is located either in intracellular space (35-40%), interstitial space (between cells, 11-15%) or intravascular space (blood plasma, 4-5%). Fluid acts as a transportation vehicle for metabolic wastes and nutrients between the body and the surrounding environment. It is possible to move fluid and materials between intravascular and extravascular space through capillaries via diffusion, vesicular transport and filtration-reabsorption. In the case of water immersion, mechanism of interest is filtration-reabsorption (or autotransfusion) since it causes a net movement of fluid through capillary-interstitial pressure gradient (Figure 18). Because water immersion even at moderate depths causes a pressure gradient between interstitial and intravascular compartments, it leads to a fluid shift from the former to the latter. Therefore, plasma volume increases, and blood is displaced away from the periphery through venous and lymphatic system towards the centre of the body (Wilcock et al. 2006, Becker 2009, Ayme et al. 2013). Plasma volume increases in the first 20 to 25 minutes of water immersion and then remains on constant level. This change occurs equally in different water temperatures (32, 34,5 and 36 °C) (Yamazaki et al. 2000). As a result, cardiovascular stretch receptors are mechanically stimulated, the reflex activation leads to increased stroke volume and cardiac output. Note that the increase in cardiac output is due to increased stroke volume as heart rate decreases (Pendegast et al. 2015). In addition, water immersion is known to cause fluid shifts that aid the return of fluid from the muscles into the blood (Wilcock et al. 2006).

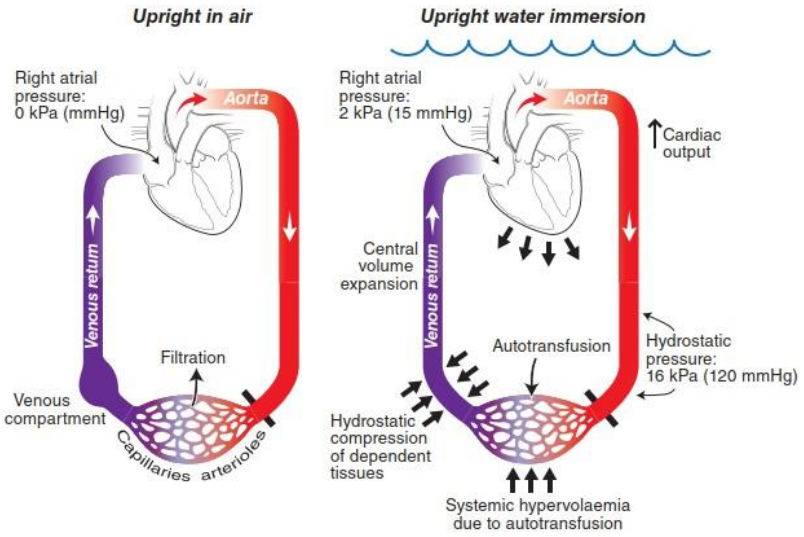

FIGURE 18. Hydrostatic pressure compresses the dependent tissues causing capillary reabsorption (or autotransfusion) in dependent limbs. This leads to increase in central volume with increasing right atrial pressure and ultimately, the cardiac output. From: Pendergast et al. 2015

Subjects immersed in water have also reported to perceive less fatigue. Because the water has a buoyant force that reduces gravitational forces acting on the human body, it could provide a relief for the gravitational muscles and thus help to conserve energy. The reduction in the perceived levels of fatigue can be attributed to the above neuromuscular mechanisms via increased inhibitory pathway activity or just by providing a psychological element (Wilcock et al. 2006).

5.3 Neuromuscular responses to water immersion

Water immersion also causes responses in the neuromuscular system in order to adapt to the environmental challenge. It has been reported that EMG activity and maximal voluntary contraction has been reduced in plantarflexion muscles when compared immersed condition to dry land (Pöyhönen & Avela 2002). Similar findings have been reported with knee extensor muscles regarding the EMG activity, but not in force output. These reduced EMG amplitudes could be due to electromechanical alterations caused by the water environment or because of

neurophysiological alterations (Pöyhönen et al. 1999). Another study that compared muscle activity of lower extremities and trunk muscles during a sit-to-stand task during water immersion and on dry land reports equally reduced EMG responses in other lower extremity muscles, including the long head of biceps femoris, tibialis anterior and gastrocnemius medialis. However, trunk muscle activity in rectus abdominis and erector spinae muscles was found to be higher (Cuesta-Vargas et al. 2013). However, Colado and colleagues (2013) found no differences in trunk muscle activity (latissimus dorsi, erector spinae and rectus abdominis) when doing shoulder flexions with different devices and depths at different velocities. Only difference found was higher activity of the latissimus dorsi muscle at xiphoid level depth. Shoulder muscle activity was not measured.

There is contradicting literature reporting that EMG activity underwater is like that of measured on dry land. It is speculated that instead of neurophysiological mechanisms, the differences between the results are due to inefficient water proofing (Silvers & Dolny 2011, Rainoldi et al. 2004, Veneziano et al. 2006). However, one of the studies used a manual muscle testing method was used on both immersion and dry land condition that could result in different EMG activity during a maximal voluntary contraction (Silvers & Dolny, 2011). In the studies by Rainoldi et al. (2004) and Veneziano et al. (2006) the immersion protocol differed from those of in the previous studies. Rainoldi and colleagues (2004) used only a partial immersion was used so that only the muscle group in question was immersed compared to a head out of water immersion in the study by Pöyhönen (1999). Methodologically more robust study in terms of force measurements was done by Pinto and colleagues (2010). When comparing MVC force and EMG values in upper extremity (m. biceps brachii and m. triceps brachii) and lower extremity (m. rectus femoris and m. biceps femoris) they found no differences in either force or EMG values between dry and immersed conditions (Pinto et al. 2010).

The effects of water immersion onto stretch reflex excitability has been studied using H-reflex and stretch reflex measurements. The results have varied between the studies. An early study by Dietz and colleagues (1989) observed a reduction in stretch reflex responses caused by balance perturbation under water but the responses increased linearly with increasing loading on the subjects. Similar observations were found with increasing depth levels where smallest stretch reflex amplitudes due to perturbation were found with neck level depths (Dietz &

Colombo 1996). Pöyhönen & Avela (2002) reported a trend towards diminished H-reflexes and stretch reflexes from Achilles tendon (Pöyhönen & Avela 2002). However, when using H-reflex measurements, also opposite results have been obtained. In a study by Nakazawa et al. (2004) the H-reflexes were measured with altering the gravitational load on both ankle and knee joints (Figure 19). They found out that as the gravitational loads were increased in either ankle or knee joint, the H-reflexes diminished. Also, when the gravitational loads were decreased from either joint, the H-reflexes increased. They hypothesized that because of the buoyancy of the water, gravitational input from joint receptors are reduced leading to diminished inhibitory mechanisms and therefore increased reflex sensitivity (Nakazawa et al. 2004). Similar results have been reported by Cronin et al. (2016); they found out that the H-reflexes increased in the acute phase of water immersion. After returning to dry land there was a non-significant reduction in the H-reflexes compared to pre-immersion values (Cronin et al. 2016). A key difference in studies between Pöyhönen & Avela (2002) and Cronin et al. (2016) is that in the former waterproof trousers, or waders, were used. This would cause the hydrostatic pressure to compress the trousers around the extremity providing tactile feedback that could have inhibitory effects on reflex excitability, as observed by the reduction in H-reflex amplitude (Pöyhönen & Avela 2002, Cronin et al. 2016). In functional movements, such as walking, H-reflex has been shown to retain its' phase-dependent modulation under water, but the reflex gain is higher than that of on land (Miyoshi et al. 2006).

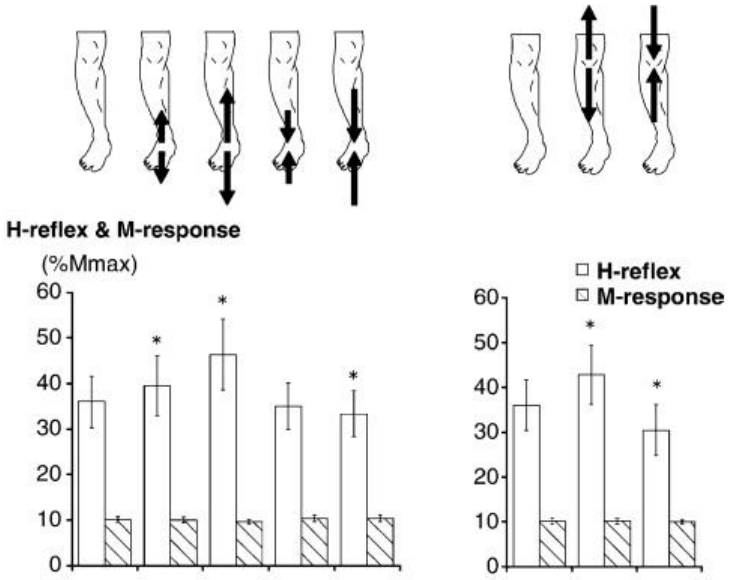

FIGURE 19. H-reflexes and M-waves during water immersion with altered joint loads. Arrows around the joint describe whether a small or large increase/decrease was applied to the joint. Bar graphs indicate their corresponding H-reflex responses. From: Nakazawa et al. 2004.

Further evidence for the altered proprioceptive feedback during water immersion is provided by MU studies. Sugajima et al. studied hip flexor muscles and their recruitment during shoulder level water immersion. Hip flexion under water resulted in larger motor unit spike amplitudes compared to dry land hip flexion which reflected the more traditional recruitment from small to large motor units. They concluded that as proprioceptive feedback can alter the orderly recruitment especially in static contractions, weightlessness reduced said feedback by releasing muscles from their weightbearing task (Sugajima et al. 1996). Research by Dalecki & Bock also demonstrates a degrading effect of water immersion on the proprioceptive feedback for isometric tasks and passive arm positioning. However, during an active movement with a purposeful position task the proprioceptive feedback seems to increase perhaps because of the high viscosity of the water. They found out that during active arm movements precision was increased (Dalecki & Bock 2013). Isometric force exaggeration has also been reported during water immersion. By using a joystick, subjects were instructed to match low force levels (5, 15 and 25N) to different directions without visual feedback. A 24% increase in the peak force

production was reported in the study, a result which the authors explained with a lack of proprioceptive feedback (Dalecki et al. 2012). In a continuation study, the same authors compared the isometric force matching tasks with and without visual feedback. A similar increase in the peak forces was observed that was drastically reduced with the use of visual feedback, a known compensation for the lack of proprioception. However, this compensation was not enough as there was a small but significant difference between the force matching tasks in dry and immersed conditions (Dalecki & Bock 2014).

Dalecki & Bock (2013) suggested that water immersion could lead to changes in the muscle tone while passively seated in the water (Dalecki & Bock, 2013). However, Kubo et al. (2004) examined the mechanical properties of the triceps surae muscle before and after warm and cold-water immersion; they compared muscle fascicle, Achilles tendon and aponeurosis behaviour while the muscle-tendon complex was at rest or during an isometric contraction. Based on their results, there were no changes in any of the parameters in either of the muscle conditions (Kubo et al. 2005). Change in muscle tone could be related to fluid shifts as shifts in blood volume (independent from vestibular, visual or proprioceptive feedback) can alter the sense of posture (Vaitl et al. 1997).

5.4 Effects of water immersion at the cortical level

Because water environment has specific physical properties it can be inferred that water immersion activates several distinct somatosensory modalities such as tactile, pressure and thermal sensations. Sato and colleagues have studied the effects these modalities caused by water immersion on cortical processing. Somatosensory input from periphery activates several cortical areas and can be evaluated by somatosensory-evoked potentials. In their study they examined the somatosensory-evoked potentials caused by median nerve stimuli were recorded with electroencephalography from nine scalp electrodes. They found out that these potentials are attenuated under immersed condition, suggesting a gating mechanism on the short-latency somatosensory-evoked potentials, providing evidence for water immersion to be capable of influencing cortical processing of somatosensory inputs. Other tactile stimuli, such as rubbing of the palm have been found to have a similar effect (Sato et al. 2012). In a different study by

the same author, they utilized functional near infrared spectroscopy (fNIRS) that is non-invasive and uses intrinsic optical imaging to measure cerebral blood flow. Compared to dry land, water immersion increased blood flow in sensory- and motor-related areas (Figure 20). Initially increases were found at the S1 and the parietal association area and as the immersion progressed changes were also recorded in the M1 and supplementary motor area (Sato et al. 2012).

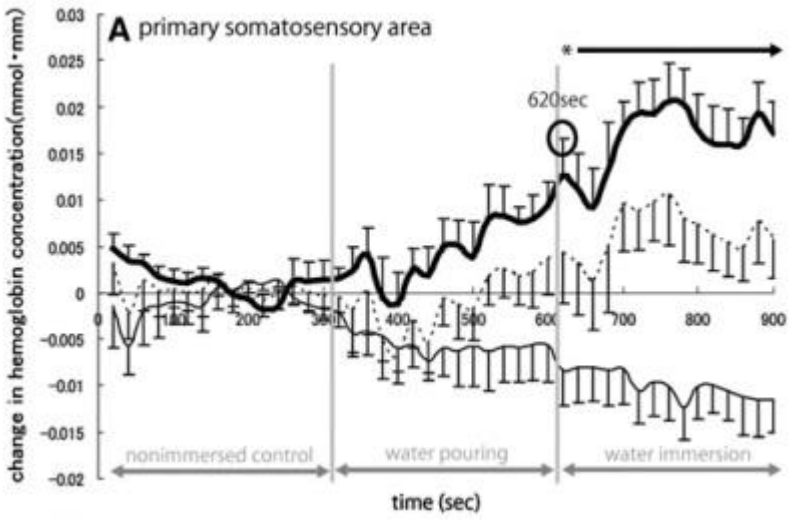

FIGURE 20. Increase in blood flow in the primary somatosensory area. Heavy line presents oxygenated haemoglobin, thin line indicates deoxygenated haemoglobin. Dashed line indicates the total haemoglobin. From: Sato et al. 2012.

Cortico-cortical behaviour under water immersion has also been studied. In a paired-pulse paradigm, Sato et al. (2013) examined the inhibitory circuits within the motor cortex activated via afferents of the target area, at two different latencies, 20 ms ISI for short-latency afferent inhibition (SAI) and 200 ms ISI for long-latency afferent inhibition (LAI). SAI and LAI are hypothesized to result from cortico-cortical inhibitory transmission that originates from the somatosensory cortex. In addition to SAI and LAI, SICI and ICF were examined. Electromyography responses were recorded from first dorsal interosseous muscle. It was discovered that both SAI and LAI decreased significantly during water immersion but returned to baseline afterwards, meaning that the amount of inhibition caused by the afferent volley

reaching somatosensory cortex was decreased during water immersion. SICI and ICF remained unchanged by the environmental change. However, this could be because the whole limb was not underwater in the study due to electrical stimulation of the median nerve required for SAI and LAI (Sato et al. 2013). To counter this limitation, Sato and colleagues devised another study in which they would immerse the extremity under investigation completely (Sato et al. 2015). Conditions under which the examinations were conducted were dry land (control), whole-hand water immersion and whole-hand water flow, 15 minutes per condition. Parameters measured with TMS included MEPs, IO-curves and SICI from target muscle (first dorsal interosseous). The findings show that corticospinal excitability assessed via IO-curve was unchanged in water immersion condition but was significantly increased in the water-flow condition (Figure 21). Also, SICI was decreased and ICF increased in the water-flow condition but remained unchanged in the water immersion condition (Figure 22). They concluded that water immersion and hydrostatic pressure alone would not be enough to alter corticospinal excitability whereas increased cutaneous stimulation from the water flow stimulation exceeds the required threshold (Sato et al. 2015, Sato et al. 2014).

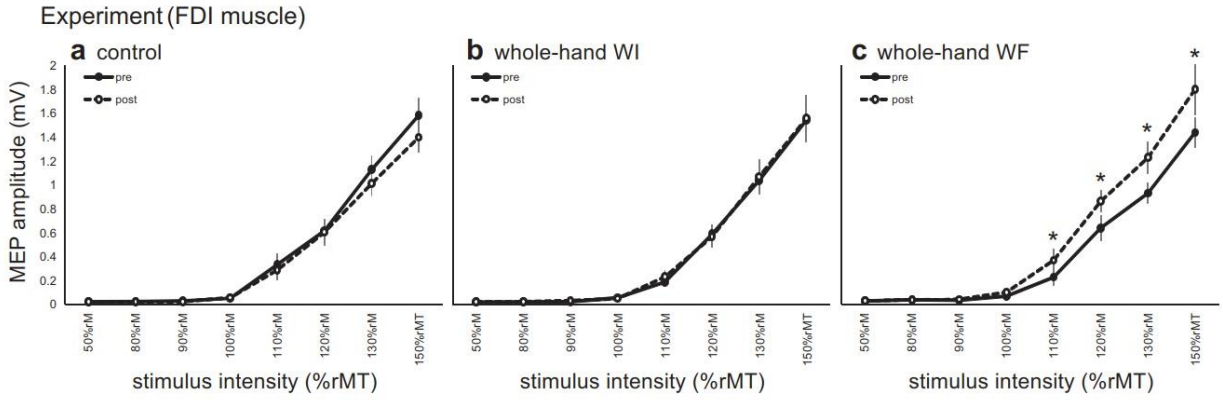

FIGURE 21. IO-curves obtained from the first dorsal interosseous muscle during dry, water immersion and water flow conditions. Asterisk indicates statistically significant findings. From: Sato et al. 2015.

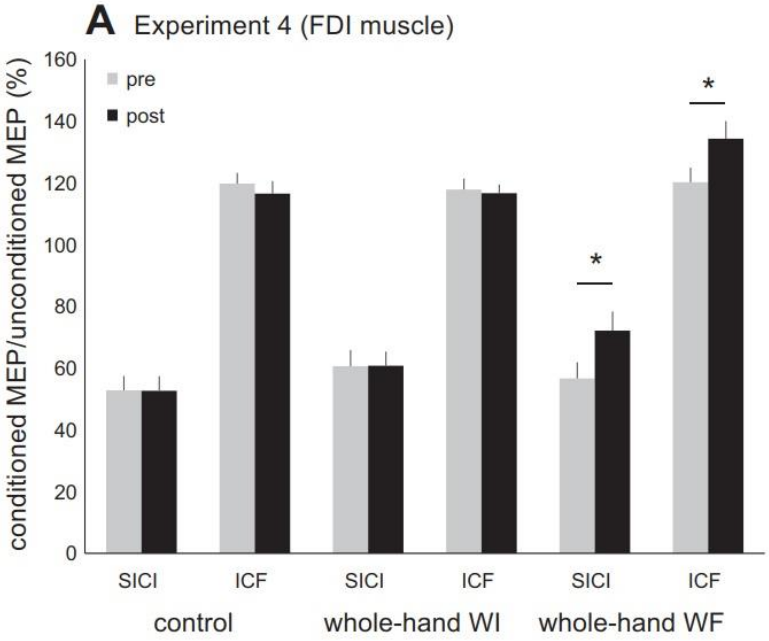

FIGURE 22. SICI and ICF obtained from first dorsal interosseous muscle during dry land (control), water immersion (WI) and water-flow (WF) condition. Asterisk indicates statistically significant findings. From: Sato et al. 2015.

6 PURPOSE OF THE STUDY

Given that aquatic environment is used for both rehabilitation and as a training environment for the athletes (Park et al. 2014, Stevens et al. 2015) despite having limited information on how the water immersion alters our motor control, this study aims to investigate whether water immersion changes corticospinal excitability of the soleus muscle. As all previous examinations of water immersion on corticospinal excitability have been done on upper extremity muscles, we targeted soleus specifically for its' role in gait and as the motor control of lower extremity muscles differs from that of upper extremity. Lastly, this is the first study that assesses corticospinal excitability of when target muscle is voluntarily activated during water immersion. Therefore, it sheds light whether the control of an isometric, steady-state contraction differs between dry land and during immersion when the subject is seated.

Based on the literature we hypothesize that passive sitting in the water would not be enough to alter corticospinal excitability on afferent feedback alone (as was concluded by Sato et al. 2015) caused by still water immersion. During voluntary activation, we hypothesized that water immersion might have an excitatory effect on cortical excitability due to fluid shifts in the muscles and as there is evidence that spinal excitability is increased in water immersion.

7 METHODS

Nine (9) healthy male subjects (age: 26 ± 2, weight (kg): 85 ± 15, height (cm): 180 ± 9) volunteered for the study. The subjects were recruited via e-mail and word-of-mouth advertisement to participate in two separate experiments: one active session (Active Experiment, AE) and one passive session (Passive Experiment, PE). Both experiments consisted of two conditions: "DRY" for measurements obtained on dry land and "WATER" for measurements obtained during water immersion. All participants were without any known neurological, cardiovascular disease or musculoskeletal disease. Prior to data collection all participants provided a written and informed consent and answered a TMS safety questionnaire. All the experimental procedures were approved by the University of Jyväskylä ethics committee (29.01.2019) and conducted according to the Declaration of Helsinki at University of Jyväskylä laboratories.

7.1 Experimental setup and data recording

7.1.1 Protocol

The subjects were randomized to start with either AE or PE. Protocol used for both AE and PE is schematically represented in Figure 23. In the AE, after EMG preparation and waterproofing, the subjects were seated into a custom-built chair that allows for plantarflexion force recording. The subjects began with three (3) warm-up contractions with increasing force levels, each separated by 2 min. After the warm-up contractions three maximal voluntary trials were performed. If the force increased for more than 10% between the first and the highest, fourth trial was included to ensure that the true maximum was found. From the maximal force level, the 20% MVC force line was drawn on the computer screen for the subjects to follow in rest of the experiment. Force trials were followed by the hotspot determination during the 20% MVC contraction and after which the aMT was determined. To counter for fatigue development the subjects were given a rest period after each contraction/stimulation setting. After aMT measures either IO-curves or paired-pulse stimulations were recorded according to randomization. Figure 24 shows the the experimental setup for "DRY" condition.

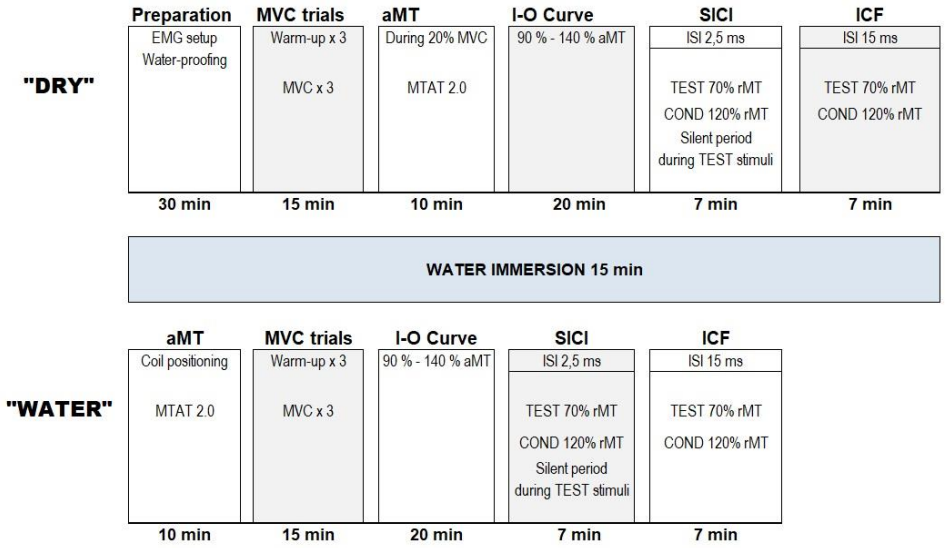

FIGURE 23. Schematic representation of the protocol in both AE and PE. Times reported are inclusive of the resting times between the stimulation protocols.

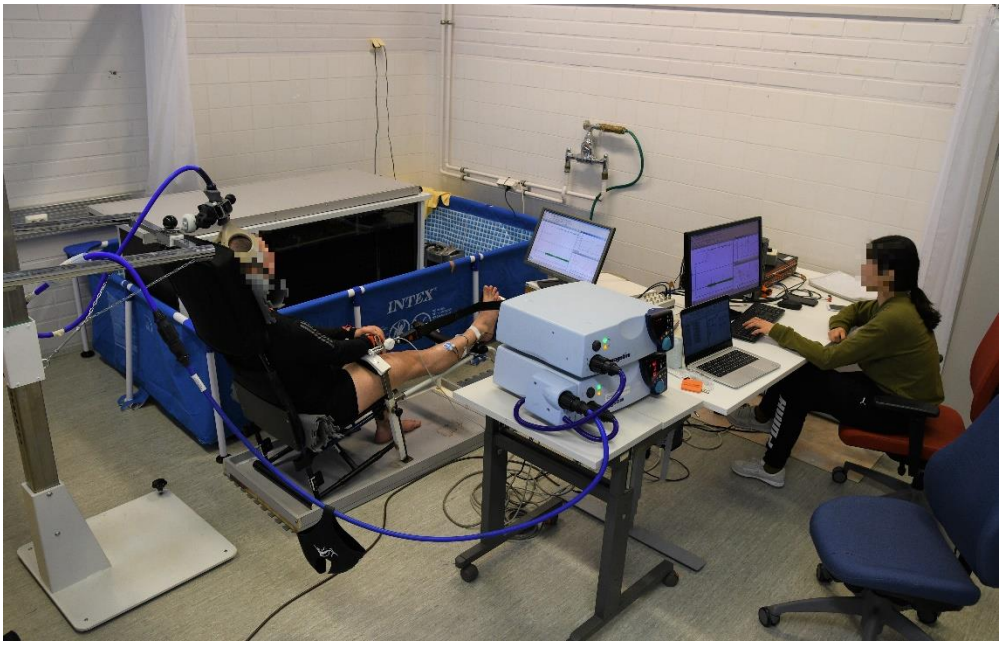

FIGURE 24. Experimental setup during “DRY” condition.

After “DRY” measures the chair was moved into the water tank and the subject was seated on the chair in the tank (Figure 25). After immersion a 15-minute waiting period was begun prior

to starting measurements. The EMG levels were monitored throughout the immersion and measurements were stopped if the data seemed corrupt due to water infiltration. The water tank was covered with a lid, on top of which the computer screen was moved to allow for the observation of the force level. The TMS coil was placed on top of the subjects' head and secured in place. The stimulation site was verified with a few test stimulations. After the 15-minute waiting period the subjects again performed the three warm-up contractions followed by the MVC trials. Again, based on the MVC result the 20%MVC line was drawn for the subjects for the rest of the experiments. The measurements followed with aMT determination like that of dry land after which the IO-curves or paired-pulse stimulations were given according to the randomization. The AE session lasted approximately 2-2,5 hours.

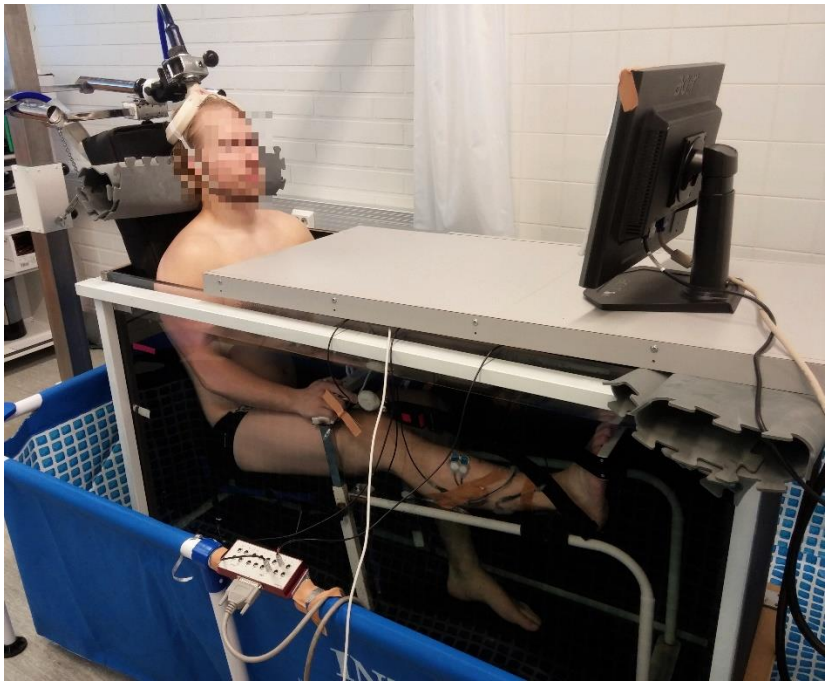

FIGURE 25. Water-immersion in AE with TMS coil-holder in place.

The PE protocol followed an identical setting as the AE. The measurements started with subject preparation and "DRY" measurements. Measurements followed the same pattern starting with the warm-up and MVC trials. The resting motor threshold was determined next and according to randomization followed with either IO-curves or paired-pulse stimulations. All the measurements were conducted with soleus muscle at rest. During stimulations, subjects were

given an attention task in which they were asked to count from 200 backwards. After "DRY" measures the subject moved to the water tank and after the 15-minute waiting period the measurements began with warm-up and MVC trials, followed by the rMT investigation and finished with either IO-curves or paired-pulse stimulations based on the randomization. The PE session lasted approximately 1,5-2 hours.

In both AE and PE sessions the subjects began with "DRY" measures followed by "WATER" measurements. The subjects remained in the water tank for the whole measurement session without exiting the tank as this would have been a practical inconvenience that would have elongated already a long measurement protocol. The duration of the immersion was approximately one hour for the AE and 45 minutes for the PE.

7.1.2 Electromyography

Electromyography data was recored from two muscles, m. tibialis anterior (TA) and m. soleus via bipolar setting with Ag-AgCl, surface electrodes (Ambu BlueSensor N-00-S/25, diameter 6 mm). The skin underneath the electrodes was shaved, abraded and cleaned with alcohol. The resistance between the two electrodes was monitored to be lower than 2kΩ. The soleus electrodes were placed in a bipolar configuration on top of the soleus muscle, above the Achilles tendon. This configuration has been shown to result in best H-reflex responses (Botter & Vieira 2017). Tibialis anterior electrodes were placed according to SENIAM on the muscle belly, two-thirds of the line between medial malleolus and medial condyle of tibia. The ground electrode was placed on tibia. The EMG signals were amplified (3dB) and band-pass filtered (10-1000Hz) (EISA 16 – 2, Freiburg, Germany). Force (1000 Hz) and EMG (2000 Hz) were sampled with a CED1401 computer interface and Spike2 software (CED Ltd, Cambridge, UK).

A compromise from the usual belly-tendon configuration was made in order to have a secure waterproofing of the EMG setup (Figure 26). Waterproofing was done according to Silvers & Dolny (2011). Each electrode set was covered with a 10 x 8 cm strip of waterproof, transparent, adhesive film (OpSite^{TM}). In the first layer there were holes cut to the film to allow access electrode snaps. Next, the electrode wires were connected, and the buttons were covered with

silicone plugs (Cirrus Healthcare Products LLC, USA). After connecting the wires and covering the electrode buttons a large, 12 x 12 cm film of OpSite was placed on top of the electrodes. Finally, to further improve the waterproofing, thin strips were placed on the seams of the top-most layer of OpSite. Participants went through both dry and immersed measurements with the waterproofing in place.

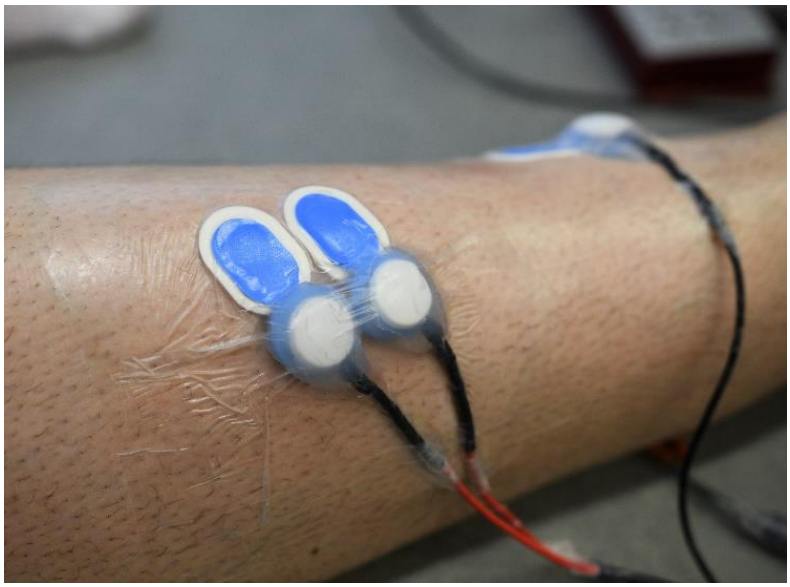

FIGURE 26. Water-proofed TA EMG setup.

7.1.3 Force-measurements and the water immersion

Participants were seated in upright position in a custom-made chair (University of Jyväskylä, Faculty of Sport and Health Sciences). The chair kept the hip and knee angle constant at 90° and 150°, respectively. The chair was fitted with a water-proof force transducer from which a strap was connected to the foot (Figure 27). The tightness of the strap was adjusted so that the ankle angle was 90°. In passive experiment the ankle was fixed at 120°.

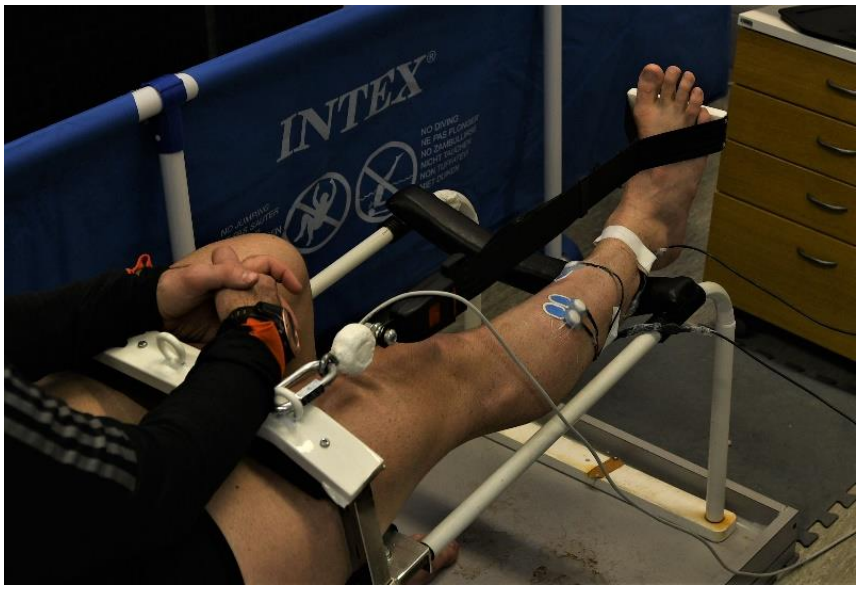

FIGURE 27. Water-proofed force transducer.

The chair was made to fit into a custom-built water tank (University of Jyväskylä, Faculty of Sport and Health Sciences), in order to allow controlled immersion. The tank (160 x 80 x 70 cm) fit all the participants comfortably. Subjects were immersed to sternum level. One side of the tank walls was made from transparent plastic to allow for observation. The tank was filled with regular tap water and the water was kept clean by changing it every week and by using commercial water cleaning system (Cristalprofi e1501, JBL, Germany, Neuhofen). Water temperature was kept at thermoneutral 34 °C (3619 Aquarium Heater, EHEIM, UK, Edmunds) and the temperature was monitored during the experiments with a thermal gauge.

7.1.4 Transcranial magnetic stimulation

The motor cortex was stimulated with a double-cone coil (2 x 126 mm in diameter) attached to two Magstim 200 stimulators connected via Bistim unit (Magstim, Dyfed, UK). Pairing of two stimulator units allows the other stimulator to deliver conditioning stimulus and the other the test stimulus. The coil was positioned over the vertex and moved in small (0,5 cm) increments to localize the hotspot eliciting MEPs in the right soleus. MEPs were elicited by monophasic, 1 ms rectangular pulses. The hotspot was determined to be the spot where there was the greatest

soleus MEP while the TA MEP remained as small as possible. Once the hotspot had been localized, it was marked on the scalp of the subject and the coil was secured in place with a custom-built coil-holder (University of Jyväskylä). Still, the position of the coil was monitored by the experimenters.

Measures of cortical excitability were motor thresholds (MT), IO-curves, SICI and ICF. In the AE, SP was also recorded. Motor threshold was determined by the MTAT program (Motor Threshold Assessment Tool v. 2.0). The cut-off for motor evoked potential (MEP) value was 0,05mV in peak-to-peak amplitude. For the aMT, the same tool was used, and it was determined to be a MEP when the response was above the background EMG (BG EMG) level (Soto et al. 2001). MT was expressed as percentage of maximal stimulator output (%MSO). IO-curves were obtained with ten stimulations at each intensity level in respect to the MT starting from 90% MT and ending at 140% MT (60 stimulations in total). Time between each stimulation at each intensity level was randomized to 5 - 8s and the order of stimulation trains at separate intensities was randomized. Paired conditioning-stimulus technique (Kujirai et al. 1993) was used to measure SICI and ICF in soleus muscle. The stimulation parameters in both were conditioning stimulus intensity 70% MT and 120% for the test stimulus with 2,5 ms ISI for SICI and 15 ms ISI for ICF (Perez et al. 2004). For both stimulation methods 10 unconditioned stimuli and 10 conditioned stimuli were delivered (40 stimulations in total for both SICI and ICF). The order of conditioned and unconditioned stimuli trains was randomized. Time between each stimulation at each stimulation setup was randomized to 5 - 8s.

7.2 Data analysis

Spike2 software was used to determine all measures during off-line analysis. In total three participants had to be excluded from analysis due to technical difficulties that occurred during measurements. The analysis was carried out with $N = 6$, who completed both AE and PE. Force data was measured as kilograms and converted into Newtons (N) for the analysis. For the MVC trials, peak force value was identified and 500 ms of mean force was analysed as the MVC force. Same time window was used to determine MVC root mean square (RMS) EMG from the rectified EMG trace. In the AE, background EMG RMS was analysed for 500 ms prior to each

stimulus and averaged from each contraction. Background EMG was normalized to that of obtained during MVCs. Bipolar EMG setting resulted in some of the MEP responses being polyphasic, therefore areas of MEPs were measured. Areas were determined from between cursors marking the first deflection from the baseline or BG EMG to when the EMG activity returned to baseline or a silent period began. Time of the area analysed remained the same despite this approach. MEPs obtained on "DRY" condition were normalized to MVC EMG area analysed from the same 500 ms window as RMS and similarly MEPs obtained on "WATER" condition were normalized to MVC EMG area of immersed MVC trials. SICI and ICF was calculated as ratio ($SICI_{RATIO}$ or ICF_{RATIO}, respectively, expressed as percentage) between the areas of conditioned and unconditioned MEP areas with the following formula:

$$SICIratio\ or\ ICFratio = \frac{conditioned\ MEP\ area}{unconditioned\ MEP\ area}\ x\ 100$$

The paired pulse stimulation paradigm was deemed successful if the result was lower than 100% for SICI and higher than 100% for ICF (Brownstein et al. 2018).

Silent period was also analysed in the AE. Silent period was determined as the duration between the cursors where first cursor was placed on the end of MEP response and second cursor at the time spot where EMG activity resumed to the level that occurred prior to stimulus. Silent period was measured from the EMG activity that occurred during SICI test stimulus contractions as the relative stimulus intensity was same for every subject (120% of MT).

7.3 Statistical analysis

SPSS version 24.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. All data are presented as means ± standard deviation (SD) (or ± standard error of the mean, SEM where stated). Significance was set at an α-level of 0,05. Shapiro-Wilk test was used to assess the normality of the data. In the case of non-normal distribution, a logarithmic transformation was computed. After the transformation, the data was analysed again both visually and statistically. If the transformation did not influence visual presentation or statistics, non-transformed data

was used in the analysis. For the IO-curves two-way repeated measures analysis of variances (ANOVA) were conducted. The within-subject factors were “condition” (two levels: dry and water) and “intensity” (six levels: from 90% MT to 140% MT). In AE another two-way repeated measures ANOVA was conducted for BG EMG activity. The two within subject factors were “condition” (two levels: dry and water) and “contraction” (eight levels: each contraction during 90% of aMT to 140% of aMT and $SICI_{TEST}$ and $SICI_{COND}$ contractions, eight in total). If Mauchly’s test of sphericity showed a significant result indicating that the sphericity assumption was violated, a Greenhous-Geisser correction was used. If a significant difference was observed, a follow-up paired t-test was conducted. Paired t-tests were also conducted on MT values (expressed as % of MSO), MVC force, MVC RMS EMG and $SICI_{RATIO}$. In active experiment further paired t-tests were conducted on SP duration and conduction time. Wilcoxon-Signed rank test was used for not normally distributed data points when pairwise comparison was conducted. Statistics are reported in detail for significant differences.

8 RESULTS

8.1 Passive experiment

ICF stimulation protocol was unsuccessful to facilitate test MEP ($SICI_{RATIO}$ was <100%) and was therefore excluded from the analysis. Data was normally distributed normally for all variables (Shapiro-Wilk $p > 0,05$) but 140% of rMT, MVC EMG and $SICI_{RATIO}$ (Shapiro-Wilk $p < 0,05$). Table 1. Illustrates the results for MVC F and MVC RMS EMG in PE. There were no statistical differences between "DRY" and "WATER" conditions in either force or EMG.

TABLE 1. Passive experiment. Individual maximal voluntary force (F) and root mean square electromyography (EMG RMS) recordings. Measures obtained both in "DRY" condition and in "WATER" condition. No statistically significant differences.

	FORCE (N)		EMG (RMS)	
SUBJECT	**DRY**	**WATER**	**DRY**	**WATER**
A	741,4	823,8	0,244	0,276
B	1131,7	1222,0	0,133	0,175
C	1121,9	1188,6	0,181	0,17
D	1505,4	1384,7	0,137	0,131
E	1271,0	1375,9	0,17	0,175
F	1083,7	855,2	0,141	0,082
MEAN	1142,5	1141,7	0,168	0,169
SD	250,2	247,3	0,042	0,064

The individual rMT and stimulation intensities for paired-pulse paradigms are presented in Table 2. Paired T-test did not reveal statistically significant differences between "DRY" and "WATER" in any of the parameters.

TABLE 2. Passive experiment. Individual resting motor threshold (rMT) values (as percentage of maximal stimulator output, MSO) and stimulation intensities used for paired-pulse paradigm. Measures obtained both in "DRY" condition and in "WATER" condition. Statistical tests revealed no significant differences between the conditions.

	rMT (% of MSO)		**$SICI_{TEST}$ (% of MSO)**		**$SICI_{COND}$ (% of MSO)**	
SUBJECT	**DRY**	**WATER**	**DRY**	**WATER**	**DRY**	**WATER**
A	38	36	46	43	27	25
B	53	53	64	64	37	37
C	50	58	62	70	35	41
D	44	41	56	53	31	29
E	42	41	50	49	29	29
F	48	46	58	58	34	32
MEAN	46	46	56	56	32	32
SD	6	8	7	10	4	6

IO-curves are drawn in Figure 28. The IO-curve values had not normally distributed data points. Analysis were carried out with logarithmic transformation that resulted a normal distribution. Transformation had no effect on either statistics or visual representation of the curves, therefore non-transformed values are reported. Two-way repeated measures ANOVAs revealed imain effects as follows: for "intensity" there was a significant effect (F[1.462, 7.311] = 14,378, p = 0,004, η^2 = 0,742) and for "condition" (F[1, 5] = 9,270, p = 0,029, η^2 = 0,650). Due to significant changes found on "condition", further paired T-tests were conducted separately on the intensity levels between the "DRY" and "WATER". T-tests revealed significant differences between 120% of rMT (± SD) (1,019 ± 0,473 vs. 0,723 ± 0,427, t[5] = 6,560, p = 0,001) and 140% of rMT (1,893 ± 1,029 vs. 1,197 ± 0,681, t[5] = 4,394, p = 0,007) stimulation intensities, indicating higher responses in "DRY" condition.

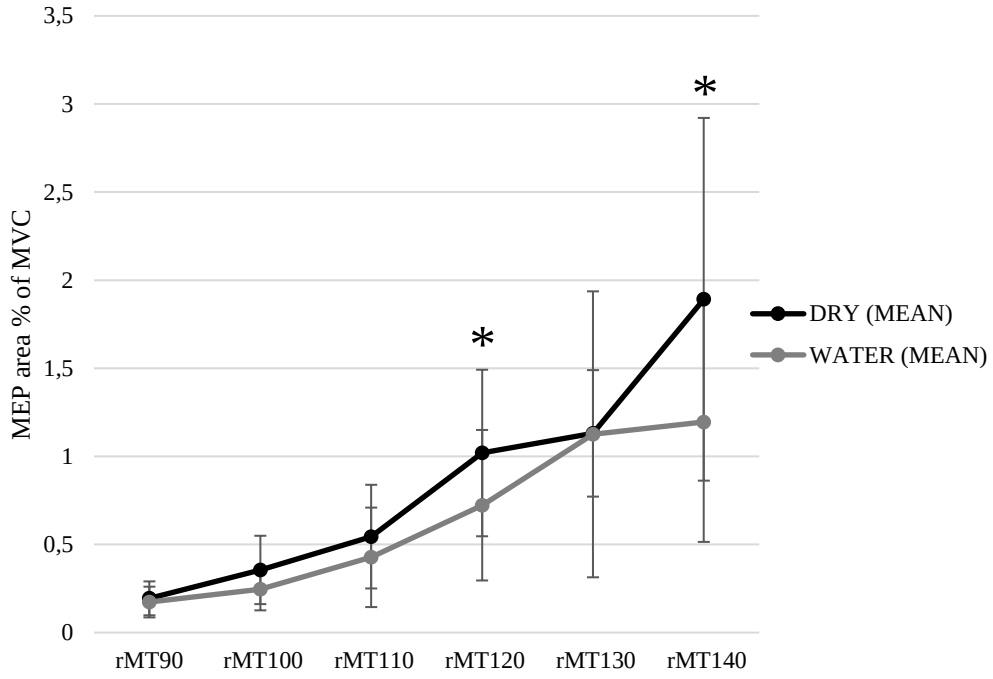

FIGURE 28. IO-curves in PE with standard deviation. Black dotted-line represents the IO-curve obtained on dry land and grey line with triangles during immersion. Statistically significant ($p < 0,05$) differences indicated by *-symbol at 120 % and 140% of rMT.

$SICI_{RATIO}$ is expressed as bar graphs in Figure 29. Wilcoxon-Signed rank test revealed no statistically significant difference between the two conditions for $SICI_{RATIO}$ (DRY: 31,8 ± 14,9; WATER: 38,1 ± 11,6, Z = -0,734, $p = 0,463$).

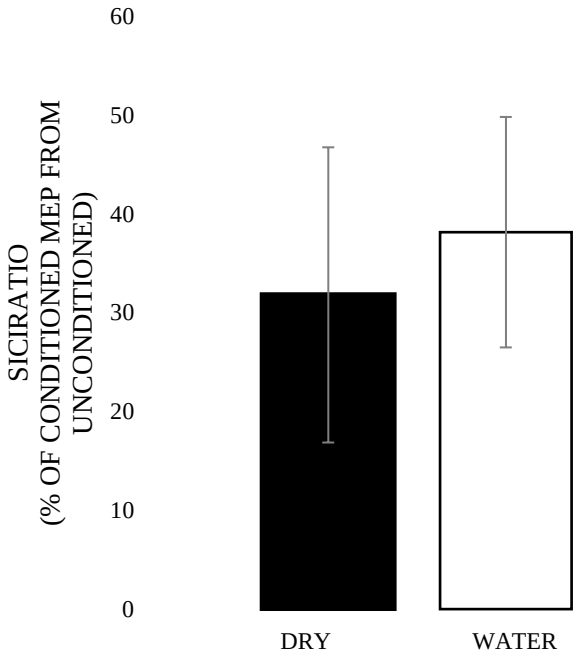

FIGURE 29. $SICI_{RATIO}$ with SD in PE. Smaller the ratio (further from 100%), higher the inhibition caused by the conditioning stimulus. No statistical differences between the two conditions.

8.2 Active experiment

Intracortical facilitation (ICF) stimulation protocol was unsuccessful to facilitate test MEP ($SICI_{RATIO}$ was <100%) and was therefore excluded from the analysis. Data were distributed normally for all variables (Shapiro-Wilk $p > 0,05$). Table 3 illustrates the results for MVC F, MVC RMS EMG per subject. Paired samples T-tests revealed no significant differences in MVC or MVC EMG. BG EMG activity is drawn in Figure 30. Two-way repeated measures ANOVA for BG EMG activity revealed no statistically significant differences in either "contraction" ($F[1.936, 9.679 = 2,032$, $p = 0,184$, $\eta^2 = 0,289$) or "condition" ($F[1, 5] = 0,542$, $p = 0,494$, $\eta^2 = 0,098$). Stimulation intensities used and aMT did not differ significantly between "DRY" and "WATER" (Table 4).

TABLE 3. Active experiment maximal voluntary contraction (F) (N) and corresponding root mean square (RMS) electromyography (EMG) activity. Individual values per subject and as group means for both “DRY” and “WATER” condition.

	FORCE (N)		EMG (RMS)	
SUBJECT	**DRY**	**WATER**	**DRY**	**WATER**
A	839,5	769,9	0,296	0,329
B	1200,4	1279,8	0,176	0,139
C	1138,5	1073,9	0,442	0,268
D	1357,3	1194,5	0,117	0,115
E	1582,9	1565,2	0,181	0,115
F	1111,1	1019,9	0,08	0,062
MEAN	1205,0	1150,5	0,216	0,173
SD	250,1	267,7	0,133	0,103

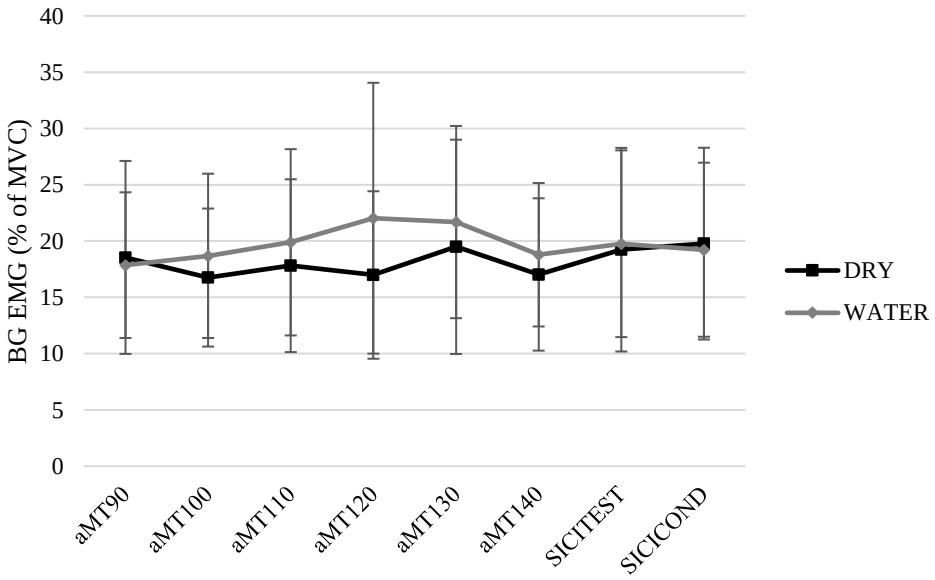

FIGURE 30. BG EMG from each contraction (named after stimulation protocol) with SD, normalized to MVC EMG. No statistically significant differences in the amount of background activity between the two conditions.

TABLE 4. Active experiment. Individual active motor threshold (aMT) values (as percentage of maximal stimulator output, MSO) and stimulation intensities used in the paired-pulse paradigm. Mean values used for statistical analysis. Measures obtained both in "DRY" condition (D) and in "WATER" condition (W).

	aMT (% of MSO)		**$SICI_{TEST}$ (% of MSO)**		**$SICI_{COND}$ (% of MSO)**	
SUBJECT	**DRY**	**WATER**	**DRY)**	**WATER**	**DRY**	**WATER**
A	33	33	40	40	23	23
B	47	45	57	55	33	32
C	44	39	53	47	31	27
D	33	32	42	42	23	23
E	37	37	44	44	26	26
F	38	38	46	46	27	27
MEAN	39	37	47	46	27	26
SD	6	5	7	5	4	3

IO-curves are drawn in Figure 31. The IO-curve values had not normally distributed data points. Analysis were carried out with logarithmic transformation that resulted a normal distribution. Transformation had no effect on either statistics or visual representation of the curves, therefore non-transformed values are reported. Two-way repeated measures ANOVA revealed a significant interaction of "stimulus intensity" (F[2.237, 11.184] = 21,441, p < 0,0001, η^2 = 0,811) but not for "condition" (F[1, 5] = 0,155, p = 0,710, η^2 = 0,03).

Silent period duration was longer in "DRY" condition (161,78 ± 29,0) than in "WATER" (129,67 ± 29,98) but the difference did not reach statistical significance (t[5] = 2,224 p = 0,077). $SICI_{RATIO}$ is expressed as bar graphs in Figure 32, showing no statistical difference between the two conditions (75,8 ± 12,5 for DRY and 68,6 ± 21,9 for WATER).

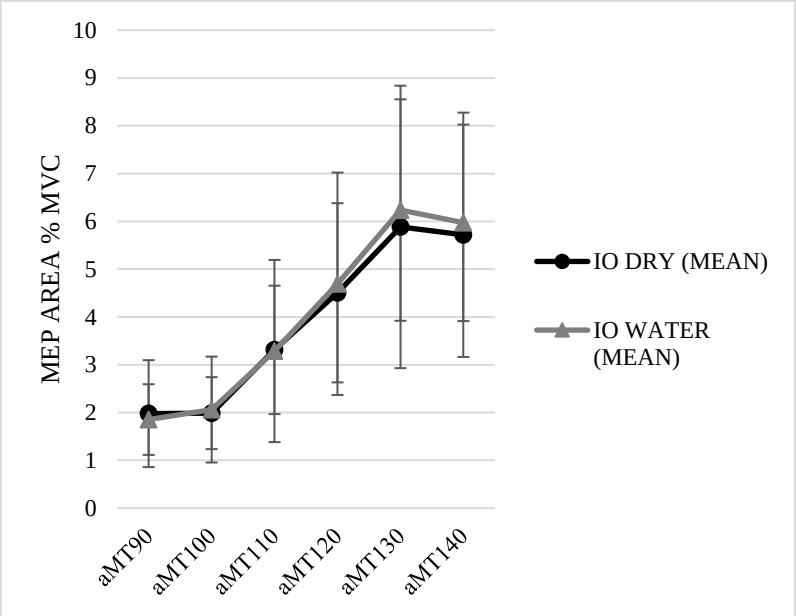

FIGURE 31. IO-curves in the AE with SD. Dotted black line represents the mean IO-curve obtained on dry land and dark grey with triangles during immersion. No statistically significant differences between the conditions.

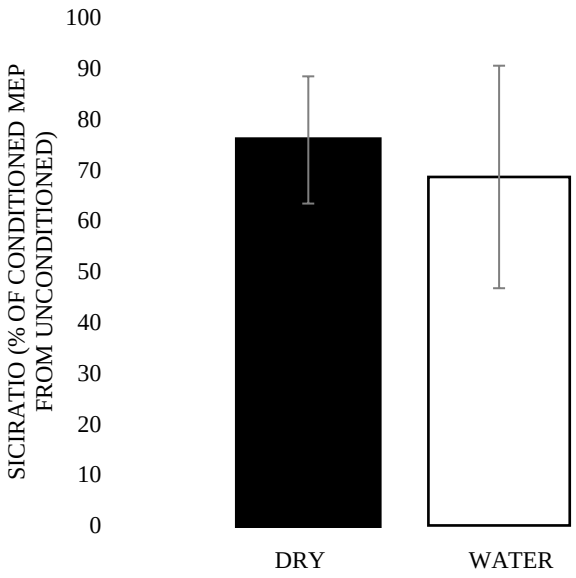

FIGURE 32. Active experiment $SICI_{RATIO}$ as bar graphs with SD. Smaller the ratio (further from 100%), higher the inhibition caused by the conditioning stimulus. No statistical differences between the two conditions.

9 DISCUSSION

In this study it was investigated how immersion in still, thermoneutral water up to sternum level would influence corticospinal excitability of the soleus muscle in either at rest or during a submaximal isometric contraction. Main findings showed that both EMG levels and MVC remained similar in dry and water immersion conditions in both experiments. Further, in the passive experiment there was a significant difference in IO-curves at 120% of rMT and 140% of rMT, while in active experiment the IO-curves were similar. Lastly, the intracortical inhibition and silent period had non-significant differences in both experiments between the conditions. Based on the results, the immersion in still water is not enough to induce a change in the corticospinal excitability or in the intracortical circuitry during neither of the activity conditions.

9.1 Corticospinal excitability of soleus muscle at rest

In PE the IO-curve measures at 120% and 140% of rMT showed statistically significant differences towards higher excitability in dry land. This effect was not mirrored in the SICI measures. Even though the SICI measures remained statistically not significant, this data would not support the effect of cortical inhibitory mechanisms being responsible for the observed difference in corticospinal excitability since on dry land the inhibition caused by the conditioning stimulus was higher (but not significant) in dry land than in water. It has been previously reported that being immersed in still water is not enough to change either corticospinal excitability or the excitability of the intracortical circuitry in the upper extremities (Sato et al. 2015, Sato et al. 2014).

Since transcranial magnetic stimulation measures the excitability of the whole corticospinal tract, it is not possible to distinguish the changes occurring independently at the spinal level or cortical level without incorporating additional methods. However, as the data above hints at unaltered (or even slightly more efficient intracortical inhibition in dry land), an indirect hypothesis towards a change spinal excitability is discussed. Dietz and colleagues (1989) observed a reduction in stretch reflex amplitudes caused by balance perturbation to be

diminished when immersed to water. Further, the stretch reflexes continued grow smaller in size when immersion depth was increased (Dietz et al. 1989). Following the same pattern, Pöyhönen & Avela (2002) observed also a reduction in H-reflexes and Achilles tendon reflexes when subjects were immersed in water. Therefore, a plausible mechanism to explain the above findings that corticospinal excitability is higher in dry land is that the spinal contribution is reduced during water immersion, especially since intracortical inhibition remains unaltered as was observed in this study.

However, the H-reflexes have also been shown to increase during water immersion, suggesting either a gain spinal excitability or a reduction in inhibitory mechanisms acting presynaptically (Cronin et al. 2016, Nakazawa et al. 2004). In the study by Nakazawa and colleagues (2004), they attributed the observed increases in H-reflexes to reduction in gravitational input from the joints of the lower extremities. Cronin et al. (2016) observed similar increases especially in the acute phase of the water immersion, where reduced gravitational (or reduction in joint loading) input is hypothetically the "highest" as e.g. buoyancy might limit said input, before the system adapts to the new environment.

Since the H-reflex responses have varied in previous studies, a brief exploration is made into as what physiological phenomena could explain the variations in the results observed and if water immersion could have an effect through said mechanisms. H-reflex is not a direct measure of spinal excitability as it is heavily modified by presynaptic inhibition of Ia afferent terminals (Misiaszek 2003). Spinal cord receives a constant stream of afferent input from various locations such as skin, muscles, tendons and joints, as is arguably the case in water immersion caused by the physical characteristics of water. Therefore, to produce a voluntary motor task, this sensory feedback needs to be managed by either inhibition or reduced facilitation (Knikou 2008). This inhibition of afferent information is most economically done at the earliest possible sites, before the afferent activity has produced an effect in the central nervous system. Presynaptic inhibition is a dynamic process that can inhibit the flow of information in some axonal branches but not in other of the same afferent. Therefore, it may address the excitation to specific neuronal targets based on the needs in motor control or sensory gating. Thus, the presynaptic inhibitory synapses of afferent terminals of αMNs are the prime targets for such neural activity in order to inhibit the afferent input (Rudomin and Schmidt 1999). These presynaptic terminals receive inputs from neurons in motor

centres in the brainstem and cerebral cortex and therefore is a location at which central neurons can regulate the strength of spinal reflexes (Kandel et al. 2013, 801). According to McComas (2016), this reduction of the sensory information could serve two functions depending on the state of the system: 1) preventing an overload of information not needed in various sensory pathways of the brain and spinal cord and 2) tuning spinal reflexes down that would otherwise interfere in resting state or in reflex and voluntary contractions (McComas 2016). Taken together, presynaptic inhibition allows the CNS to functionally modulate spinal excitability based on the current situation. Extrapolated to observed discrepancies in H-reflex studies conducted during water immersion, varying effect of presynaptic inhibition could be responsible for the observed changes. Further, between the studies the positions in which the subjects were tested (standing vs. seated), water-proofing methods used, or depth of immersion could all be factors that influence the amount of presynaptic inhibition acting on Ia afferent - αMN terminal.

9.2 Corticospinal excitability during submaximal isometric contraction

This was the first study attempting to uncover possible changes in corticospinal excitability of the soleus muscle during an active contraction while immersed to water. Based on the data collected, water immersion does not elicit changes in corticospinal excitability measures or in the excitability of the intracortical circuits of healthy male subjects. Further, no changes were observed in maximal force produced, in the EMG activity during MVC trials nor in the background EMG levels of submaximal isometric contractions. The steady EMG measures can most likely be attributed to the successful water-proofing methods.

Varying EMG amplitudes have previously been reported. Pöyhönen et al. (1999) observed a reduction in knee extensor muscle activity during water immersion. Also, Pöyhönen & Avela (2002) found out that the EMG activity of plantarflexors is lower during water immersion. However, Silvers & Dolny (2011) introduced a water-proofing protocol in which they showed that when proper waterproofing is applied, EMG levels are similar to those of obtained on dry land (Silvers & Dolny 2011). Similarly, EMG levels have been shown to be comparable to those of measured during dry land contraction also in upper extremity muscles (Veneziano et al. 2006, Rainoldi et al. 2004). In the article by Pöyhönen et al. (1999) the possible effect of electromechanical factors in the form of water in the electrode attachment or wires are discussed as possible causes for detected reduction in EMG activity. However, this methodological issue was

fixed in the later study by Pöyhönen & Avela (2002) where waterproof trousers (waders) were worn by the subjects in order to prevent any leakage to the electrodes. With an adequate waterproofing, the authors still observed a reduction in both MVC force and EMG. The use of waterproof trousers could be a potential cause for different measures of EMG activity between the studies. As the hydrostatic pressure compresses the trousers around the extremity, they act as compressive joint support that can modulate proprioception that in turn trigger inhibitory mechanisms reducing H-reflex amplitudes (Pöyhönen & Avela 2002). Moreover, Pinto and colleagues (2010) found no differences in either force or EMG values during MVC trials of the upper and lower extremity (Pinto et al. 2010). Therefore, it can be argued that when EMG electrodes are water-proofed and when the immersion depth is relatively low, fluid-shifts caused by hydrostatic pressure is not enough to alter the mechanical architecture of the muscle and thereby the EMG activity. Mechanical adaptation of the muscle architecture was examined by Kubo et al. (2004) in a relatively low immersion and they found no changes between dry and immersion conditions (Kubo et al. 2004). Indeed, it seems that as fluid leaves the interstitial space, it is rapidly replaced by intracellular water (Stocks et al. 2004).

Despite no significant differences in the IO-curves or SICI measures, a nearly statistically significant difference (p = 0,07) was observed on SP and it is therefore briefly discussed. As it was previously shown, SP consists of both spinal and cortical mechanisms. To facilitate the discussion, SP is considered alongside another inhibitory mechanism that has cortical origins but could be modulated through spinal mechanisms also. Sato and colleagues (2013) observed that when the subject is immersed into water, both SAI and LAI were decreased while SICI and ICF values remained unchanged (Sato et al. 2013). Afferent inhibition, whether due to short (SAI) or long (LAI) ISI, occurs when a sensory afferent volley inhibits a motor response of a given muscle. It is studied by combining non-invasive nerve stimulation with TMS over M1. Since TMS activates corticospinal neurons transsynaptically, afferent volley may inhibit or facilitate the MEP when preceding the TMS pulse. Functionally, SAI and LAI represent inhibition from sensory cortex to motor cortex and therefore is an essential component in sensorimotor integration. Working hypothesis for the dynamics of SAI between S1 and M1 cortices is that increased GABAergic activity in S1 would reduce the excitation of S1 pyramidal neurons. Because these cells are inhibitory towards I-wave interneurons in M1, they would reduce the inhibition from S1 towards I-wave interneurons and thereby facilitating M1 pyramidal neurons, leading to a higher MEP (and reduced SAI) (Turco et al. 2018). Indeed, it has been shown that SAI operates via distinct yet reciprocally connected GABAergic inhibitory interneurons that converge onto corticospinal

neurons (Alle et al. 2009, Miyaguchi et al. 2017). Pharmacological studies have shown that SAI is mediated through $GABA_A$ as administration of lorazepam (enhancing $GABA_A$ activity) decreased SAI (Di Lazzaro et al. 2005). Silent period has also been studied using the same pharmacological agent and it was shown that the duration of silent period decreases while having no effect on rMT or active IO curves (Kimiskidis et al. 2006; but see also Ziemann et al. 1996). When combined with the finding that water immersion increases blood flow in the S1 (Sato et al. 2012), one functional explanation for the observed reduction in SP duration could be the gating of ascending afferent information (Moritani et al. 1998). As the S1 engages in processing the afferent information, it would lead to depression of SAI and since SAI and SP are operated via pharmacologically similar GABAergic activity, also to reduced silent period (Turco et al. 2018).

An alternate mechanism is briefly discussed. Besides in gating afferent information, SAI has been shown to be reduced in planning or executing finger movements. It was hypothesized that this decrease could be due to increased spinal excitability especially in tonic movement of the finger (Asmussen et al. 2013). Škarabot and colleagues (2019) challenged the understanding that changes into the SP would arise solely from cortical origins and suggest that spinal mechanisms, such as disynaptic facilitation, recurrent inhibition or afferent firing, behind SP might not be as static as is currently understood (Škarabot et al. 2019). Therefore, as SAI and LAI has been shown to be decreased during water immersion, a sign of decreased inhibition from S1 to M1 that could result from increased spinal excitability and as SP may be altered also through spinal mechanisms independently from cortical mechanisms, it could be that the observed reduction (although not statistically significant) is due to increased spinal excitability that was caused by the water immersion. Spinal excitability has shown to be variable during water immersion, as previously discussed.

Above explanations of the possible mechanisms are entirely hypothetical as the modulation of SAI or LAI has not been investigated during water immersion with active motoneuron pool and was not measured in this study either. In addition, the dynamics between SP and SAI/LAI were considered on a pharmacological basis. In practice, one study compared the duration of silent period and SAI between healthy subjects and subjects with chronic shoulder pain. Their findings showed that shoulder pain patients had both decreased SAI and increased SP duration (Bradnam et al. 2016). Also, when SAI measures have been combined to H-reflex studies, it was shown that reduction in SAI was a result of both spinal and supraspinal mechanisms (Asmussen et al. 2014).

Several limitations remains in the current study. As was discussed earlier, the MEP represents both the cortical and spinal excitability without the possibility to differentiate between the two unless the methodology is expanded. In this study there were no measures that could capture changes in the spinal excitability (such as H-reflex measures). This is mainly due to the fact that a safe application of electrical stimulation during immersion wouldn't have been possible without including waders and this might have had an effect to the measures on its' own. Therefore, to keep the immersion condition as natural as possible to mimic therapeutic conditions, all external clothing except swimming trunks were not allowed. Secondly, despite the hypothesis relying on mechanical properties of the muscle caused by the fluid shifts, no method to actually investigate this was not included into the study. Before, ultrasound has been used to quantify the behaviour of muscle fascicles during different experimental conditions. However, in the current study the immersion tank was designed so that it fits one person at a time. In addition, operating an ultrasound probe reliably would not have been possible from outside the water tank as the cover and the screen would have prevented the manipulation of probe placement. This limitation could be countered by designing the water tank so that fits a pre-attached ultrasound probe (with a long enough cord) comfortably and allows the experimenter to adjust the probe when necessary. Lastly, the study is limited by the number of subjects recruited. Based on six subjects it is impossible the draw conclusions on a wider scale. Allowing more time for the recruitment of the participants and taking extra precautions to ensure reliable water-proofing so that data would not be lost would have improved this.

Further studies should incorporate methods that allow for the examination of spinal mechanisms of motor control. In this study, the cortical mechanisms of isometric soleus contraction remained largely unchanged. This could also be due to the position in which the subjects were tested. It allowed for controlled force and EMG measurements but possibly limited the effect that e.g. buoyancy could have on motor control. While including methods to assess spinal mechanisms, also different postures should be examined to uncover how the water medium changes the control of balance, for example. Additionally, to study more dynamic movements where drag would increase the effort, an underwater cycling could be utilized. That being said, water-proofing procedures would be an obstacle to overcome.

10 CONCLUSION

To conclude, this study aimed to uncover possible changes in the corticospinal excitability caused by water immersion, both motoneuron pool at rest and during a submaximal isometric contraction when soleus muscle is targeted. At rest, there was a reduction in corticospinal excitability in during water immersion at mid to high ranges of IO-curve, possibly due to adaptations at spinal level. During active contraction water immersion elicited no changes to any of the measured parameters compared to measures obtained on dry land. It could be argued that the protocol used in this study did not adequately represent functional conditions typically used in either aquatic training or rehabilitation. Namely, activities performed standing. The experimental setup was selected to examine the effects of possible fluid shifts from the extremities to excitability of the corticospinal tract. Therefore, in order to establish reliable measures, the position of the subject was heavily fixed. This resulted in eliminating two physical characteristics of water as possibly effecting the corticospinal tract: buoyancy and viscosity. Further studies should incorporate these physical characteristics into the protocol design in addition to methods that would allow exploration of spinal excitability during water immersion, both at rest and during activity.

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