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KANDIDATSPECIALE, IDRÆT

Study proposal: The effects of local and remote ischemic preconditioning on motor unit properties in the tibialis anterior muscle of healthy adults

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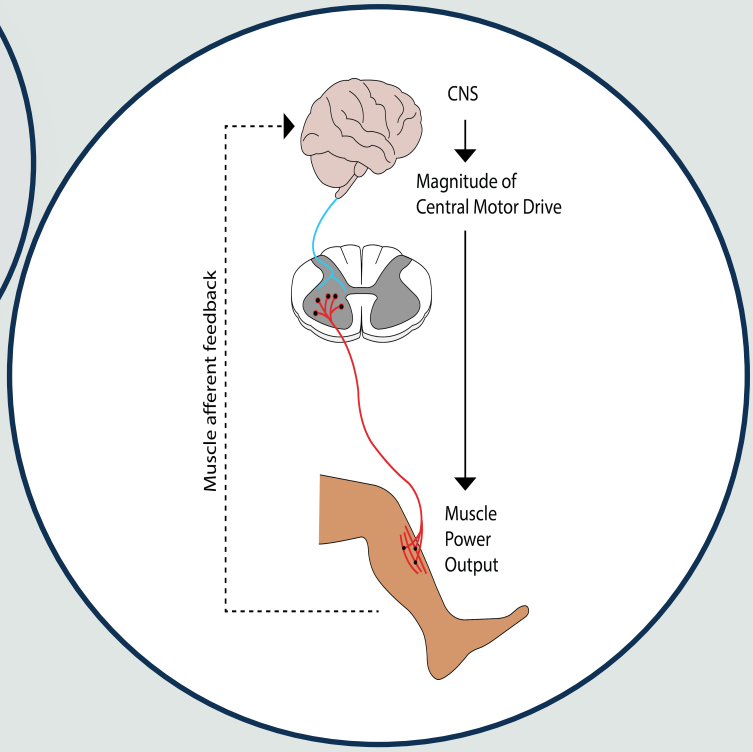

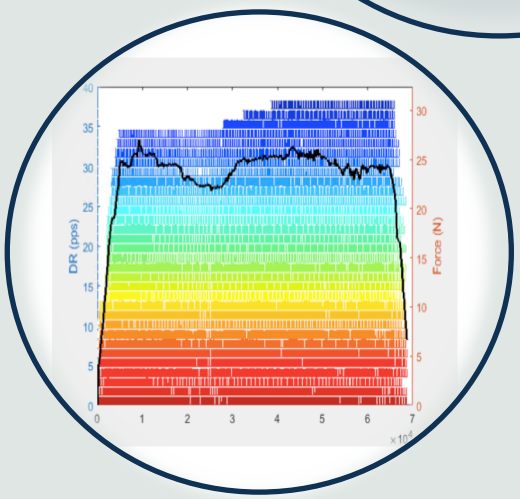

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PREFACE

This Master's thesis consists of i) worksheets, ii) the study proposal: "*The effects of local and remote ischemic preconditioning on motor unit properties in the tibialis anterior muscle of healthy adults*" and finally iii) the Appendix. The worksheets provide fundamental background information to the study proposal and therefore constitute the first part of the thesis in order to assist the reader. The worksheets ground the thesis in the theory and provide the theoretical basis for the potential influence of ischemic preconditioning on motor unit properties. They cover the following topics in detail; ischemic preconditioning, neuromuscular control, the structure and function of motor units, and electromyography (EMG). The study proposal includes an introduction, proposed methods, data analysis, expected results, and a discussion of possible outcomes of the present study proposal. Finally, the Appendix includes the complex procedure of the decomposition of motor units, which is fundamental to our method and to identify and track motor units before and after an intervention, which are necessary to answer our hypotheses.

The global situation with the spread of the COVID-19 virus (the coronavirus disease) in the spring of 2020 has led to a reorganization of the originally intended experimental project including a complete protocol of an intervention in the laboratory and data processing. Due to these circumstances and restrictions it has not been possible to access the laboratory and collect our own data. Instead, this thesis has been restructured into a study proposal conceptualized through a description of the rationale of the thoughts underlying the same intended study design.

The rearrangement has been challenging, but definitely educational and resulted in a study proposal which aims to quantify the effects of local and remote ischemic preconditioning on motor unit recruitment measured with high-density surface EMG.

The study design was created in collaboration with another group of students (20gr8102) with the original intention of working in the laboratory on the data collection together. The rest of the worksheets and study proposal were entirely completed by Mathias and Trine.

RESUMÉ

Der er udarbejdet et studieforslag, hvis formål er at undersøge, hvorvidt motoriske enheder (ansvarlig for muskelaktivering) kan optimeres hos raske voksne efter en intervention med iskæmisk prækonditionering (IPC). IPC er en intervention karakteriseret ved korte perioder med iskæmi og efterfølgende fri blodgennemstrømning. Tidligere undersøgelser har vist, at anvendelse af IPC kan have en forbedrende effekt på nervesystemet og dermed bevirke en forbedret muskelaktivering, men de bagvedliggende mekanismer er endnu ikke fuldt afdækket. Eftersom IPC som behandling er ikke-invasiv, billigt og let anvendelig, er der derfor et stort potentiale i anvendelsen af IPC, hvis der kan opnås en dybere forståelse af de underliggende mekanismer.

I tillæg til at undersøge hvorvidt motoriske enheder kan optimeres hos raske voksne efter en intervention med IPC, vil studieforslaget desuden kunne belyse, om der er forskel mellem IPC placeret lokalt på den ekstremitet, der udfører muskelkontraktionen (LIPC), og på IPC placeret på en anden ekstremitet end den der udfører muskelkontraktionen (RIPC), hvilket vil kunne forbedre anvendelsen af IPC.

Dette foreslås undersøgt med high density surface electromyography (hd-EMG), som er en ny teknologi indenfor muskelfysiologi, der muliggør undersøgelsen af IPC's effekt på de motoriske enheder før og efter en intervention, hvilket vil kunne danne grobund for fremtidige undersøgelser.

Studieforslaget indebærer rekrutteringen af 16 raske voksne, der vil gennemgå tre interventioner; LIPC, RIPC og kontrol i en tilfældig rækkefølge. Hver forsøgsgang indebærer måling af maksimale frivillige kontraktioner (MVC), hvorefter en submaksimal isometrisk rampekontraktion op til 40% af MVC udføres med skinnebensmusklen og måles med hd-EMG både før og efter hver intervention. På baggrund af tidligere undersøgelser og den eksisterende viden på området, forventes det, at såvel LIPC som RIPC vil forbedre aktiveringen af de motoriske enheder signifikant sammenlignet med kontrol-interventionen. For kontrol-interventionen forventes det, at resultaterne før og efter interventionen vil være de samme. Derudover forventes LIPC at have en større positiv effekt på aktiveringen af de motoriske enheder sammenlignet med RIPC.

WORKSHEETS

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These worksheets should be considered as supplementary material to the study proposal and provide the foundation for a deeper understanding of the theory and method related to the project.

CLARIFICATION OF DIFFERENT METHODS OF ISCHEMIC CONDITIONING

The application of ischemic conditioning has been performed using various methods. To clarify the methods of ischemic conditioning mentioned in the present thesis, the differences will be elaborated in Figure 1. Furthermore, as the present thesis will use the right leg as the test leg, the right leg will always be referred to as the exercising limb.

INVASIVE ISCHEMIC CONDITIONING BY DIRECT ARTERIAL OCCLUSION

Ischemic conditioning (invasive) is illustrated in Figure 1A.

Remote ischemic conditioning (invasive) is illustrated in Figure 1B.

NON-INVASIVE ISCHEMIC CONDITIONING INDUCED BY A BLOOD PRESSURE CUFF ON A LIMB

IPC: Overall description of non-invasive ischemic conditioning.

LIPC: Local IPC (exercising limb) illustrated in Figure 1C.

RIPC: Remote IPC (non-exercising limb) illustrated in Figure 1D.

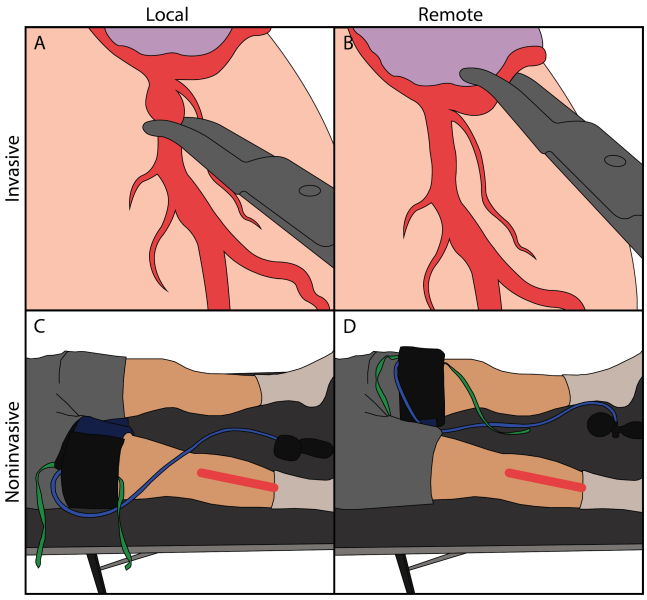

Figure 1: Illustration of the different techniques of ischemic conditioning. A) Invasive local ischemic conditioning of the artery. B) Invasive remote ischemic conditioning of the artery. C) Non-invasive local limb ischemic preconditioning of the exercising limb, induced by a blood pressure cuff. D) Non-invasive remote limb ischemic preconditioning of the non-exercising limb, induced by a blood pressure cuff. The exercising limb is indicated by the red mark on the right leg (Modified Figure from Hess et al. 2015).

ISCHEMIC CONDITIONING

INTRODUCTION TO ISCHEMIC CONDITIONING

The concept of ischemic preconditioning was first presented by Murry et al. (1986), who proved ischemic conditioning as a strategy to protect the myocardium against longer periods of subsequent ischemia in canine hearts. Since then, ischemic conditioning has become an endogenous phenomenon, whereby organs are exposed to a controlled, short-term local, non-lethal ischemia conditioning that protects against lethal ischemia by reducing the cellular damage in the same organ (Stokfisz et al. 2017).

Ischemic conditioning is typically applied by 3-5 cycles of 5 minutes of full arterial occlusion each separated by 5 minutes of reperfusion (Cocking et al. 2019). A study by Przyklenk et al. (1993) showed a remote effect of ischemic conditioning when applied to one organ, which revealed the same protective effect on another organ. This procedure is called remote ischemic conditioning (Stokfisz et al. 2017). A later modification by Kharbanda et al. (2002) revealed an easy and non-invasive application of ischemic conditioning by using a blood pressure cuff on limbs. This method is referred to as ischemic preconditioning (IPC).

MECHANISMS OF ISCHEMIC CONDITIONING

The mechanisms of ischemic conditioning are still not fully understood, but several different mechanisms and signaling molecules have been reported to contribute to the protective effects (Heusch, 2015). However, several studies suggest the protective effect of remote ischemic conditioning to be caused by activation of neural, humoral (Hess et al. 2015; Hausenloy & Yellon, 2008; Stokfisz et al. 2017; Lim et al. 2010) and/or systemic pathways (Hess et al. 2015; Hausenloy & Yellon 2008; Caru et al. 2019) *(See Figure 2 for an overview of these pathways)*. The neural and humoral pathways are suggested to depend on endogenous metabolites generated in the conditioned tissue, such as adenosine, bradykinin, opioids, angiotensin1 and endocannabinoids (Hausenloy & Yellon, 2008). In the neural pathway, it is proposed that these metabolites activate a local afferent nerve, which stimulates an efferent nerve, which terminates at the heart and mediates protection of the myocardium (Hausenloy & Yellon, 2008). In the humoral pathway, the protective effect is thought to be caused by the metabolites being released

into the bloodstream and activating receptors in the myocardium (Hausenloy & Yellon, 2008). In the systemic pathway, it is suggested that the stimuli from remote ischemic conditioning trigger a systemic protective response, which suppresses inflammation and apoptosis (Hausenloy & Yellon, 2008). When the neural signal or humoral metabolites from the conditioned tissue arrives at the heart, it initiates intracellular mechanisms. The binding of metabolites to G-protein cell surface receptors is thought to activate intracellular kinases like Protein Kinase C and other signaling components such as reactive oxygen species and the mitochondrial K_{ATP}-channel, which are all supposed to elicit a protective effect of remote ischemic conditioning (Hausenloy & Yellon, 2008).

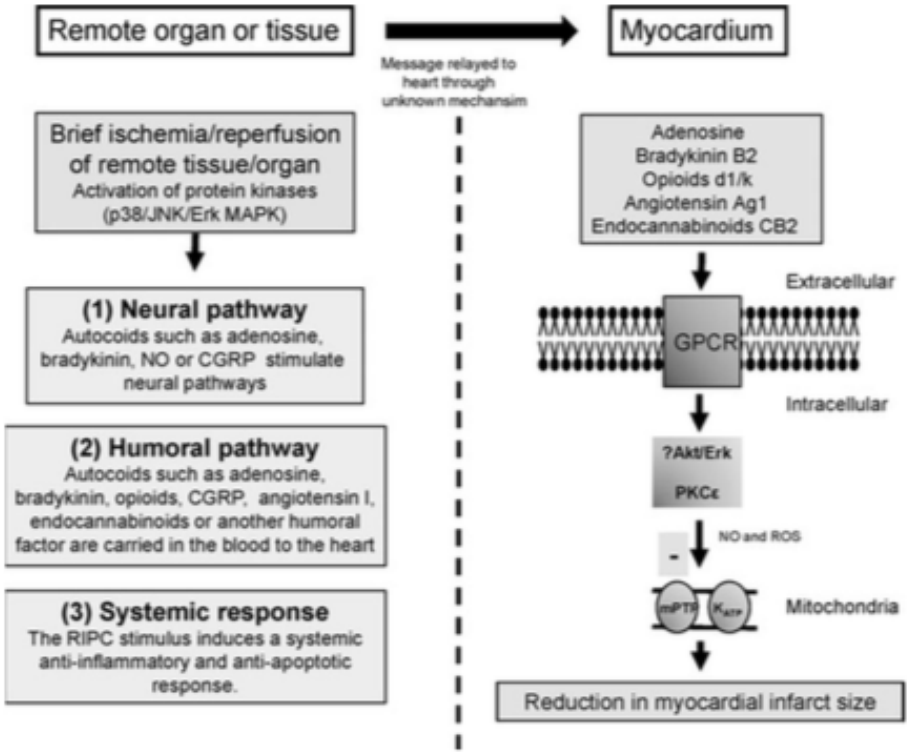

Figure 2: Illustration of the potential pathways of cardioprotection induced by remote ischemic conditioning (Hausenloy & Yellon, 2008).

The neural and humoral pathways were originally thought of as mechanistically distinct. However, it has recently been suggested that the two are interdependent (Pickard et al. 2016). A study on rats examined the effect of remote ischemic conditioning on myocardial infarct size with or without femoral vein occlusion, femoral nerve resection and/or sciatic nerve resection. They

found that plain remote ischemic conditioning (i.e. no vein occlusion or nerve resection) displayed the largest effect, whereas the effect was completely abolished or partially abolished when occlusion and/or nerve resection were applied (Lim et al. 2010). These findings suggest the protective effect of remote ischemic conditioning could be due to interplay between the neural and humoral pathways. Furthermore, it has been demonstrated that neural activity in the conditioned tissue is necessary for the release of the protective factors (Michelsen et al. 2012).

Hess et al. (2015) stated that IPC could be a physical exercise equivalent in terms of similar mechanisms and metabolites. This was reasoned by a study examining apoptosis in rabbit hearts perfused with dialysate from a human donor who underwent either IPC or high intensity exercise (Michelsen et al. 2012). Dialysates from both groups showed a protective effect by reducing myocardial infarct size in rabbit hearts, which implies a common humoral factor (Michelsen et al. 2012). Furthermore, it was suggested that the protective effect on the myocardium from both IPC and vigorous exercise could be dependent on opioid receptor activation (Michelsen et al. 2012).

EFFECTS OF IPC ON PERFORMANCE

Recently, IPC has been applied as a possible ergogenic aid for improving performance in sports and exercise settings (Cruz et al. 2017; Halley et al. 2019; Cocking et al. 2019). IPC has been shown to provide an overall small but beneficial effect on exercise performance (Cocking et al. 2019), ranging across modalities, time domains and intensities (Halley et al. 2019). Most studies in the performance domain have used cuff positioning directly on the exercising limb, referred to as local IPC (LIPC), whereas other studies have used a remote cuff positioning on the non-exercising limb, referred to as remote IPC (RIPC) (Salvador et al 2016). However, both methods have shown to be beneficial compared to a sham-group (Cocking et al. 2018).

De Groot et al. (2010) were the first to prove a performance enhancing effect of IPC. They used bilateral leg occlusion to increase maximal power output and maximal oxygen consumption, in a maximal incremental cycle ergometer test performed by trained cyclists (De Groot et al. 2010). Since then, improvements have also been reported in maximal voluntary knee extensions on an isokinetic dynamometer (LIPC on tight) (Paradis-Deschênes et al. 2016) and fatigue and time to task failure tested with handgrip exercise (RIPC on arm) (Barbosa et al. 2015). Furthermore, a

review by Salvador et al. (2016) found the beneficial effect of IPC on performance to be greater for aerobic exercises compared to anaerobic, whereas power and sprint performances seemed not to be influenced by IPC.

In the literature, the performance effects related to IPC have been attributed to changes in aerobic metabolism and blood flow during exercise (Cocking et al. 2019). However, another potential effect of IPC on exercise performance suggests that IPC may influence the registration of fatigue signals in the body (Crisafulli et al. 2011; Cruz et al. 2015). Cruz et al. (2015) found that IPC increased the time to exhaustion by 8% compared to a sham group, in a cycling exercise with sustained load. Furthermore, during the cycling exercise, attenuation in the subjects' rate of perceived exertion and an increase in the myoelectrical activity of the vastus lateralis muscle was found (Cruz et al. 2015). These results from Cruz et al. (2015) suggest that IPC reduces the fatigue signals in the body, which thereby provide a possible explanation for the ergogenic effect of IPC on endurance performance (Cruz et al. 2015). Desensitization of fatigue signals has been suggested to involve group III/IV muscle afferents (Halley et al. 2019).

NEUROMUSCULAR CONTROL

AFFERENT CONTROL

The actions of thinly myelinated (group III) and unmyelinated (group IV) muscle afferents have substantial importance for the exercising human, as they function as a component of a proposed central feedback loop (Amann et al. 2011). The central feedback loop is illustrated in Figure 3. When exercising, contraction-induced mechanical and chemical stimuli start to activate molecular receptors on the terminal end of both group III/IV muscle afferents, located within skeletal muscles (Amann et al. 2011). The activation increases the spontaneous discharge of the muscle afferents, which project via the lumbar dorsal horn of the spinal cord to various sites within the central nervous

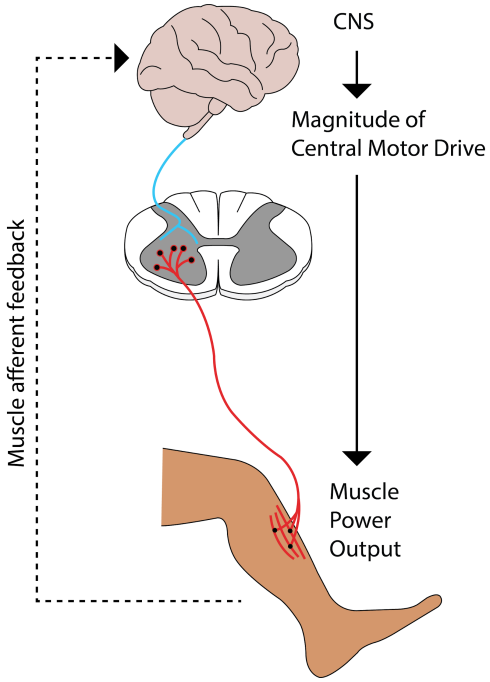

Figure 3: Simplified illustration of the central feedback loop, modified from Amann, 2011 and Sartori et al. 2017.

system (Amann et al. 2011). During high-intensity endurance exercises, the increased discharge from muscle afferents is proposed to provide inhibitory feedback to the central nervous system, which regulates the magnitude of central motor drive (Amann, 2011). These effects on central motor drive can lead to a limitation of development of exercise-induced peripheral muscle fatigue, and is thought to prevent extensive alterations of muscle homeostasis and potential harm (Amann, 2011).

Furthermore, group III/IV muscle afferents are well known to adjust cardiovascular and respiratory activities. Group III/IV muscle afferents transmit information via the spinal cord to the cardiovascular and ventilatory control centers within the brainstem, which induce an increase of activity in the sympathetic nervous system (Laurin et al. 2015).

VOLUNTARY CONTRACTION

The human motor function is created by the neuromuscular and musculoskeletal systems and their interaction (Sartori et al. 2017). When a voluntary muscle contraction occurs, the central nervous system delivers synaptic input to the motor neuron, which causes a change in the membrane potential, resulting in an output of spike-trains of action potentials (Farina & Negro, 2012; Farina et al. 2016).

A motor neuron and the muscle fibers it innervates, is called a motor unit. The motor unit is the final pathway, which delivers the electrical output (the spike-trains of action potentials) from the central nervous system to muscle fibers (Duchateau et al. 2006). This output provides information to produce muscle contraction and thereby generate the force needed for movement (Farina & Negro 2012; Heckman & Enoka, 2012). The connection from the axon to the muscle fibers in the motor unit is said to have a one-to-one relation, since a discharge of action potentials creates a depolarization at the neuromuscular junction, which leads to an action potential in the innervated muscle fibers (Del Vecchio et al. 2017). Thereby the generation of force is achieved by recruitment and modulation of the discharge rate of the motor units (Del Vecchio et al. 2017). The discharge rate and the recruited motor units are the main strategies controlled by the central nervous system to regulate the voluntary contraction and force output of the involved muscle (Konrad, 2006).

When a stimulus from the central nervous system arrives at the motor unit, the action potential will only be evoked if the strength of the electrical impulse/stimuli is strong enough to reach above the recruitment threshold. If not, no fibers in the motor unit will be activated (Liu et al.

2002). This is often referred to as an all-or-none principle (Cannon, 1924). When the spike train of action potentials is above the recruitment threshold, the motor unit is being activated and it will cause a contraction of all the fibers it innervates synchronously (Liu et al. 2002). Furthermore, the recruitment threshold is different for each motor unit, and the muscle fibers in the motor unit consist of the same metabolic profile and behave in the same manner once they are activated (Liu et al. 2002).

MOTOR UNIT ACTION POTENTIAL

The representation of the electrical changes generated by the activity of a motor unit during a contraction is called the motor unit action potential, and can be intercepted by electrodes inserted in the muscle mass or placed on the skin above the muscle using electromyographic devices (Rodrigez-Carreno et al. 2012) *(See Electromyography)*.

As illustrated in Figure 4, the motor unit action potential can be described as a superposed signal of the whole motor unit, because it is generated by a summation of the action potentials of all muscle fibers innervated by an individual motor unit present in the detection site of an electrode (Rodrigez-Carreno et al. 2012). The shape of each motor unit action potential differs according to the anatomical characteristics and the number of the innervated fibers (Heckman & Enoka, 2012).

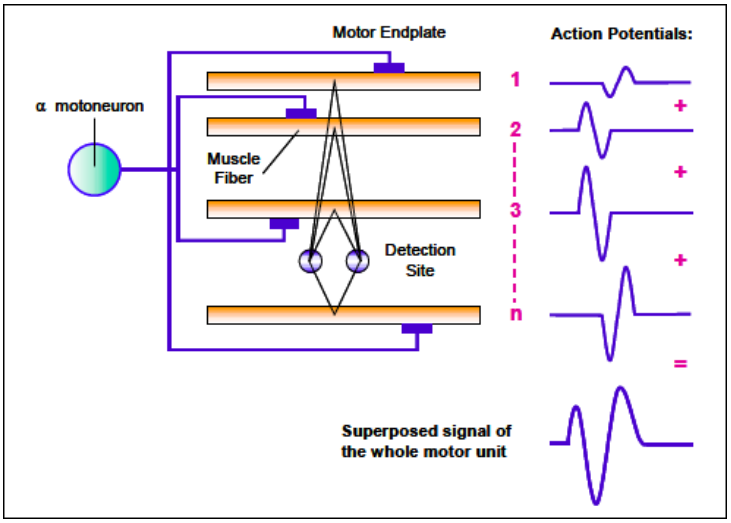

Figure 4: Illustration of the summation of action potentials of individual muscle fibers near the detection site generates the motor unit action potential as a superposed signal of the whole motor unit (Konrad, 2006).

THE STRUCTURE AND FUNCTION OF MOTOR UNITS

There are two important terms to describe the anatomical relationship between motor neurons and muscles: one being the motor neuron pool, the other being the motor unit. The motor neuron pool is motor neurons which all innervate the same muscle. They are collectively arranged in a cluster

in the ventral horn of the spinal cord (Duchateau et al. 2006; Heckman & Enoka, 2012). Motor units are essential in force generation and they work together in coordinating contractions of a muscle (Heckman & Enoka, 2012).

By cadaveric dissections, it has been possible to conclude that the size of the motor neuron pool provides information about the average number of innervated muscle fibers by the motor neuron (Heckman & Enoka, 2012). The total number of motor units within a muscle depends on the function and size of the muscle, in a wide range from a few motor units to over a thousand in the large muscles (Liu et al. 2002). The number of motor units is difficult to estimate in vivo, since the available methods to measure it have limitations. By cadaver dissection, the number of motor units in the tibialis anterior muscle was estimated to be 445 and by an electrophysiological method the number was estimated to be 118±50 (Heckman & Enoka, 2012). While the size of the motor neuron pool and number of motor units is useful for a between-muscle comparison, it is however, the range within the individual motor unit that is crucial in order to fully understand the nervous system and force generation (Heckman & Enoka, 2012).

FORCE PRODUCTION

As mentioned in the section *Voluntary contraction*, the increase of force in a muscle during a voluntary contraction depends on two mechanisms; one being an increase of the number of active motor units, known as motor unit recruitment, the second being the discharge rate of action potentials to the motor units (Milner-Brown et al. 1973; Enoka & Duchateau, 2017).

The discharge rate indicates the frequency at which a motor neuron discharges, and thereby generates force in the innervated muscle fibers (Petersen & Rostalski, 2019). When a muscle contraction occurs, the recruitment and the discharge rate vary depending on the force a muscle exerts, and the muscle that is performing the contraction (Duchateau et al. 2006). Within a muscle, many motor units generate small forces and a few motor units generate large forces, this distribution of the innervation number is often referred to as ‘an exponential distribution of recruitment thresholds within a motor unit pool’ (Heckman & Enoka, 2012; Enoka, 2019). Due to the exponential distribution it is suggested that the recruitment of motor units is the significant factor of force generation at low forces, since most motor units have a low recruitment threshold (Enoka & Duchateau, 2017; Duchateau et al. 2006). In experimental studies the upper limit of the motor unit recruitment is seen at 85% of maximal force for most muscles (Duchateau et al. 2006), including the tibialis anterior, where motor units are recruited up to 80-90% of maximal

voluntary contraction (MVC) (Van Cutsem et al. 1998). Therefore, the final 20-10% of force produced in a maximal voluntary muscle contraction is accomplished entirely by an increase of the discharge rate (Duchateau et al. 2006).

SIZE PRINCIPLE

Motor unit size measured by the number of innervated muscle fibers is proportional to the recruitment threshold and the force twitch amplitude (Heckman & Enoka, 2012; Petersen & Rostalski, 2019). This is also called the Henneman's size principle, where motor units are recruited in an orderly manner according to size. The motor neurons with small cell bodies are being recruited before motor neurons with large cell bodies (Henneman et al. 1965). The motor neurons with large cell bodies have a large axon diameter and many dendrites that innervate large motor units, and therefore have a greater motor axon conduction velocity. Large motor units are therefore able to generate great maximal tension, where smaller motor units are recruited first and produce lower maximal tension (Del Vecchio et al. 2018). As seen in Figure 5, Del Vecchio et al. (2018) showed that motor unit conduction velocity was positively correlated with the recruitment threshold measured for a large sample of motor units in the tibialis anterior muscle. This principle depends on the intensity of a certain contraction and the large motor units will only be activated at a higher level of muscle force (Heckman & Enoka, 2012).

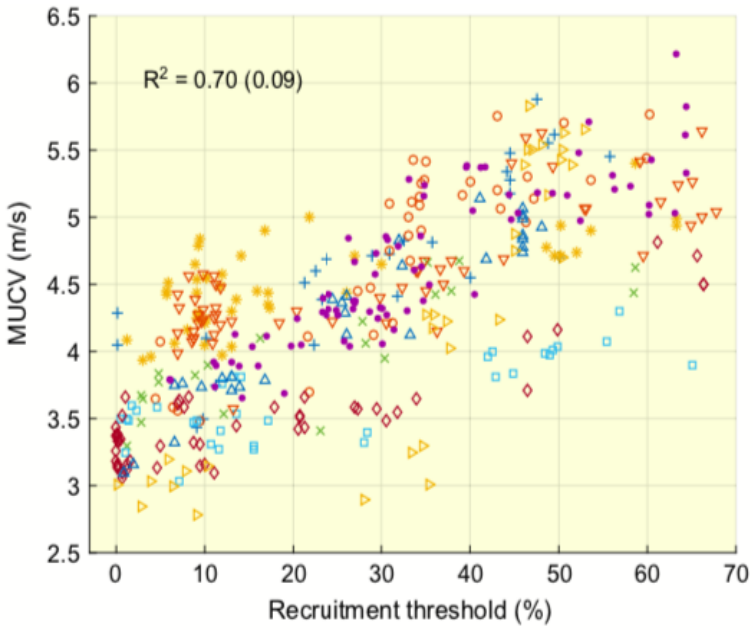

Figure 5: Shows a correlation of R^2= 0.7 (0.09) (mean ± SD across 10 subjects) between motor unit conduction velocities (MUCV) and the respective recruitment threshold (% of maximal voluntary force). Each colored symbol represents a subject (*n*=10). The data are reported for all measured motor units in tibialis anterior (*n*=406). From Del Vecchio et al. (2018).

ELECTROMYOGRAPHY

Electromyography (EMG) is a measure of the electrical activity from motor unit action potentials in muscles (Petersen & Rotalski, 2019).

Generally, there are two types of EMG recordings: intramuscular EMG and surface EMG, which are recorded by invasive- and non-invasive electrodes, respectively (Farina & Negro, 2012). Subsequently, surface EMG can be divided into two categories: bipolar surface EMG (a single bipolar signal from two electrodes placed on the skin over the muscle) and high-density surface EMG (hd-EMG) (a relatively new technique with 64 closely spaced electrodes placed on the skin over the muscle) (Farina et al. 2016; Drost et al. 2006). These three methods (intramuscular-, bipolar surface EMG, and hd-EMG) will be elaborated below.

EMG signal provides information about the neural system of the individual. However, the assessment of the neuromuscular function of individuals is difficult to measure (Sartori et al. 2017). The recorded motor unit action potentials with surface EMG provide a global measure of muscle activation. When force production increases, the number of motor units involved increases, which results in an increase in the amplitude of the EMG signal (Heckman & Enoka, 2012).

The behavior and properties of motor units give insight into the neural code, which is responsible for human movements. For this reason, motor units have been investigated for decades. However, only a few studies have analyzed the motor unit properties after a training intervention, this being a result of earlier methodological limitations in the measurements (Duchateau et al. 2006; Martinez-Valdes et al. 2017b). These limitations have been due to measurements of small samples of motor units, and a large variability in the positioning of needle electrodes *(see intramuscular EMG)* and difficulty of separating individual motor units from the global EMG recordings *(see bipolar surface EMG)*. Recently, a new method has overcome these limitations and made it possible to track individual motor units across sessions *(see high-density surface EMG)*. Figure 6, presents an overall illustration of the application area for the three described techniques in the present study.

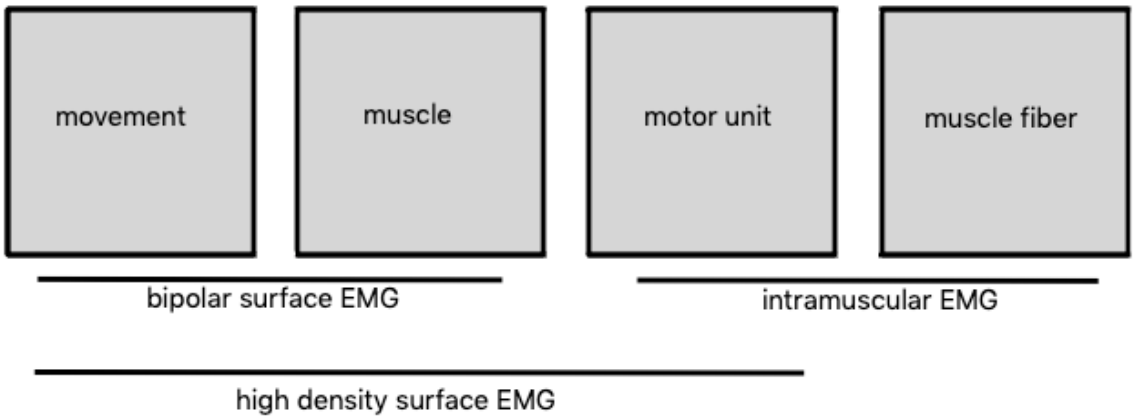

Figure 6: Illustration of the optimal application area of the various EMG techniques described in the worksheets. Bipolar surface EMG is limited in detecting motor units and muscle fibers, and is often used in movement studies. Intramuscular EMG recordings are limited in the global measure of the muscle, though it provides information from the single motor unit and muscle fiber level. The hd-EMG technique provides a global measure of the activity in the muscle, and with data processing it is possible to separate single motor unit action potential and track them across sessions (Modified Figure from Drost et al. 2006).

INTRAMUSCULAR EMG

The first method of EMG recording was demonstrated in 1928 with intramuscular invasive electrodes that recorded action potentials discharged by single motor units (Adrian & Bronk, 1929). Methods with intramuscular fine wire electrodes or concentric muscular needle electrodes only record action potentials from a few motor units, which is not representative for the activity of a whole muscle. The method is limited in tracking the same motor unit across experimental sessions, since it is almost impossible to locate the exact same motor units across sessions. Furthermore, the small sample of detected motor units will be too variable to make conclusions about adaptations in the motor unit across sessions (Martinez-Valdes et al. 2017a).

BIPOLAR SURFACE EMG

A non-invasive method for EMG recording is by bipolar surface EMG, that provides a global measure of muscle activation (Enoka, 2019) by comprising a single bipolar signal from two electrodes placed on the skin above the muscle (Drost et al. 2006). The amplitude of the signal in the global signal (Figure 7) magnifies as the activated number of motor units increases (Heckman

& Enoka, 2012). It is, however, difficult to separate the shapes of the detected motor unit action potentials in the global interference signal (Heckman & Enoka, 2012).

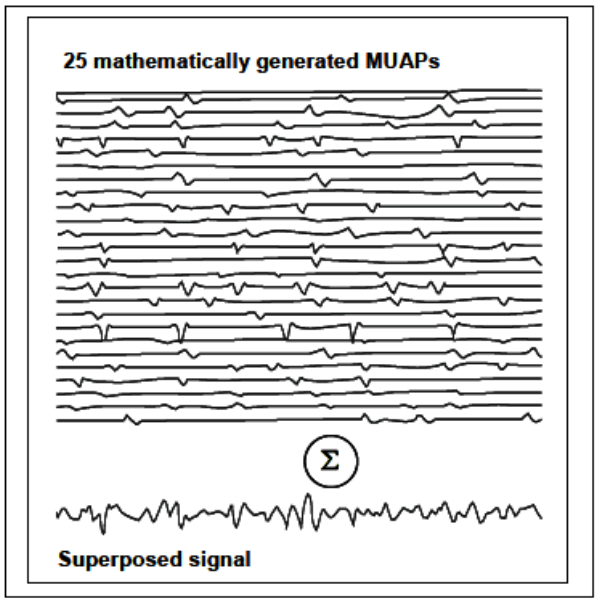

Figure 7: Illustratration of the global (superposed) EMG signal. All motor unit action potentials from detected active motor units are summed in a superposed bipolar signal, also called an interference pattern, where positive and negative amplitudes are symmetric distributed (mean value equal to zero) (Konrad, 2006).

The interference signal is often rectified with positive and negative phases of motor unit action potentials that overlap in time, and therefore reduces the size of the averaged motor unit action potentials. This is termed amplitude cancellation (Keenan et al. 2006). A variation in size of the motor unit action potentials, can contribute to a variation in the EMG amplitude, which could be hidden in the global measure of the bipolar surface EMG (Enoka, 2019). The absolute amplitude of interference rectified signal is therefore less than the motor output from the central nervous system, and bipolar surface EMG is therefore not a valid measure of the relation to the central motor drive (Enoka, 2019), but still provides a representative association (Heckman & Enoka, 2012). The recordings of bipolar surface EMG are difficult to interpret (Enoka, 2019) and it is not possible to detect the characteristics of motor unit action potential with the bipolar surface EMG (Heckman & Enoka, 2012).

HIGH-DENSITY SURFACE EMG

Hd-EMG is a relatively new and non-invasive surface EMG method to measure motor unit action potentials within the muscle with multiple, closely spaced electrodes. Hd-EMG consists of a matrix of 64 electrodes that are arranged in 13 rows and 5 columns. Each electrode is 1 mm in diameter with 8 mm between adjacent electrodes. With this method, the matrix with 64 electrodes

allows for identifying unique shapes, discharge times and locations of motor unit action potentials (Enoka, 2019). It is even possible to identify several motor units with hd-EMG at both low and high forces (close to MVC force) (Martinez-Valdes et al. 2017b). Motor units can be identified across experimental sessions, even separated by weeks, because it is possible to track the behavior and properties of the same motor unit (Martinez-Valdes et al. 2017b). The study of each motor unit activity depends on the separation of the action potentials from the hd-EMG signal. This method enables the identification for each time the motor unit action potential discharge and this is referred to as EMG decomposition (Farina et al. 2016). There are different methods for decomposition of EMG, where the gold standard is by full manual decomposition by experts, the hd-EMG decomposition can also be decomposed with a non-manual decomposition framework (Negro et al. 2016). The procedure of tracking motor units across sessions, measured with hd-EMG, is illustrated in Figure 8.

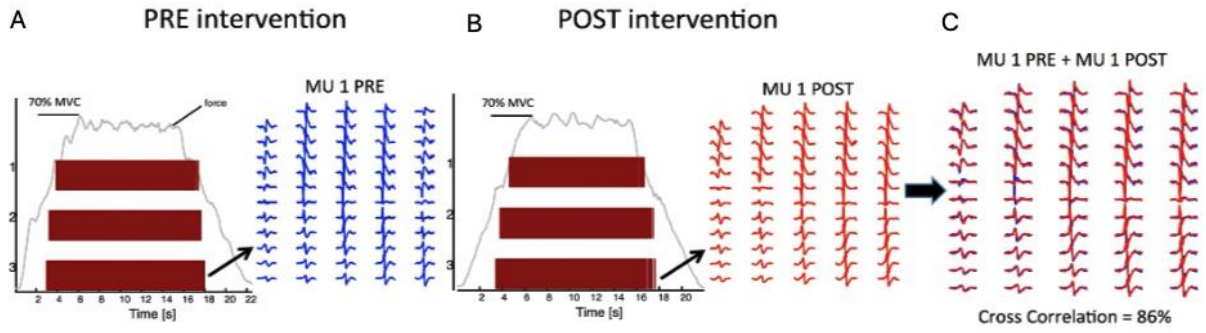

Figure 8: Illustration of the procedure of tracking motor unit pre and post intervention. A) In the left half of the figure the identification of three motor unit action potential spike trains at 70% of MVC before (PRE) the intervention, is seen. In the right half of the figure the motor unit action potentials shapes from the first motor unit are shown in blue. B) After the intervention (POST) the same motor units that could be tracked and were similar with motor unit number one from PRE, indicated by red. C) The two identification of motor unit number one were compared by cross correlation and were regarded as the same motor unit, since the cross correlation were 86% (Martinez-Valdes et al. 2017b).

With hd-EMG the number of motor units identified is increased, compared with intramuscular EMG and contrary to the bipolar surface EMG, it is possible to identify several motor units with hd-EMG (Farina et al. 2016). For that reason, the captured motor unit action potentials with hd-EMG provides a realistic and representative number of the motor units (Heckman & Enoka, 2012; Sartori et al. 2017), which reveal information about how the nervous system controls muscle force (Enoka, 2019).

Figure 9 is presented to illustrate how hd-EMG can detect an optimization of the central motor drive, after an intervention with one session of local IPC on chronic stroke survivors (Hyngstrom

et al. 2018). As seen in Figure 9, the interspike interval (time between motor unit action potentials in ms) has increased at 40% of MVC, indicating that the motor unit action potential had reached force recruitment threshold earlier (Hyngstrom et al. 2018).

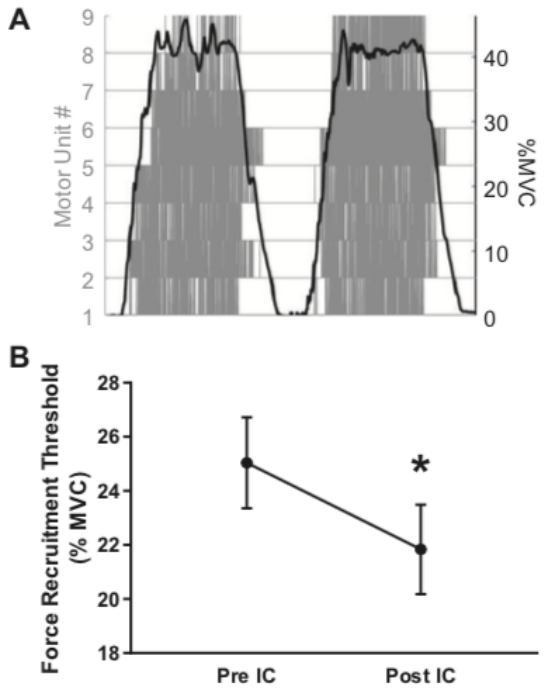

Figure 9: Illustration of motor unit firing and recruitment during a submaximal ramp contraction at 40% of MVC. A) A single chronic stroke survivor subject's nine tracked motor units Pre (left) and Post (right) a single intervention of IPC. The plot is a raster plot with torque generated during the submaximal ramp (up to 40% of MVC) and the firing rates of the action potentials from each of the nine motor units matched between time points. B) An indication of all subjects' average force recruitment threshold (% MVC), were decreased after an intervention of local IPC, reflecting an increase of the central motor drive (Hyngstrom et al. 2018).

Figure 10 is presented to illustrate how hd-EMG can detect an optimization of the central motor drive after a strength intervention, where the discharge rate is increased compared to the control group.

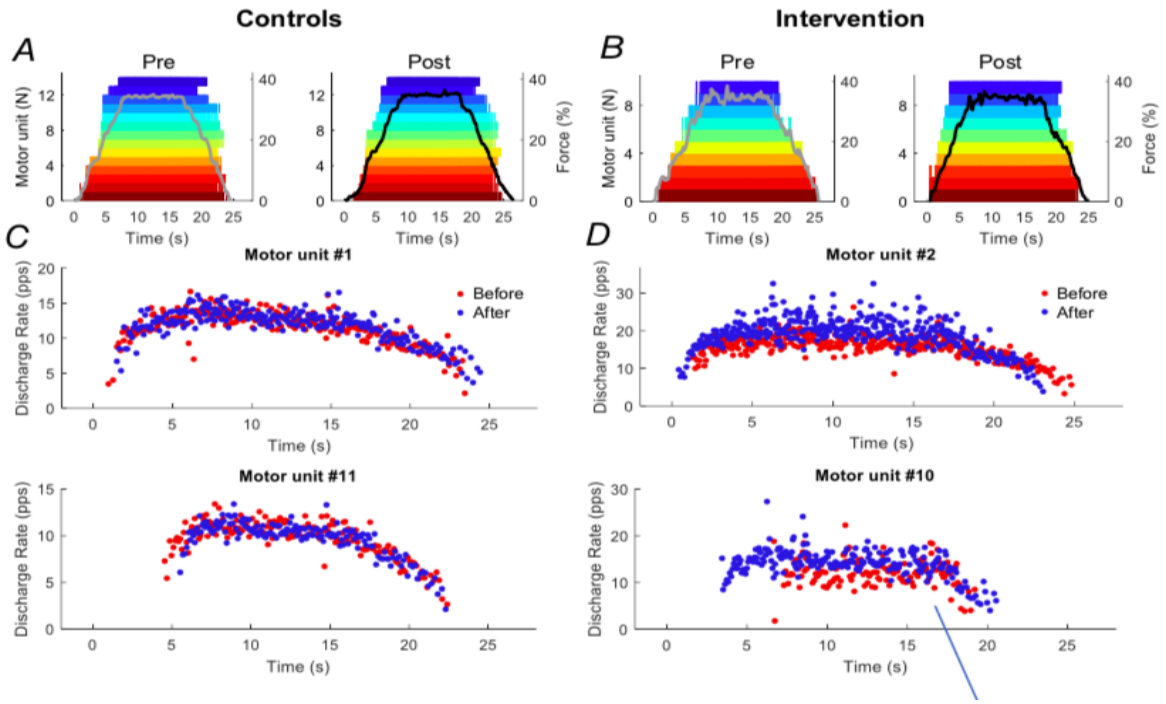

Figure 10: Illustration of the discharge times for each motor units (indicated by different color) tracked across the 4-weeks of strength intervention pre and post for A) control group and B) intervention group. The gray line is pre and the black line is post the produced force of the ankle dorsiflexors in an isometric contraction until 35% of maximal voluntary force pre. C) and D) represent the discharge rates for two representative motor units from the control group and intervention group, respectively (Del Vecchio et al. 2019).

THE POSSIBLE INFLUENCE OF IPC ON MUSCLE ACTIVATION DURING EXERCISE

As mentioned in the section *Mechanisms of ischemic conditioning,* IPC has been associated with an increase in endogenous metabolites, including opioids. Furthermore, IPC have been suggested to influence the registration of fatigue signals during exercise, due to involvement of group III/IV muscle afferents (Crisafulli et al. 2011; Cruz et al. 2015).

A study by Amann et al. (2011) examined the influence of group III/IV muscle afferents on peripheral fatigue, central motor drive and endurance capacity during high-intensity leg-cycling. Subjects performed a constant-load cycling exercise (80% of peak power output), with impaired

opioid receptor-sensitive group III/IV muscle afferents (Amann et al. 2011). By blocking opioid receptor-sensitive muscle afferents, central motor drive, estimated via bipolar surface EMG on the quadriceps, was increased by 9%, however the cardiovascular and ventilatory responses were compromised, which were expressed in a shorter time to exhaustion and an increase in peripheral fatigue development of nearly 70% (Amann et al. 2011). These results suggest that group III/IV muscle afferents influence performance in two contradictory ways. On the one hand, group III/IV muscle afferent feedback ensures adequate optimized muscle O_2 transport via circulatory and ventilatory responses to the working muscles. On the other hand, afferent feedback inhibits central motor drive and thereby restricts the neural excitation in the exercising musculature (Amann et al. 2011).

Based on this knowledge, it has been suggested that IPC facilitated an increase in muscle activity via a disruption of the central feedback loop, through inhibited signaling from ischemia sensitive group III/IV muscle afferents, due to the metabolites produced during the IPC cycle leading to preservation of skeletal muscle function (Halley et al. 2019). Recent studies have shown IPC to increase muscle activity, measured with EMG, in a clinical population (Hyngstrom et al. 2018) and in both aerobic and anaerobic cycling (Cruz et al. 2015; 2016).

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STUDY PROPOSAL: THE EFFECTS OF LOCAL AND REMOTE ISCHEMIC PRECONDITIONING ON MOTOR UNIT PROPERTIES IN THE TIBIALIS ANTERIOR MUSCLE OF HEALTHY ADULTS

ABSTRACT

Ischemic preconditioning (IPC) refers to a technique that includes brief rounds of ischemia and reperfusion and which has recently emerged as an intervention to improve muscle activity. IPC can be applied either locally to the tissue of interest (LIPC) or on the tissue of a remote limb (RIPC). The underlying mechanisms of IPC are still not fully understood, but it is suggested that IPC inhibits the ischemia sensitive group III/IV muscle afferents, which leads to an increase of the excitability of the spinal motor neurons. The effects from IPC on motor unit properties has only been investigated in chronic stroke survivors, and the study showed that LIPC increased muscle strength through improved muscle activation.

The objective for the present study proposal is to investigate if IPC has an effect on the discharge rate and recruitment threshold for motor units in healthy adults when performing a submaximal isometric muscle contraction. An additional objective is to investigate whether the effect of LIPC and RIPC respectively differ.

It is hypothesized that LIPC increases the discharge rate and decreases the recruitment threshold for the motor units to a larger extent than RIPC, and that both of these interventions will lead to a higher discharge rate and lower recruitment threshold of the motor units compared to a sham intervention.

The proposed study will be carried out as a randomized counterbalanced crossover study with a required sample size of 16 healthy adults. During the proposed study, subjects will undergo three interventions: LIPC, RIPC and sham. Each intervention will consist of four cycles of 5x5 min occlusion/reperfusion, with a pressure of 225 mmHg for both LIPC and RIPC, and 25 mmHg for sham. Before and after each intervention, the subjects will perform three maximal voluntary contractions (MVC) and two submaximal isometric ramp contractions at 40% MVC with the dorsiflexors. During the experimental protocol, measurements of central motor drive (motor unit discharge rate and recruitment threshold) from the right tibialis anterior muscle will be conducted using high-density surface electromyography.

Carrying out the study in practice can contribute with a unique perspective on the central motor drive after an IPC intervention and knowledge of the possible effects on motor unit properties.

INTRODUCTION

Ischemic conditioning refers to a phenomenon where brief rounds of ischemia and reperfusion directly induced on the coronary arteries cause endogenous mechanisms that have been shown to protect against ischemia-reperfusion injuries in the myocardium (Murry et al. 1986). Subsequently, ischemic conditioning was applied to remote tissue and revealed a remote effect of ischemic conditioning, which also provides cardioprotection (Przyklenk et al. 1993). Later, the method of ischemic conditioning was modified into an easy and non-invasive application by using blood pressure cuffs inflated to 200 mmHg on limbs, referred to as ischemic preconditioning (IPC) (Kharbanda et al. 2002), resulting in the same amount of cardioprotection.

Despite ischemic conditioning being introduced over 30 years ago, the exact mechanisms involved are complex and not yet fully understood (Heusch et al. 2015). In addition to the beneficial effects of IPC in clinical settings, the effects from IPC on vessels, muscles and heart raised the possibility that IPC could also be a possible ergogenic aid for improving physical exercise performance (Sharma et al. 2015; De Groot et al. 2010). This has led to various studies focusing on the effect of IPC on sports performance. Overall, IPC has shown small enhancement of performance effect in various modalities; incremental cycle ergometer test (bilateral IPC on tights) (De Groot et al. 2010), maximal voluntary knee extensions on an isokinetic dynamometer (LIPC on thigh) (Paradis-Deschênes et al. 2016) and fatigue and time to task failure tested with handgrip exercise (RIPC on arm) (Barbosa et al. 2015). In relation to performance effects both LIPC and RIPC have shown to be beneficial compared to a sham-group (Cocking et al. 2018).

There is still a lack of clarity with regards to the optimal protocol for cuff positioning in order to enhance performance (Salvador et al. 2016; Incognito et al. 2016). In addition, several studies have used bilateral blood pressure cuffs positioned on both thighs or arms or unilaterally directly on one limb (Salvador et al. 2016). IPC on the exercising limb is referred to as local IPC (LIPC), or with the cuff positioning on a non-exercising limb referred to as remote IPC (RIPC). The inconsistency of the reported methods of cuff application, amount of tissue occluded, and number of IPC cycles makes it challenging to analyze an effect of IPC on performance due to a wide variety of factors influencing the results (Salvador et al. 2016).

In addition to the focus on the ergogenic effects of IPC, a growing body of literature has recently focused on IPC as a potential stimulus in enhancing the central motor drive and thereby

facilitating performance (Cruz et al. 2015; Halley et al. 2019). More specifically, it has been proposed that the cycles of IPC induce a metabolic accumulation, which inhibits signaling in ischemia sensitive group III/IV muscle afferents (Halley et al. 2019). The inhibition of group III/IV muscle afferents is thought to disrupt the central feedback loop (Cruz et al. 2017) and thereby influence the registration of fatigue signals in the body (Crisafulli et al. 2011; Cruz et al. 2015). This can lead to a preservation of skeletal muscle function (Amann, 2011; Halley et al. 2019). Furthermore, this inhibition is thought to engage brainstem centers that release neuromodulators, which increase the excitability of the motor neuron pool (Heckman & Enoka, 2012; Sharma et al. 2015). Importantly, this can lead to a greater force output due to increased motor unit discharge rates and a decrease of the recruitment threshold (Hyngstrom et al. 2018).

One study that did investigate muscle function after an intervention of LIPC was Hyngstrom et al. (2018), which examined the effect on chronic stroke survivors. They applied LIPC on the paretic leg of the subjects who subsequently performed a maximal knee extension and at isometric ramp contraction up to 40% of maximal voluntary contraction (MVC). The study found an increase in both MVC and in muscle activity of the paretic leg after the intervention compared to a sham group. Hyngstrom and colleagues (2018) speculated whether ischemia induced during IPC engages autonomic centers in the brainstem responsible for force generation and muscle activation, possibly due to the influence of the ischemia sensitive group III/IV muscle afferents. Consistent with the result from Hyngstrom et al. (2018), an increase in muscle activation in healthy subjects has also been observed in cycling during aerobic and anaerobic conditions following intermittent bilateral IPC (Cruz et al. 2015; 2016). Halley and colleagues, however, did not find any improvements in muscle activity after a 2-min isometric leg extension task (Halley et al. 2018) or 3-min isokinetic leg extension task after an IPC intervention (bilateral leg occlusion) (Halley et al. 2019). Based on their findings, Halley et al. (2018; 2019) suggested that IPC does not have an effect on the central motor drive in healthy adults.

The studies by Halley et al. (2018; 2019) and Cruz et al. (2015; 2016), however, all used bipolar surface electromyography to measure EMG amplitude, which only provides an estimate of the gross nervous system output to the muscle (Enoka, 2019). The absolute amplitude detected with the bipolar surface EMG is less than the motor output, partially due to amplitude cancellation, and for that reason bipolar surface EMG does not provide a valid measure of the central motor

drive (Enoka, 2019). Furthermore, electromyographic signals from neighboring muscles may also be detected by the electrodes and influence the signal amplitude (Konrad, 2006).

The recent development of high-density surface electromyography (hd-EMG) can provide measures of central motor drive such as discharge rate and recruitment threshold (Del Vecchio et al. 2019). Especially motor unit recruitment and discharge rate are two of the key factors of neuromuscular control, as these factors play a crucial role in modulation of the force output of the involved muscle (Konrad, 2006). Furthermore, because it is possible to track and identify several motor units across sessions (Martinez-Valdes et al. 2017b; Enoka, 2019), hd-EMG allows for a deeper mechanistic insight in the properties of the motor units after an IPC intervention.

To the best of our knowledge, the effects of IPC on motor unit recruitment during submaximal contractions has only been examined in chronic stroke survivors in the study by Hyngstrom et al. (2018). Using hd-EMG, they found an increase in muscle activity and a decrease of the force recruitment threshold after an intervention of LIPC and it was suggested that the effect of IPC could be due to inhibition of the ischemia sensitive group III/IV muscle afferents. However, Hyngstrom et al. (2018) only examined LIPC, whereas the effect of RIPC on motor unit properties is still unknown.

Examining whether IPC has an effect on motor unit properties and whether there is a difference between LIPC and RIPC can provide important, new information concerning the use of IPC as an ergogenic instrument. Furthermore, if IPC intervention is found to have the same effect on motor unit properties in healthy adults free from neuromuscular deficits as the effect found by Hyngstrom et al. (2018) in chronic stroke survivors, this would open up new avenues for the study of the optimal application of IPC and its influence on motor unit properties.

The aim of this study proposal is to present a study design that in a future experimental setting will be able to provide a deeper understanding of how IPC influences motor unit properties. In the proposed study design, healthy subjects will perform submaximal isometric ramp contractions in order to examine whether an IPC intervention enhances central motor drive and whether the location of IPC stimuli impacts the motor unit properties. Three interventions are included in this counterbalanced within-subject crossover study design: LIPC = exercising leg, RIPC = non-exercising leg, and sham conditioning on the exercising leg. The target force will be the same for

all three interventions as to 40% of their MVC and an optimization would be seen if the discharge rate is increased and/or the recruitment threshold is decreased.
It is hypothesized that LIPC increases the discharge rate and decreases the recruitment threshold for the motor units more than RIPC, and that both of these interventions will have a higher discharge rate and lower recruitment threshold of the motor units compared with a sham-intervention.

MATERIALS AND METHODS

PARTICIPANTS

16 adults who meet the following criteria will be recruited; healthy, right leg dominant, non-smokers, uninjured, without any neurological disorders and do not take any forms of medication influencing their nervous system. The sample size is based on a 95% confidence interval, a power of 0.8, and an effect size of 0.47 (Cohen's d). Cohen's d was used to estimate the effect size based on data of submaximal motor unit force recruitment threshold from Hyngstrom et al. (2018) who had a similar intervention with IPC on muscle activation, although on a clinical population. The effect size in the study by Hyngstrom et al. (2018) was 0.94. For the present study, the effect size is anticipated to be lower due to an intact nervous system of the subjects. This will be accounted for by dividing the effect size from Hyngstrom et al. (2018) by two, resulting in an effect size of 0.47 for the present study, giving an estimated sample size for this study of 12 subjects. Furthermore, loss of data due to withdrawal from participants or poor data quality will also be accounted for by adding 30% (4 subjects) to the estimated sample size.
All potential participants will be pre-screened using a questionnaire prior to the experiment to assure they fulfill the recruitment criteria (Appendix B). Furthermore, there will be a familiarization session where the participants will receive a verbal and written explanation of all test procedures, after which they have to provide their written informed consent to proceed to the test sessions. The participants will be able to withdraw at any time during the experiment.
The participants will have to refrain from vigorous training 48 hours prior to each test session. Furthermore, consumption of alcohol and caffeine will not be allowed 24 hours prior to each test session. The present study is approved by the Research Ethics Committee of North Denmark, Denmark (N-20130029) and will be performed in accordance with the Declaration of Helsinki.

STUDY DESIGN

In order to investigate the local (right leg) and remote (left leg) effects of IPC on motor unit properties in the right tibialis anterior muscle, a counterbalanced randomized within-subject crossover design will be applied. Figure 1 shows an illustration of the study design. The study will consist of one familiarization session and three test sessions (with three different interventions: LIPC, RIPC and sham) in a counterbalanced order and all participants will report to the laboratory on these four separate occasions. The familiarization session and the first test session will be separated by a minimum of 24 hours and in between test sessions a washout period of at least seven days will be utilized. The time of day each session takes place will be standardized.

In order to avoid any placebo effects, the participants will not be informed of the purpose of the study. Instead, the participants will be informed that the purpose of the study is to examine whether different pressures (225 mmHg and 25 mmHg) and different locations (local = exercising limb, remote = non-exercising limb) have an effect on submaximal force generation.

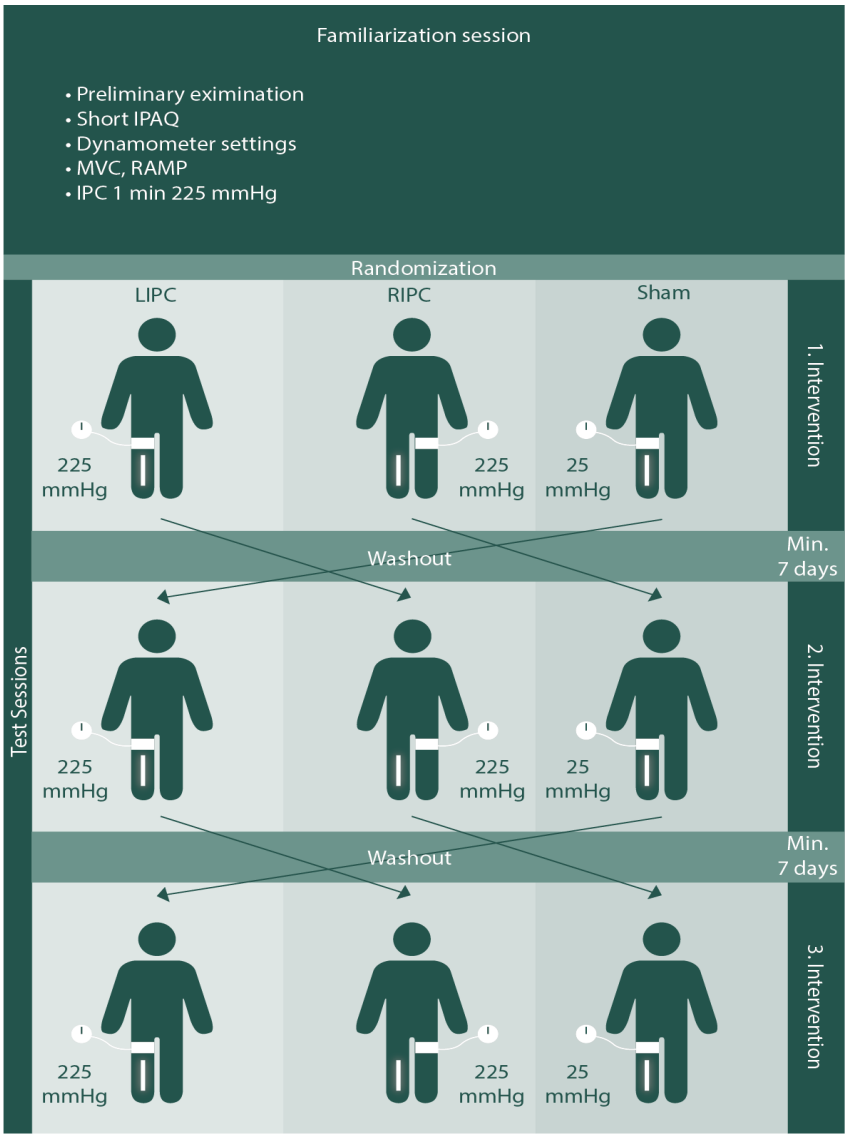

Figure 1: Illustration of the study design. Following the familiarization session, the subjects will be randomized into three different intervention orders (illustrated by the arrows). The three interventions differ by the positioning of the cuff in relation to the exercising limb (indicated by the white mark on the right tibialis anterior muscle) and the pressure provided by the cuffs.

FAMILIARIZATION

During the familiarization session, the participants will undergo a preliminary examination including anthropometric measurements and asked to answer a short International Physical Activity Questionnaire (IPAQ) to assess their level of physical activity and examine the homogeneity of the subjects. Subsequently, the individual setting of the dynamometer for each participant will be documented in order to ensure the same position for each participant at each session. At the familiarization session, the participants will receive a thorough explanation of the test procedures, the test protocol, and an introduction to the MVCs and submaximal trapezoidal contractions (ramp contractions) of the right leg. After which the subjects will be asked to perform three MVCs and two isometric ramp contractions of 40% of their MVC with the right tibialis anterior muscle (~100° in the ankle joint), to ensure a clear understanding of the test protocol. During the familiarization session and the following three test sessions, the participants will be instructed to focus on the movement of the ankle dorsiflexors and to isolate the activation of the tibialis anterior muscle as much as possible (Del Vecchio et al. 2019). Subsequently, the procedure for the hd-EMG placement will be explained. Finally, the subjects will receive a brief (one minute) leg occlusion on the right leg, at the highest pressure (225 mmHg) as used in the test sessions.

EXPERIMENTAL PROCEDURES

At the three test sessions, the participants will have the hd-EMG matrix mounted on the right tibialis anterior muscle, afterwards they will position themselves in the dynamometer and then undergo a standardized warm-up before proceeding to the exercise protocol. The warm-up will consist of eight isometric contractions with the dorsiflexors at increased intensities of the perceived maximal voluntary torque (4x50%, 3x70%, 1x90% with 15-30s between each contraction) (Del Vecchio et al. 2019).

As illustrated in Figure 2, the exercise protocol will consist of two sections, one pre and one post the intervention. The recordings from the first section prior to the intervention will function as a baseline and will be compared to the recordings from the second section after the intervention. Each section consists of a warm-up and three MVCs, followed by two isometric submaximal trapezoidal contractions. The three MVCs will be separated by 90s rest. During the MVCs the participants will be instructed to pull as hard as possible for 5s and will receive strong verbal

encouragement to ensure maximal effort during each contraction. During the submaximal ramps the participants will perform contractions up to 40% of their MVC (Hyngstrom et al. 2018). The isometric ramps consist of a 4s graded contraction, 10s hold at 40% of MVC, and a 4s graded relaxation. The two isometric ramps will be separated by 180s rest. While performing the submaximal ramps, the participants will receive verbal instructions to ensure a correct force output. Throughout the sessions the participants will get feedback on the generated force, which will be displayed on a computer screen placed two meters in front of the participants' eyes.

After the intervention, the participants will perform the same procedure as in section one. Additionally, at the end of the second section, three MVCs separated by 90s will be added to test for fatigue. As with the first section, the 40% of the isometric ramps in section two are based on the MVC from section one.

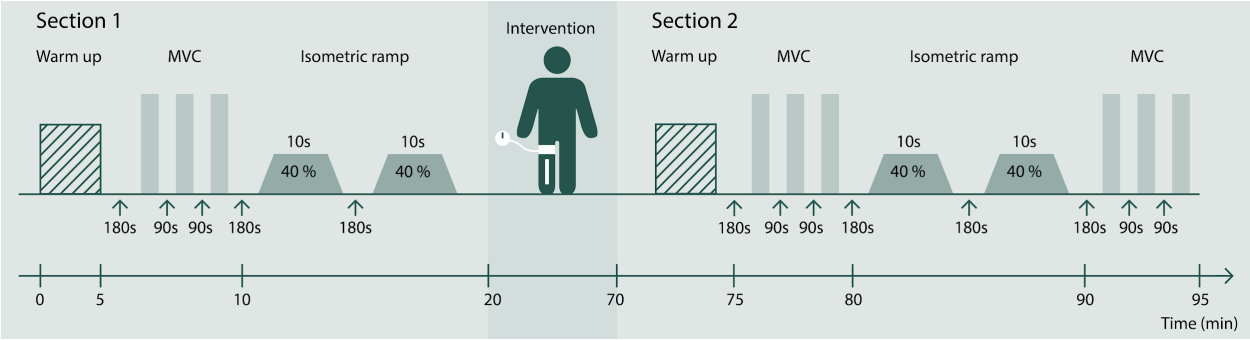

Figure 2: Illustration of the experimental protocol divided in two sections, separated by an intervention. Section 1 consists of a warm-up period, three MVCs, two isometric ramps with 10 seconds hold at 40% of MVC, thereafter an intervention of either LIPC, RIPC or sham. Section 2 consists of the same as section 1, but with three MVCs in the end to test for fatigue. Each arrow indicates rest in seconds. An approximate timeline in minutes is shown at the bottom.

ISCHEMIC PRECONDITIONING AND SHAM PROTOCOL

The LIPC, RIPC and sham protocol will be executed with the participants lying comfortably in a supine position. During each intervention, the participants will be instructed to move as little as possible and stay calm. A blood pressure cuff (Tourniquet Cuff, REF: 20-54-522, Size: 61cm / 24in. VBM, Germany) will be used for the intervention protocol, which consists of four cycles of five-minutes with cuff inflation and five-minutes of cuff deflation, resulting in a total duration of 40 minutes. The cuff will be placed proximally on the left upper thigh, under the gluteal line of

the non-exercising limb for RIPC, whereas both LIPC and sham will be placed in the same position on the exercising limb (right thigh).

The blood pressure cuff will be inflated to 225 mmHg for LIPC and RIPC and 25 mmHg for the sham intervention (Hyngstrom et al. 2018). The pressure for sham conditioning is chosen in order to avoid any ischemia and in order for the participants to feel a pressure on the thigh. Within the last 15 seconds of each inflation during the intervention the participants will be asked to rate their pain on a numerical pain scale ranging from 0-10, where 0 is "no pain at all" and 10 is "worst imaginable pain" (Ferreira-Valente et al. 2011). If answers are above 6, participants will be excluded from the study to eliminate influence of pain as a possible effect (Cherry-Allen et al. 2017).

DYNAMOMETER

The test of isometric contractions will be performed with the dorsiflexor muscles in a dynamometer (HUMAC2015®, NORM^{TM}, CSMi Solutions, Stoughton, Massachusetts). Participants will be placed in a sitting position in the dynamometer (Figure 3) with their hip flexed at ~120° (180°= supine position) and the right knee will be positioned over a knee stand with a knee flexion at ~90° (180° = supine position). The right foot will be placed in a footplate and the dynamometer will measure the anatomical zero (~100° angle in the ankle joint), which will be the position of the ankle when performing MVC and isometric ramps. The ankle will be positionally aligned with the axis of the rotation of the dynamometer and the right foot will be tightly attached with velcro straps to the footplate, in order to secure the position throughout the test. Furthermore, the participants will be strapped around the right thigh, hips and shoulders to avoid any unnecessary movements and isolate the movement of the tibialis anterior muscle. The left leg will rest on a foot support in a sitting position with the knee extended at ~90°. Before each test, the dynamometer will be calibrated to account for the weight of the participant's limb. When performing MVC's, the participants will be instructed to stay in place by holding on to the handlebars on the side of the chair and only use the tibialis anterior muscle for force generation.

Figure 3: Illustrates the experimental setup for the MVCs and isometric ramb contractions as explained above.

HD-EMG RECORDINGS

High-density surface electromyography (hd-EMG) will be recorded with a matrix of 64 electrodes [13 rows x 5 columns; 1 mm diameter; 8 mm inter-electrode distance, ELSCH064R3S, OT Bioelettronica, Italy]. First, the skin will be prepared for placing the matrix through shaving, light skin abrasion and cleaned using disinfection swaps with 70% ethanol (Alcoswaps (MEDIQ, Denmark)). The matrix will then be positioned on the muscle belly of tibialis anterior; one third of the distance between the tibial tuberosity and the intermalleolar line in accordance with Barbero et al. (2012), and with the electrode columns following the muscle fibers.

The placement will be marked with a marker pen and, in order to be able to replicate the position of the matrix, the participants will be instructed to remark the line until the next session (Martinez-Valdes et al. 2017b). To prepare the matrix for each session they will have an adhesive foam layer (KITAD064, OT Bioelettronica, Italy) to make the matrix disposable. The skin-contact layer of the matrix will be prepared with conductive gel (AC-Cream, Adhesive, Conductive cream, CC1, OT Bioelettronica, Italy) in order to optimize the signal of the electrode. Two ground electrodes will be placed distally as a strap electrode on the right ulna and on the

medial malleolus of the right leg, respectively (Wrist & Ankle strap with male clip connector; WS1, WS2, OT Bioelettronica, Italy). In addition, a reference electrode (Ambu, Neuroline 720) will be placed on the dynamometer to ensure minimal noise on the signal output (Del Vecchio et al. 2019).

The electrode matrix on the tibialis anterior muscle will be connected with cables (AD1x16, OT Bioelettronica, Italy) to the multi-channel amplifier (EMG-USB, OT Bioelettronica, Italy). The hd-EMG signals will be amplified, sampled at 2048 Hz and band pass filtered (10–500 Hz). The gain will be adjusted regularly at each trial in order to maintain the highest gain possible throughout all trials to ensure a maximum amount of resolution, given that the process of the decomposition requires a high gain for extracting motor units effectively. The force signal will be recorded with Software (OT-Biolab 2.0.6) and synchronized with the hd-EMG signal.

MOTOR UNIT IDENTIFICATION AND TRACKING

The convolutive blind source separation method will be used to extract motor units and identify the action potential shapes from the hd-EMG recordings and track motor units from pre to post for each intervention (Martinez-Valdes et al. 2017b). For quality control, the threshold of the silhouette will be set at >0.9. Silhouette is a normalized measure and represents a decomposition accuracy index and the higher the value, the greater is the likelihood of identical motor units (Negro et al. 2016). After the decomposition algorithm, a manual analysis of the identified motor units will be made to ensure only the motor units with a high pulse-to-noise ratio will be kept (Negro et al. 2016). To track motor units from pre to post for each intervention, a semi-blind algorithm will be used in Matlab version R2017b, where all motor units identified with a normalized cross-correlation >0.9 will be kept as a match (Martinez-Valdes et al. 2017b). From the decomposition procedure, the output of the mean discharge rate and recruitment threshold for all the identified motor units from each subject during each intervention will be extracted. Mean discharge rate is defined as the mean firing frequency (pulses per second (pps)) of the identified motor unit and the recruitment threshold is defined as the torque value (N) of when the motor units begin the discharging of action potentials (Martinez-Valdes et al. 2020). These data will be divided into two sub-sets. The first sub-set; matched motor units (tracked across time points; pre/post) providing an accurate insight of changes in the motor unit properties after an

intervention. The second subset: all identified motor units (all identified motor units in either pre or post) for a comparison of motor unit properties between interventions (Martinez-Valdes et al. 2017b). The statistics will be run on both sub-sets across the three interventions (time points: pre/post) (see Statistical analysis).

The procedure of decomposition and motor unit tracking is thoroughly described in Appendix A.

STATISTICAL ANALYSIS

All statistical tests will be performed in SPSS Statistics version 25 (IBM, Armonk, NY, USA). The criterion for statistical significance will be set at $\alpha \leq 0.05$. For all data, the normality of distribution will be tested with the Shapiro-Wilk's normality test. A Mann-Whitney U test will be used for data, which do not assume normality of distribution. MVCs from the start of the first (pre) and second section (post) and the end of second section (fatigue) of each intervention will be compared using a two-way repeated measures analysis of variance (ANOVAs) with two within subject factors (intervention: LIPC, RIPC and sham) and (time: pre, post and fatigue). On the following outcome variables; mean discharge rate and mean recruitment threshold from both matched and all identified motor units, separate two-way repeated measures ANOVAs with two within subject factors (intervention: LIPC, RIPC and sham) and (time: pre and post) will be performed to determine any main effect of the intervention, time, or intervention*time interaction. If the assumption of sphericity is not met, Greenhouse-Geisser's correction of degrees of freedom will be employed. Levene's test of equality of error variances will be measured. If a significant difference in the ANOVA is found, post hoc Bonferroni pairwise comparisons will be used to test for differences between interventions.

EXPECTED RESULTS

The expected results are based on the hypothesis of the present study proposal (Figure 4).

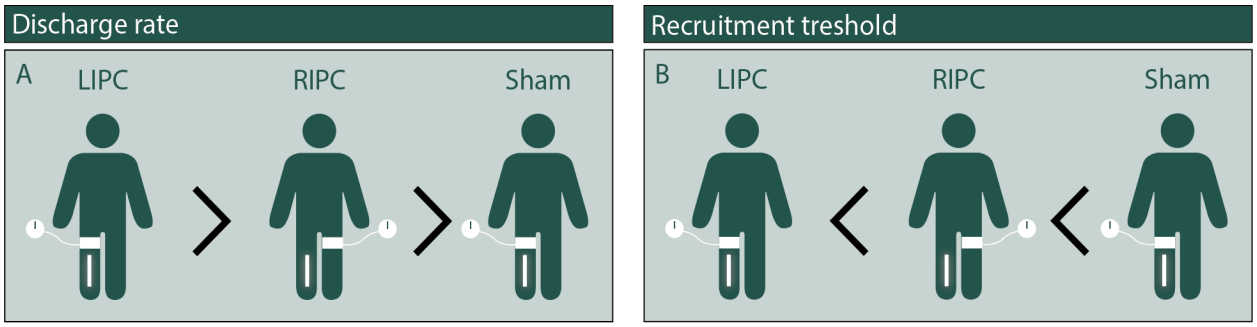

Figure 4: Simplified illustration of the hypothesized effects on the motor unit properties after the three interventions. (A): Discharge rate and (B): recruitment threshold. It is hypothesized that both LIPC and RIPC have a significantly higher mean discharge rate and a significantly lower recruitment threshold. No changes are expected for the sham intervention. Furthermore, LIPC is expected to have a greater increase of discharge rate and a greater decrease in recruitment threshold compared to RIPC.

The LIPC and RIPC interventions are expected to result in a significantly higher mean discharge rate and a significantly lower recruitment threshold during the post-trial compared to the pre-trial, whereas mean discharge rate and recruitment threshold for the pre-trial and post-trial in the sham intervention are expected to be the same. Furthermore, it is expected that LIPC display both a higher increase of mean discharge rate and a greater decrease in recruitment threshold compared to RIPC (Figure 4). Both matched and all identified motor units are expected to show the same behavior and the expected results of the three interventions are illustrated from pre to post in Figure 5.

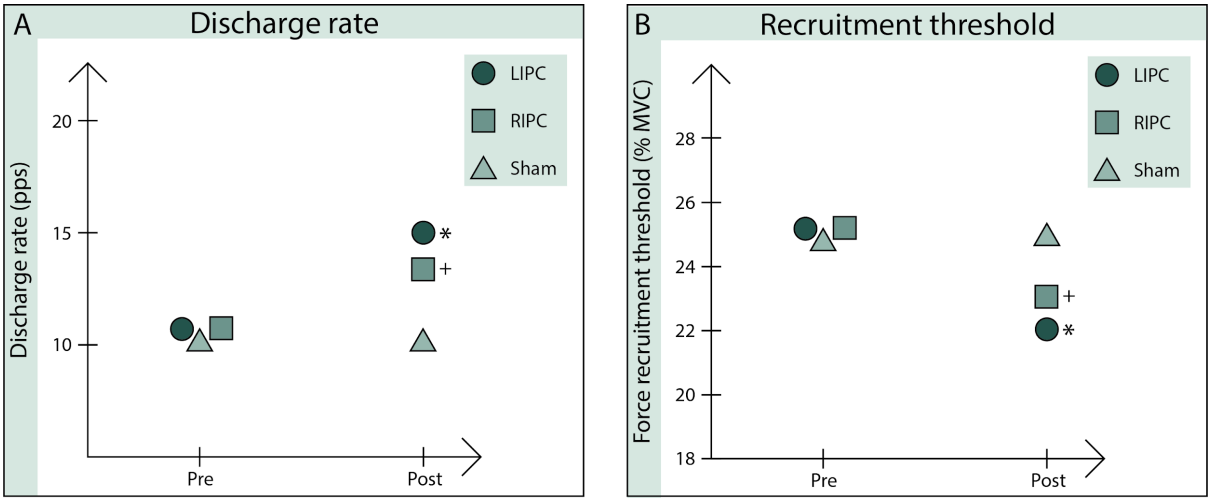

Figure 5: Illustration of the expected results in both the matched and all identified motor units (A): discharge rate, and (B): recruitment threshold for both pre and post for all three interventions; LIPC, RIPC and sham. * And + Indicates a significant change for LIPC and RIPC, respectively. To avoid an overlapping of the intervention-symbols at pre, where the values are expected to be the same, they are placed side by side.

GENERAL CHANGES IN THE MOTOR UNIT PROPERTIES

Figure 6 illustrates how either an effect or no effect in the central motor drive from the interventions could be seen in the motor unit properties. The expected outcome (no effect) of the motor unit properties of the discharge rate and recruitment threshold for the sham intervention would be displayed as in Figure 6.A and 6.C, respectively. In absence of an effect on the discharge rate and recruitment threshold, the discharge rate would be unchanged from the pre-trial to the post-trial.

Both LIPC and RIPC are, however, expected to increase the discharge rate and lower the recruitment threshold from pre to post as displayed in Figure 6.B and 6.D, respectively. The effect on discharge rate would reflect an increase in the discharge rate after the intervention, whereas the lowered recruitment threshold would be reflected in an earlier onset of discharge rate of the motor unit after the intervention.

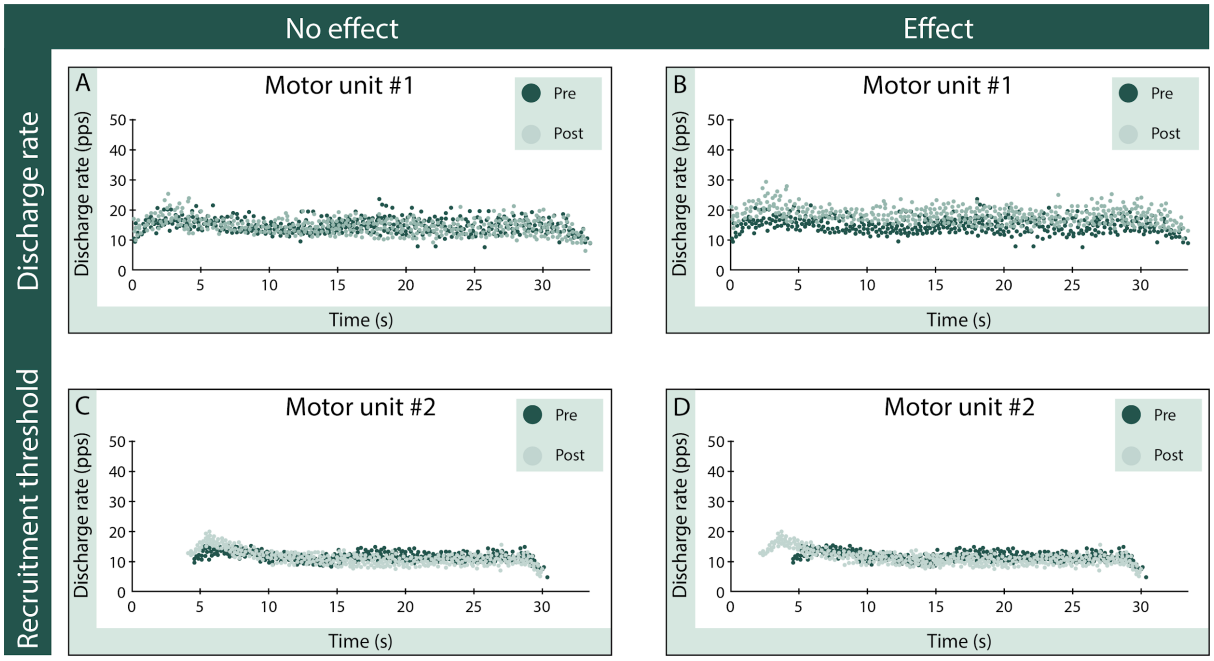

Figure 6: A simplified illustration of the changes in the motor unit properties, isolated to the specific changes in discharge rate and recruitment threshold for individual motor units. Discharge rate of motor unit #1 with A: no effect and B: an effect indicated with an increase in pulses per second (pps). Recruitment threshold of motor unit #2 with C: no effect and D: an effect indicated with an earlier onset discharge rate. The illustrations are based on data from a prior study (Appendix A).

DISCUSSION

The aim of the present study proposal is to investigate if IPC has an effect on motor unit properties in healthy adults, and whether there is a difference in the effect of RIPC and LIPC. Carrying out the study in practice can contribute with a unique perspective on the central motor drive after an IPC intervention and the possible effects on motor unit properties. It will be the first study to investigate the motor unit properties for both RIPC and LIPC in healthy adults. Furthermore, the application of IPC can contribute to the existing knowledge of optimal IPC application in order to optimize an IPC protocol.

In the study proposal it is hypothesized that an intervention of LIPC and RIPC will increase the discharge rate and decrease the recruitment threshold compared to the sham intervention and that LIPC will have a greater discharge rate and lower recruitment threshold compared to RIPC (Figures 4 & 5).

EXPECTED RESULTS

For the present study proposal, the expected results are based on the assumption that IPC inhibits group III/IV muscle afferents, which induces a disruption of the central feedback loop (Crisafulli et al. 2011). However, the effects from IPC on the involvement of group III/IV muscle afferents are difficult to examine in an experimental setting. If IPC does inhibit the signaling of group III/IV muscle afferents, it should be reflected in an increase in central motor drive (Halley et al. 2019). Therefore, measurements of central motor drive (discharge rate and recruitment threshold) will be used in the present study proposal.

The cycles of IPC are thought to induce an accumulation of metabolites, which inhibits the spontaneous discharge of the ischemia sensitive group III/IV muscle afferents during exercise (Halley et al. 2019). The inhibition of group III/IV muscle afferents is thought to disrupt the central feedback loop (Cruz et al. 2017) and influence the registration of fatigue signals in the body (Crisafulli et al. 2011; Cruz et al. 2015), which leads to preservation of skeletal muscle function expressed as an increased central motor drive; increase of discharge rate and decreased recruitment threshold (Amann et al. 2011; Halley et al. 2019).

In the section of expected results, Figure 6 shows an illustration of how discharge rate (6.B) and recruitment threshold (6.D) will be displayed concerning the effect of IPC. The rationale of the illustration is to clarify how each variable will be affected by the expected increase in central motor drive. However, it is noteworthy that the actual results of an increase in the central motor drive induced by IPC could result in a decrease in the recruitment threshold accompanied by an increase in the discharge rate, and not as isolated effects as illustrated in Figure 6.B and 6.D.

Discharge rate. As illustrated in Figure 5, both the LIPC and RIPC are expected to disrupt the central feedback loop and thereby increase the central motor drive, expressed as an increase in the discharge rate from pre to post in both matched and all identified motor units. The reasoning behind this expectation is that a sufficient pressure of 225 mmHg applied in the LIPC- and RIPC-protocol to obtain a full occlusion of the leg (Salvador et al. 2016), will lead to an accumulation of metabolites adequate to affect the muscle afferents. On the contrary, the 25 mmHg applied in the sham-protocol is insufficient to induce an accumulation of metabolites and no effect in discharge rate from pre to post is therefore expected.

The effect of IPC on the discharge rate is illustrated in Figure 5.A, which shows an expected increase from pre to post by 50% (10-15 pps) for LIPC and by 30% (10-13 pps) for RIPC.

However, the magnitude of the effect on discharge rate illustrated in the figure could be questioned, since no one has examined the effect of IPC on motor unit properties in healthy adults.

Additionally, IPC is thought to be an equivalent to high-intensity exercise due to some shared mechanisms (Hess et al. 2015). Martinez-Valdes et al. (2017a) examined motor unit properties in two weeks of high-intensity interval training consisting of six sessions with 8-12 rounds of 60s at 100% power output on a cycle ergometer. They found an increase of the discharge rate by 9.5% for the matched motor units in the vastus lateralis muscle in an isometric ramp contraction at 50% of MVC. However, the study did not show any influence on the discharge rate for the low threshold motor units, conducting the same experiment but with contractions 30% of MVC (Martinez-Valdes et al. 2017a). In comparison to the expected results of the present study proposal, it is questionable whether high-intensity interval training stimulates the group III/IV muscle afferents to the same degree as IPC.

Recruitment threshold. A decrease in motor unit recruitment threshold is expected for the LIPC and RIPC (as illustrated in Figure 4 & 5). The stimulation of the ischemia sensitive group III/IV muscle afferents is thought to engage the brainstem centers, which release neuromodulators such as serotonin (Hyngstrom et al. 2018). The increased level of serotonin is known to increase the excitability of spinal motor neurons (Heckman & Enoka, 2012; Hyngstrom et al. 2018). As illustrated in Figure 6.D, the effect will be an earlier onset of the discharge rate of the motor units after the intervention and thereby a lower recruitment threshold as illustrated in Figure 5.B. The effect of the sham intervention on recruitment threshold is expected to be the same as for the discharge rate.

Number of recruited motor units and discharge rate. Force generation is achieved by a certain number of recruited motor units and modulation of the discharge rate of the recruited motor units (Del Vecchio et al. 2017). The isometric trapezoidal ramp contractions in the present study proposal are fixed to reach an upper force of 40% of the subjects MVC. The increased central motor drive, as an effect of the disrupted central feedback loop, should lead to an increase in the number of recruited motor units or an increased discharge rate (Halley et al. 2019). However, this

would mean that if the discharge rate increases, the number of recruited motor units required to maintain the fixed force will decrease and vice versa.
Additionally, the application of hd-EMG in the proposed study provides only a mechanistic insight into a small part of all the recruited motor units during the experimental protocol. The number of recruited motor units, however, is expected to decrease if the discharge rate increases as a result of LIPC and RIPC.

LIPC vs. RIPC. Besides examining the effect of IPC on motor unit properties, the present study proposal is also designed to investigate potential differences between the effect of LIPC and RIPC. A clarification of this could be of significant importance for the optimal IPC application in both performance settings and clinical populations.
As illustrated in Figure 4, 5.A and 5.B, LIPC is expected to show a higher increase in discharge rate and a greater decrease in recruitment threshold compared to RIPC. LIPC is applied directly on the exercising limb, which is expected to provide a larger accumulation of metabolites within the exercising muscle, compared with RIPC, leading to a greater increase in central motor drive. However, RIPC is still expected to induce an increase in the central motor drive although the intervention is applied on the non-exercising limb. During the reperfusion periods in the RIPC intervention, accumulation of metabolites in the non-exercising limb will be released into the bloodstream (Hausenloy & Yellon, 2008) and some of these metabolites (a smaller amount than with LIPC) is expected to be distributed to the exercising limb and thereby inhibit the group III/IV muscle afferents leading to an increase in central motor drive.

Corpus callosum. Another, but speculative, explanation of the expected effect of RIPC, is the influence of the corpus callosum. The brain relies on the corpus callosum to transfer communication between the two cerebral hemispheres for coherent integration of cognition and behavior (Doron & Gazzaniga, 2008). When RIPC is applied on the non-exercising limb, it will lead to an accumulation of metabolites affecting the group III/IV muscle afferents in the left limb, which will send the information to the right sensory cortex. Because the two cerebral hemispheres are connected via the corpus callosum, the decrease in afferent feedback induced by RIPC resulting in an increase in central motor drive, which could be transferred from the right hemisphere to the left primary motor cortex responsible for the voluntary activation of the right

limb. Subsequently, the transfer of signal would be expressed as an increase in central motor drive to the right tibialis anterior muscle, which would be seen as an increase of the discharge rate and a lowered recruitment threshold of the matched and all identified motor units. If the latter explanation stands, the effect of RIPC would also be expected to be lower compared to that of LIPC. A possible explanation for this is that LIPC on the exercised leg stimulates the group III/IV muscle afferents, which has an uncrossed route to the exercised limb, suggesting this pathway to be faster and/or more direct than for RIPC, which has a crossed route.

METHODOLOGICAL CONSIDERATIONS

High-density surface EMG. With the tracking technique of hd-EMG it is possible to identify several motor units from pre to post intervention and to observe changes in recruitment threshold and/or discharge rate for the matched motor units for LIPC and RIPC in the present study proposal (Figure 5) (Del Vecchio et al. 2019).

Studies examining the effects of IPC and muscle activation on healthy adults have so far all used bipolar surface EMG. A study by Cruz et al. (2015) found an optimized muscle activation based on an increase of the amplitude of the bipolar surface EMG, and an increased time to exhaustion by 8% for the IPC group compared to a sham group in an incremental cycling test until exhaustion. Similar results have been reported in an anaerobic 60s sprint performance, where IPC increased the overall bipolar surface EMG amplitude and performance by 2.1% compared with a sham group (Cruz et al. 2016). On the contrary, Halley and colleagues found no effect in EMG-amplitude on isolated muscle activities measured with bipolar surface EMG for a sustained 2-min maximal effort isometric leg extension task (2018) or a 3-min maximal all-out isokinetic leg extension task after an IPC intervention (2019). Based on their results, Halley and colleagues stated that IPC does not preserve the central motor drive for the isolated muscle activation (Halley et al. 2019). These ambiguous results (Cruz et al. 2015;2016 & Halley et al. 2018;2019) are based on interpretations of bipolar surface EMG measurements and do not bring a clear understanding of the influence of IPC on muscle activation.

The global EMG measure consists of the absolute amplitude of a rectified signal and due to cancellation in the motor unit action potentials it is not a representative measure of the central motor drive (Enoka, 2019). With the application of hd-EMG in the present study proposal this limitation can be overcome, because it is possible to investigate the specific adaptations in motor

unit recruitment and discharge rate after an intervention and to measure a difference in the recruitment threshold or discharge rate. Therefore, it provides an accurate interpretation of the changes in the motor unit properties after an intervention (Martinez-Valdes et al. 2017a). Motor unit properties reveal information about how the central motor drive controls the voluntary muscle activation, which is more realistic and representative compared to both intramuscular EMG and bipolar surface EMG, which are limited to a few motor units identified at low forces (Martinez-Valdes et al. 2017b; Farina et al. 2016).

Matched and all identified motor units. In the present study proposal both matched and all identified motor units will be compared pre and post and across interventions. Ideally the number of matched motor units will be high enough for a comparison of motor unit properties across interventions, allowing for the expected increase in discharge rate and decrease in recruitment threshold of LIPC and RIPC to be seen. If this is possible, specific change exhibited by individual motor units after an intervention can also be seen (Del Vecchio et al. 2019). However, the number of matched motor units across trials depends on the number of trials. In a study by Martinez-Valdes et al. (2017b), the percentage of matched motor units for vastus medialis across two trials was approximately 40%, and across three trials it was 23% in a submaximal isometric ramp at 30% of MVC using knee extension contractions.

In the present study proposal, the motor units will be matched across six trials (pre/post across three interventions) with a total of three placements of the matrix, the same as by Martinez-Valdes et al. (2017b). With one placement of the matrix it was possible to track 47% of the matched motor units from two subsequent isometric ramp contractions performed with 10% of MVC (as shown in Appendix A). Therefore, it is expected that the percentage of matched motor units in the present study proposal will be lower than the 23% for the three trials performed by Martinez-Valdes et al. (2017b). Furthermore, it should be noted that the complex decomposition and tracking procedures require an experienced analyzer. If it is not possible to match a reasonable amount of motor units across the three interventions, the matched motor units from pre and post of each intervention will be analyzed.

By using two subsets of data (matched and all identified motor units) as in the present study proposal, it will be possible to investigate both changes in motor unit properties and make a comparison of RIPC and LIPC. Furthermore, Del Vecchio et al. (2019) showed similar results in

motor unit properties for both matched motor units and all identified motor units before and after the 4 weeks intervention. Therefore, it is expected that any effect found in matched motor units applying the present study proposal will be similar for all identified motor units.

Placebo effects. The present study proposal will be the first to compare LIPC, RIPC and sham (using the sham intervention to assess control responses) in a counterbalanced crossover study design in order to shed light on the effects on muscle activation. When investigating the effects of IPC, it is a methodological challenge to find a stimulus similar to IPC and implement it as an intervention, whereas the inability to effectively perform a sham-control treatment still remains (Angius et al. 2017). The precedent for a placebo protocol is to include a sham intervention where the cuffs are inflated to 20-25 mmHg, low enough for no physiological effects and high enough for the participants to feel pressure, though it will feel obviously different compared with the pressure of IPC (Incognito et al. 2016). In the present study proposal the participants will not be informed of the real purpose or hypothesis of the study in order to avoid any placebo effects. Given the inability to effectively blind the participants, they will be informed that the purpose is to examine the effect on a submaximal ramp contraction using different pressures and different locations of the cuff.

Studies that examine the effects of IPC on performance output have an element of competition and often a feedback in the form of results or achievements, and in these studies it will be necessary to account for the placebo effects as a possible explanation for the results. However, in this present study proposal a standardized submaximal ramp contraction is used, and it is speculated whether a manipulation of the voluntary muscle contraction can be made. Since it seems unlikely that participants have influence on how to control their own central motor drive, it is not expected to influence the results of motor unit properties in the present study proposal.

CLINICAL APPLICATION

Ceiling effect for healthy adults. To the best of our knowledge, the effects of IPC on motor unit properties during submaximal contractions have only been examined in chronic stroke patients (Hyngstrom et al. 2018). In this study, the subjects performed three maximal knee extensions and a submaximal isometric ramp contraction at 40% of MVC before and after the intervention. The IPC-protocol consisted of five cycles of 5 min occlusion (with a pressure of either LIPC: 225

mmHg or sham: 25 mmHg) and 5 min reperfusion applied on the paretic leg (Hyngstrom et al. 2018). Besides a 31±15% increase in EMG amplitude measured with hd-EMG on vastus lateralis, they found that LIPC also increased strength measured during the MVC and lead to a 5% decrease in the force recruitment threshold in motor units compared to a sham group measured during the ramp contraction (Hyngstrom et al. 2018).

The application of LIPC and RIPC in the present study is expected to have an effect on healthy adults. However, if no effect is found, this could indicate a ceiling effect of IPC for healthy adults due to an intact nervous system with an optimal neural activation of their skeletal muscles when it comes to IPC-induced improvements in motor function (Hyngstrom et al. 2018). This is based on the relatively small overall effects of IPC on performance reported for healthy adults (Salvador et al. 2016), and the fact that Hyngstrom et al. (2018) found subjects with the largest degree of motor deficits tended to have the largest effect of one session of IPC. In the present study proposal, the anticipated smaller effects were accounted for by dividing the effect size from Hyngstrom et al. (2018) by two to find the required sample size for the present study proposal. However, due to the unknown difference in effect size between chronic stroke and healthy populations in relation to the effect of IPC, the sample size required in the present study proposal could be too small to see any effects on healthy adults.

Perspectives. The expected results of an increased discharge rate and a decreased recruitment threshold for both LIPC and RIPC compared with sham are illustrated in Figure 4 & 5. These results are partially based on the assumption that IPC in a clinical setting is thought to "prime" the nervous system, which will lead to a more optimized activation of the muscle during exercise, and thereby the central motor drive is optimized (Hyngstrom et al. 2018). In addition and as discussed above in *Ceiling effects,* the effects on healthy adults are expected to be less than for a patient population with a damaged nervous system (Hyngstrom et al. 2018). Based on this, the effects from IPC might be more beneficial in a rehabilitation setting compared with performance enhancement. Furthermore, IPC is an attractive method for patients who are not able to be physically active, since it is non-invasive, cheap, easy to use, and has shown to be well tolerated even by stroke patients (Hyngstrom et al. 2018).

In the present study proposal, the effect from IPC will be examined in the tibialis anterior muscle. This particular muscle is of interest in rehabilitation settings, since it plays an important role in

the swing phase of a gait cycle (Perez et al. 2004) and a potential optimization of the muscle activation after an IPC intervention would be beneficial for a wide range of populations including the elderly and patients who experience drop foot.

In conclusion, carrying this study out in practice can contribute with a unique insight in the possible effects on motor unit properties after an IPC intervention. Furthermore, the proposed study can reveal if there is a difference between the application of LIPC and RIPC, and this would open up new avenues for the study of the optimal application of IPC and its influence on motor unit properties in both a performance and clinical perspective.

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APPENDIX

APPENDIX A: DATA PROCESSING

The procedures of decomposition and motor unit tracking performed on a prior dataset are elaborated below in 6 steps. Step 1-5 is based on codes for the decomposition and identifying motor units and are elaborated in part I. The 6th step is based on a code for the motor unit matching and it is elaborated in part II.

PART I - DECOMPOSITION AND IDENTIFYING MOTOR UNITS

The prior dataset used in this analysis consists of two data files and were collected from one subject who performed two subsequent submaximal isometric trials (10% of MVC). To illustrate how the process of decomposition and matching will be performed in the study proposal, the two data files were processed as if they were collected from one subject undergoing the sham intervention. Therefore, file 1 and file 2 will represent pre- and post-data from the sham intervention, respectively.

After data collection, the files were unpacked and processed in Matlab version R2017b, with specific codes developed for this study.

Five different codes were used for the procedure of decomposition; “Channel check”, “Data cut”, “Decomposition”, “Motor unit cleaning”, “Improve cleaning” and each one of them will be elaborated below.

Step 1: Channel check

The raw EMG data were first run through the “Channel check” code (Figure 1). All 64 channels were looked through and if channels were clearly saturated or inconsistent with the others they would be categorized as bad, and manually removed from the data set.

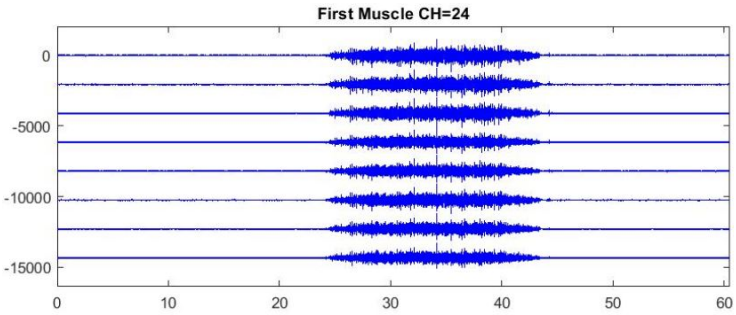

Figure 1: Illustration of the raw EMG data from channels 17-24 of the hd-EMG.

Step 2: Data cut

Subsequently the "Data cut" code was run (Figure 2), which had the purpose of only selecting the data of the voluntary contraction, and thereby removing the first and last part of the data where the subject was in a relaxed state. The full contraction was manually segmented out, for further processing by the analyzer by selecting the point where the contraction begins and ends.

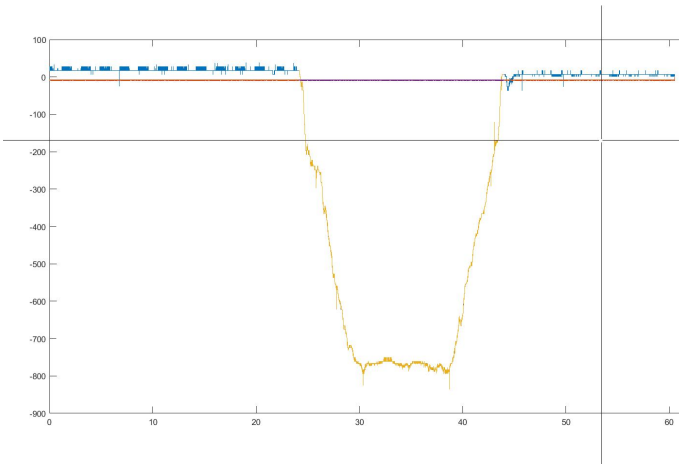

Figure 2: Illustration of the selected data of the muscle activation (marked with yellow).

Step 3: Decomposition

The segmented data were then run with the "Decomposition" code (Figure 3). The decomposition code applies complex algorithms to extract the unique motor unit shapes and separate the motor units and is based on the convolutive blind source separation method (Negro et al. 2016).

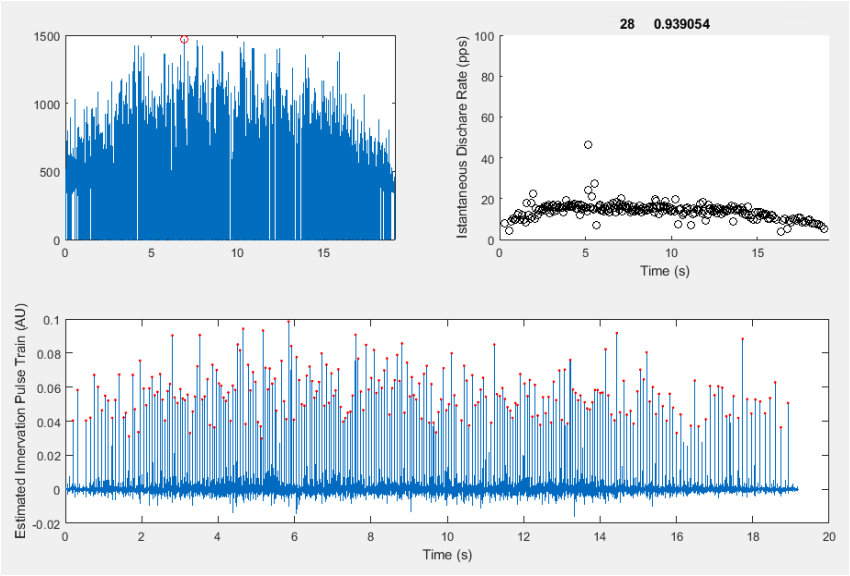

Figure 3: Screenshot of the decomposition while the computer was running code 3 "decomposition".

Step 4: Motor unit cleaning

Because the decomposition is a semi-automatic approach, a further step is required. Therefore the "motor unit cleaning" code (Figure 4) was run to manually check the motor unit decomposition and make corrections to the output from the decomposition. During the motor unit cleaning the analyzer was able to manually optimize the decomposition, add or remove small mistakes, and to remove motor units from the recording due to a lack of consistency.

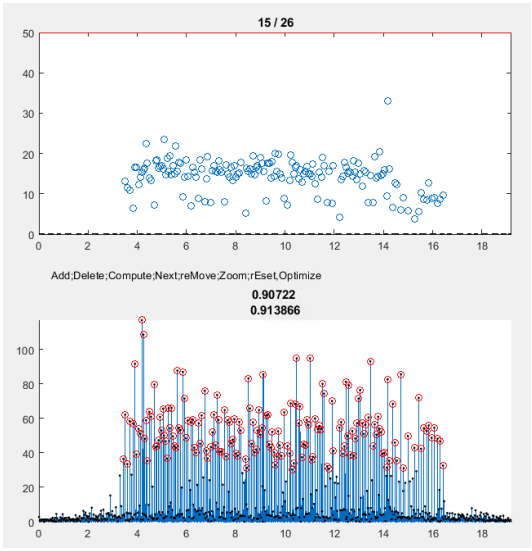

Figure 4: Motor unit cleaning of motor unit number 15 out of 26. The top image is the firing frequency of the motor unit, and the more consistent it is, the better.

Step 5: Improve cleaning

Following the first cleaning, the "Improve cleaning" code (Figure 5) was run to correct small mistakes overlooked in the first cleaning.

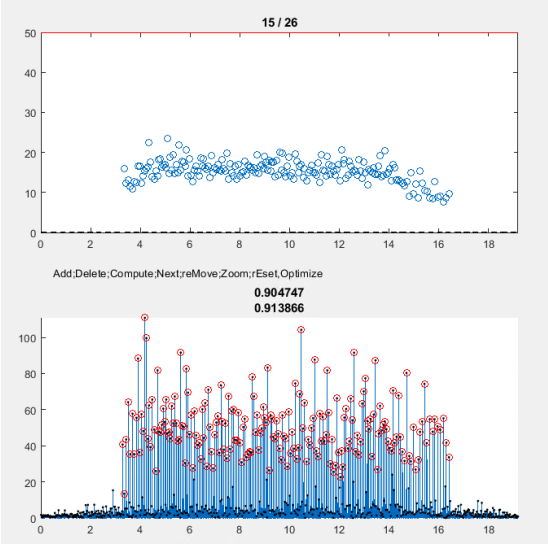

Figure 5: Improvement of the motor unit cleaning of motor unit number 15 out of 26. The top image is the firing frequency of the motor unit, and the more consistent it is, the better.

Plot of spike trains

After the motor unit cleaning, a plot of the motor unit spike trains was made (Figure 6), and the recruitment threshold and mean discharge rate for the individual motor units were extracted from each file for further use.

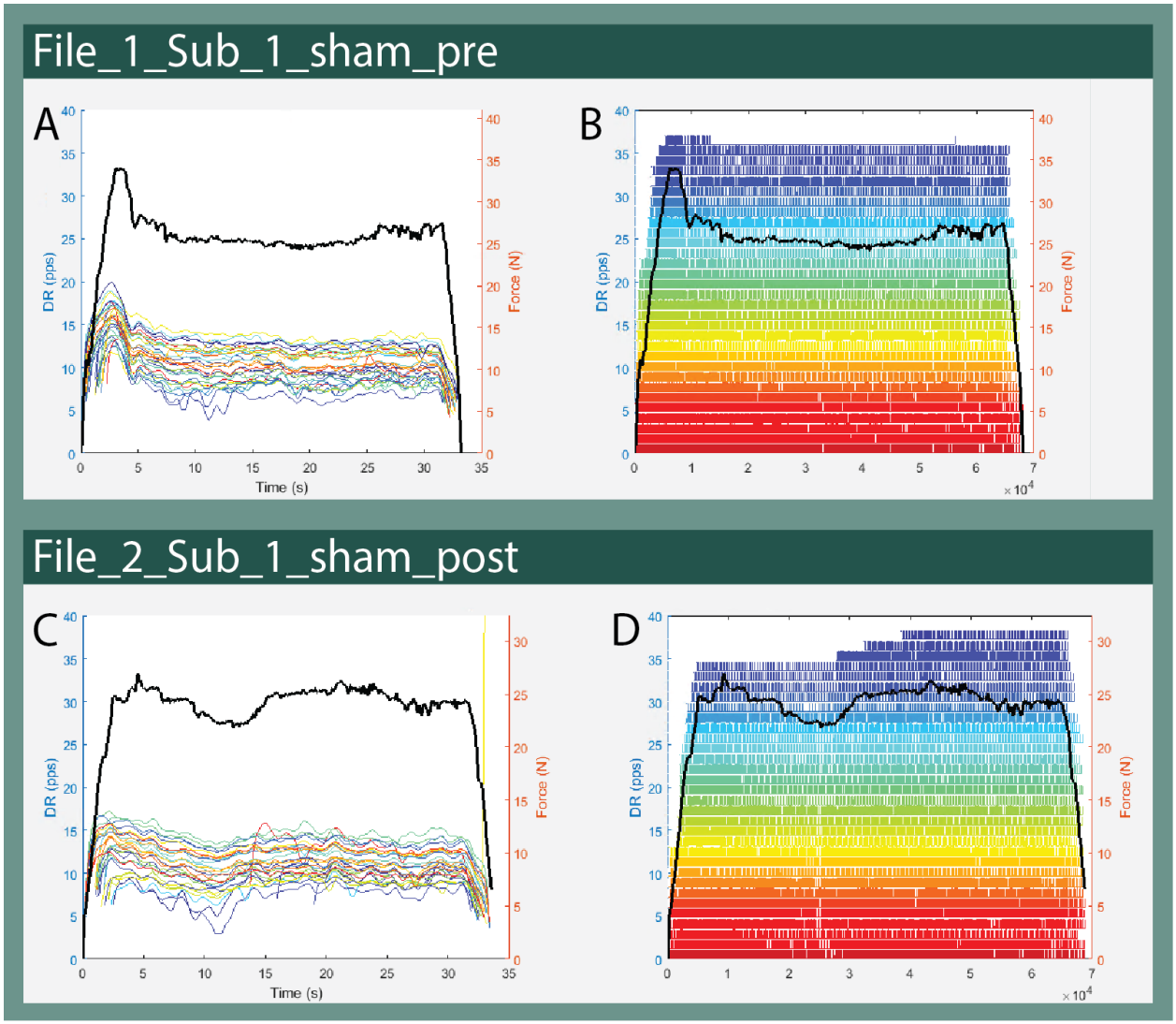

Figure 6: Illustration of the motor unit properties during a submaximal isometric contraction, which is the end result of the process of the decomposition. The force (N) exerted by the ankle dorsiflexors are marked with the black line. A & B is pre and C & D is post the intervention. Each different color represents a different motor unit. A & C is an illustration of the recruitment and derecruitment for the motor units. B & D is an illustration of the pulses per second (pps) for the motor units which indicated the discharge rate. Each pps is indicated with a vertical line for the motor units.

PART II - MOTOR UNIT MATCHING AND DATAOUTPUT

The procedures of motor unit matching are elaborated below with code 6: motor unit matching, and the classification of the data output to further use in the statistical analysis is illustrated as a flowchart in Figure 9.

Step 6: Matching motor units

After the decomposition process was completed, the motor units from pre and post were matched using the "Matching motor units" code (Figure 8). The code compares the motor unit action potential profiles and firing patterns of motor units from the two data files using a two-dimensional cross correlation to estimate the best match. Matching motor units with cross-correlations higher than 0.9 were then selected and shown in the output.

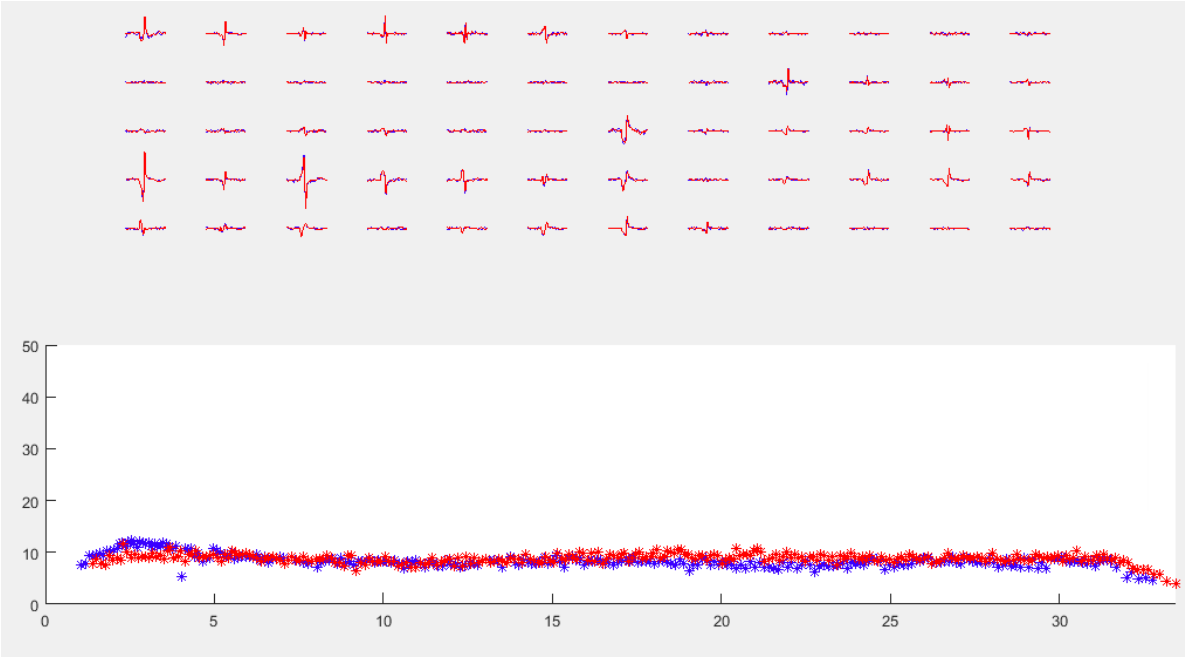

Figure 8: Illustration of the matching of the motor units where the blue is pre and the red is post. In the top are the motor unit action potentials pre and post and in the bottom are the discharge times of the motor units across time.

After the motor units have been matched, the data output (recruitment threshold and mean discharge rate) for all detected motor units was divided into two sub-sets of 1) matched and 2) all identified motor units (Figure 9.A).

The average of the mean discharge rate for the matched and all identified motor units from the pre and post were calculated and extracted to a spreadsheet (Illustrated in Figure 9.B). Subsequently, the average of the discharge rate for each subject undergoing the sham intervention will be pooled together.

In the proposed study, this procedure will be repeated on data from each intervention and subsequently performed in relation to the recruitment threshold.

When the values of recruitment threshold and mean discharge rate from all subjects in each intervention are pooled together, the data output will be ready for statistical analysis.

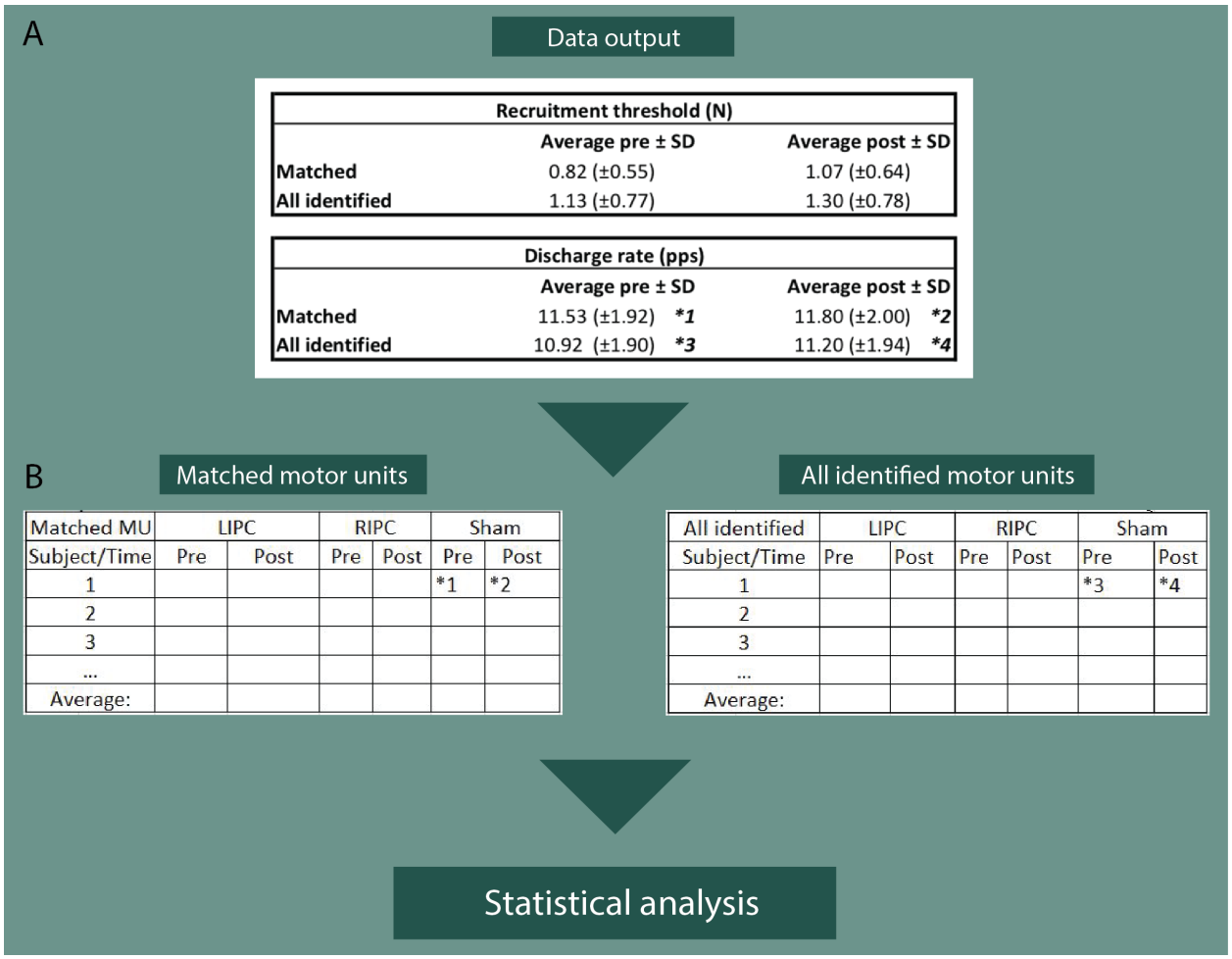

Figure 9: Flowchart of data processing from motor unit matching to data output ready for statistical analysis. A: Data output for both matched and all identified motor units. B: Illustration of the pooling of data for matched motor units and all identified motor units. *1, *2, *3 and *4 illustrates the values of discharge rate from A inserted in B. The same procedure will be performed for the values of recruitment threshold.

APPENDIX B: PARTICIPANT QUESTIONNAIRE

Participant number:_____ Date:_____

Age	
Amount of sleep (last night)	
Do you have current issues with pain that can affect your participation?	☐ Yes ☐ No
Have you had any injuries or undergone surgeries that may affect your performance?	☐ Yes ☐ No
Do you have problems with your balance, attention problems, sleep apnea, disability, or any nervous disorders?	☐ Yes ☐ No
Do you take medication that affects the nervous system or your ability to pay attention?	☐ Yes ☐ No