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How to combine the paired-pulse paradigm stimulation technique with the quadropulse (QuadS) and quintopulse stimulation (QuintS)

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Abstract

Background and Aim

Transcranial magnetic stimulation has advanced our knowledge of cortical physiology. Paired-pulse TMS allows explore intra-cortical facilitation (ICF), but the mechanism remains undetermined. Repetitive spinal motor neuron discharges (repMNDs) could contribute to ICF, which can be explored by the quadropulse (QuadS) and quintopulse stimulation (QuintS) technique (1). The objective was to establish a novel stimulation setup combining the paired-pulse stimulation paradigm with QuadS and QuintS. The ulterior objective is to explore the role of repMNDs in intracortical facilitation.

Methods

In our study we intended to find a method to measure the repMNDs in intracortical facilitation (ICF) established by the paired-pulse TMS paradigm. For this purpose, we combined the paired-pulse paradigm (PP) (2)with the quadropulse and quintopulse stimulation techniques (QuadS and QuintS) to quantify the repMNDs (1). The challenge was to set up the stimulation protocol combining multiple stimulators in a predefined sequence order and to randomize the stimulation conditions. The major challenge arose form the technical specificities of the various devices which had to be defined and explored to allow precise timing of the sequential stimuli.

Results

LabVIEW triggers the various stimulators in a sequential order, according to the experimental conditions, and acquires the data for off-line analysis. The stimulators are the Magstim BiStim^2 and the 200^2 (needed for the paired-pulse paradigm) TMS device for the motor cortex stimulation, the Grass S88 stimulator for the ulnar nerve stimulation at the wrist and the Digitimer DS7AH stimulator for the brachial plexus stimulation at the Erb's point. We use the ENMG-Viking Select IV instrument for the MEP recording. The experimental stimulation conditions are applied in a randomized order.

Conclusions

This novel stimulation protocol combines the paired-pulse stimulation and the TST, QuadS and QuintS to explore whether repMNDs contribute to the intracortical facilitation.

Introduction

A single TMS stimulus of the motor cortex can cause spinal motor neurons to discharge multiple times. To assess these repetitive discharges of the spinal motor neurons (repMNDs), we combine the triple stimulation technique (TST) with one or two additional stimuli of the ulnar nerve at the wrist (QuadS and QuintS) to study the presence of double or triple discharges, respectively. In a previous study, double discharges were recorded with a single-pulse TMS (1). Repetitive discharges of spinal motor neurons could contribute to intracortical facilitation (ICF), which to explore is the ultimate objective of our research. This study intends to develop a new method to assess repMNDs in a paired-pulse TMS paradigm.

Methods

Electromyographical recordings

We use the same Electromyographical (EMG) recording settings as previously described by Bedulli et al. (2013): A Viking Select IV EMG apparatus (Nicolet, Madison, Wisconsin, USA) records and amplifies the EMG signal. Surface electrodes are put in a belly-tendon montage on the right Abductor Digiti Minimi (ADM). Band pass filters are set at 2 Hz – 10 kHz (1). The subjects are seated in a comfortable reclining chair with the forearm on a cushion during the procedures. Cables and electrodes are fixed with sufficient tape to avoid motion artifacts during the measurement. Signal recording and off-line processing is done with LabVIEW software (National Instruments Corporation, LabVIEW 12.0f3, Austin, 2012).

Peripheral nerve stimulation

There are two supra-maximal peripheral stimulations: the first one is the stimulation of the ulnar nerve at the wrist by applying a bipolar electrode connected to a Grass S88 stimulator (Astro-Med Inc., Grass Instrument Division, West Warwick, RI, USA). The second is the stimulation of the brachial plexus at the Erb's point with a monopolar electrode (cathode) (1,3) and a copper-plate electrode (anode) on the back connected to a Digitimer DS7AH stimulator (Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). The stimulators are LabVIEW-triggered.

Transcranial Magnetic Stimulation

Transcranial Magnetic Stimulation (TMS) stimuli are applied with a figure-of-eight coil (7 mm) over the hand motor cortex, using a Magstim BiStim^2 and a 200^2 (the latter needed to obtain facilitation for the experimental conditions that require it) stimulators (The Magstim Company Limited, Spring Gardens, Whitland, UK). The localization of the optimal cortical stimulation spot for the ADM and the determination of the motor threshold are performed in accordance with the guidelines of IFCN (International Federation of Clinical Neurophysiology) (4). In short: the center of the coil is placed over the vertex or slightly lateral toward the left hemisphere. Small displacements of 1cm are made in all directions until the position yielding the lowest threshold is found (hot-spot). The coil is kept in the same position, marked on a cap, throughout the experiment. Resting and active motor threshold (RMT) are defined, as the minimum stimulus intensity that evokes MEPs of at least a 50 and 200 uV peak-to-peak amplitude (1), with the adaptive method using the TMS Motor Threshold Assessment Tool (MTAT 2.0). The TMS equipment is LabVIEW-triggered.

Triple Stimulation Technique

The triple stimulation technique was originally described by Magistris in 1998 (3). This technique consists in a succession of three stimulations that corrects for the phase cancellation phenomenon. The first stimulus is a transcranial magnetic stimulation over the motor cortex area, followed, after a delay to allow the action potential to reach the forearm, by an electrical stimulation of the ulnar nerve at the wrist and after another delay by an electrical stimulation of the brachial plexus at the Erb's point. In normal conditions, the descending and de-synchronized discharges from the TMS and the ascending action potentials from the ulnar nerve stimulation eliminate each other through a collision, and the synchronous discharges of the final third brachial plexus stimulation causes the Compound Muscle Action Potential (CMAP) corresponding to the Motor Evoked

Potential (MEP) in this case. In a pathological condition affecting the cortical motoneurons and/or the cortico-spinal tract, there is a reduced number of descending and de-synchronized discharges from the TMS which collide with the corresponding number of ascending action potentials from the ulnar nerve stimulation. This leaves a number of ascending action potentials from the ulnar nerve stimulation which then collide with the descending discharges from the final third brachial plexus stimulation. This elimination of descending discharges results in a smaller MEP which equals the reduction of cortico-spinal fibers. This collision technique, thus, generates a "blueprint" of the cortico-spinal conduction by eliminating the de-synchronized TMS discharges and "replacing" them by synchronous action potentials from the brachial plexus stimulation. This finally results in a re-synchronization of discharges and a synchronous depolarization allowing a precise quantification of the cortico-spinal conduction.

The Viking Select IV EMG apparatus has a specific TST program that triggers the stimulators with the pre-set delays between the stimulations calculated for each subject. The stimulations at the wrist and Erb's point are given at a supra-maximal intensity. We enter the calculated delays in the LabVIEW program which triggers the various stimulators in the predefined sequence.

Quadropulse stimulation technique

The quadropulse stimulation technique (QuadS) is an extension of the triple stimulation technique (TST) which consists of the same stimulation sequence as the TST with one additional stimulus of the ulnar nerve at the wrist, with a delay of 3 ms from the first wrist stimulation (1). This fourth stimulation is important because it allows explore whether a second discharge of the spinal motor neurons may be elicited after a single pulse TMS of the primary motor cortex (M1) with the TMS. The sequence of events is as follows: the descending and de-synchronized discharges from the TMS and the ascending action potentials from the first ulnar nerve stimulation eliminate each other through a collision. The second stimulation at the wrist occurs with a delay of 3 ms and the ascending action potentials collide with the descending action potentials arising from

repeated discharges of spinal motor neurons and the remaining with those from the (fourth and last) stimulation of the brachial plexus. In case of absence of repeated discharges the ascending action potentials from the second ulnar nerve stimulation collide with the (fourth and last) stimulation of the brachial plexus eliminating each other; in case of repeated discharges of spinal motor neurons the ascending action potentials from the second ulnar nerve stimulation collide with them eliminating each other, thus the descending action potentials from the brachial plexus stimulation are free to travel all the way down to the muscle. At this point there is either no MEP, as in case of absence of repMNDs, or a MEP is recorded which corresponds to a "blueprint" of the repeated discharges of the spinal motor neurons. This MEP corresponds to the CMAP generated by the presumed second discharges of the spinal motor neurons. The stimulation sequence is LabVIEW-triggered. The delays between TMS - wrist 1 and between wrist 2 – Erb's point are the same described in the TST section and calculated by the Viking Select IV EMG apparatus and inserted then in LabVIEW. The delays are: delay 1 (TMS-wrist1), equal to the minimal latency of the MEP, rounded down to the nearest millisecond, minus the latency of the potential evoked at the wrist, rounded up to the nearest millisecond; delay 2 (wrist 2-Erb's point) equal to the latency of the CMAP-Erb rounded down to the nearest millisecond, minus the latency of the CMAP-wrist rounded up to the nearest millisecond (3).

Quintopulse stimulation technique

The quintopulse stimulation technique (QuintS) is an extension of TST which consists of the same stimulation sequence as the TST with two additional stimuli of the ulnar nerve at the wrist, three wrist stimulations in total, with an inter-stimulus interval of 3 ms between them. The stimulation sequence is the same as the one for the QuadS (TMS – wrist 1 – wrist 2 –Erb's point), but with an additional stimulation of the ulnar nerve at the wrist after a delay of 3 ms (1). This fifth stimulation allows the recording of repeated discharges of the spinal motor neurons, within a time frame of 3-6 ms following the first discharge, that may or may not be elicited after the cortical TMS stimulation. The sequence of events is as follows: the descending and de-synchronized discharges from the TMS and the ascending action potentials from the first ulnar nerve stimulation eliminate each other through a collision. The second stimulation at the wrist occurs with a delay of 3 ms and the generated action potentials collide only if there are neurons discharging multiple times within a time frame of 3 ms. The third stimulation at the wrist occurs, again, with a delay of 3 ms and the generated action potentials collide only if there are neurons discharging multiple times, within a time frame of 6 ms, three times in this case. The fifth and last stimulation is always the brachial plexus stimulation applied at the Erb's point and it generates synchronous discharges that, if no multiple (third) discharges are present, collide with ascending discharges from the third wrist stimulation producing no MEP. Only if repeated spinal motor neuron discharges, beyond a time frame of 6 ms after the first discharge, have occurred and thus collided with the ascending action potentials from the third ulnar nerve stimulation, then the descending action potentials from the brachial plexus stimulation are free to travel all the way down to the muscle and a MEP is recorded. This method allows quantifying the repeated spinal motor neurons discharges and this MEP corresponds to the CMAP generated by the presumed third discharges of the spinal motor neurons. The stimulation sequence is LabVIEW-controlled. The delays between TMS - wrist 1 and between wrist 3 – Erb's point are the same described in the TST section and calculated by the Viking Select IV EMG

apparatus and inserted then in LabVIEW. For a more detailed description of the delays see the previous "Quadropulse stimulation technique" section.

Paired-pulse paradigm

In 1993 Kujirai (2) described corticocortical paired-pulse paradigms as inhibiting or facilitating motor evoked potential depending on the inter-stimuli interval (ISI). Paired-pulse paradigms consist in a pair of two transcranial magnetic stimulations (TMS) pulses. The first (conditioning stimulus, CS) is given at 80% of the resting motor threshold (RMT), though considered sub-threshold, has been shown to excite spinal motor neuron (Bedulli et al. (2013)). This first stimulation is followed after an ISI by the second stimulation (test stimulus, TS) that is given at 120% of the RMT. Both the CS and TS are given over the hand representation area of the motor cortex. If the ISI is short (1-5 ms) the evoked MEPs are smaller and, thus, considered inhibited, compared to the MEP of the test stimulus (single TMS stimulus). This is called Short Intracortical Inhibition (SICI). The paired-pulse Paradigm with a longer ISI (10-15 ms), the evoked MEPs are larger and considered facilitated (Intracortical Facilitation – ICF). The paired-pulse paradigm is also LabVIEW-controlled.

LabVIEW hardware and software

We run an application on LabVIEW equipment which was specifically developed for our lab ("EMG Triggering & Acquisition 1.8", Sci-Consulting, St-Sulpice, VD, Switzerland). For every experimental condition (see table below) we program the stimulation sequence paying particular attention to insert the subject-specific delays between TMS-wrist 1 and between the last wrist stimulus-Erb's point, previously obtained via the TST program of the Viking Select IV. The LabVIEW is responsible to trigger the stimulators at the right time and to collect

and record the data measured by the recording electrodes connected to the Viking Select IV.

Table 1 – Experimental conditions

	TMS		Peripheral stimulations			
Conditions	**CS**	**TS**	**Wrist 1**	**Wrist 2**	**Wrist 3**	**Erb**
TMS - SP		✔				
TMS - PP	✔	✔				
TST - SP		✔	✔			✔
TST - PP	✔	✔	✔			✔
QuadS - SP		✔	✔	✔		✔
QuadS - PP	✔	✔	✔	✔		✔
QuintS - SP		✔	✔	✔	✔	✔
QuintS - PP	✔	✔	✔	✔	✔	✔

Table 1 presents the different conditions that we'll test in our experiment and the kind of stimulation needed for each condition. The conditioning stimulus (CS) is set at 80% and the test stimulus (TS) at 120% of the RMT. The inter-stimulus interval (ISI) for intracortical facilitation (ICF) obtained with the paired-pulse paradigm (PP) is set at 10ms. Single-pulse TMS paradigm (SP). All the peripheral stimulations are supra-maximal. The intervals between the TS, the wrist stimulations and the Erb's point stimulation are calculated according to the TST procedure (3). The interval between the wrist stimulations is set at 3ms (1).

Laboratory's setting

Figure 1 is the global view of our laboratory with the various equipment and stimulators. The stimulation sequence for every experimental condition was controlled via the LabVIEW software and hardware.

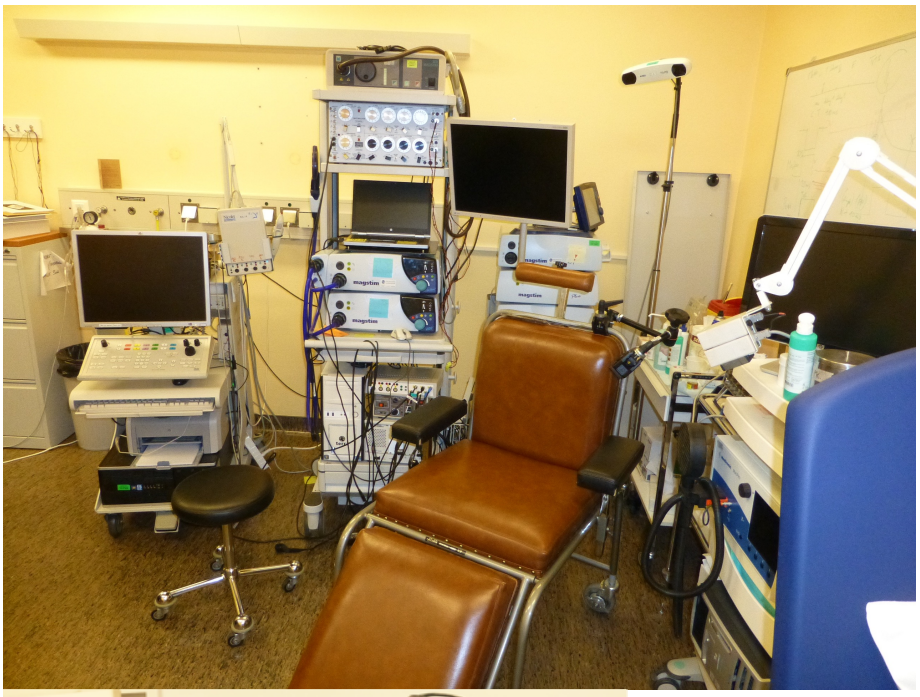

Figure 1

Laboratory overview

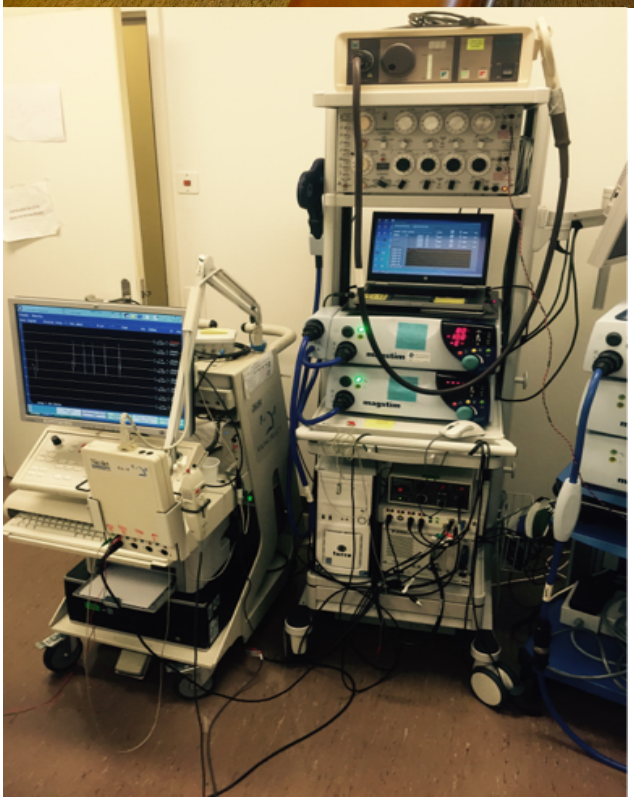

Figure 2

LabVIEW (a) triggers the various stimulators in a sequential order, according to the experimental conditions, randomizes the experimental conditions and acquires the data for off-line analysis. The stimulators are: the Magstim apparatus (b) TMS device for the motor cortex stimulation; the Grass S88 (c) for the ulnar nerve stimulation at the wrist and the Digitimer DS7AH (d) for the brachial plexus stimulation at the Erb's point. We use the ENMG Viking Select IV (e) for the MEP recording.

Discussion

This study intends to set up a new stimulation protocol combining the TST, QuadS and QuintS stimulation techniques with a paired-pulse TMS paradigm to explore the role of repMNDs in intracortical facilitation (ICF). There were a number of technical challenges and their solutions are summarized here:

LabVIEW hardware and software

We choose LabVIEW to trigger the peripheral stimulators, the TMS in both single-pulse and paired-pulse paradigms, to record the experimental data and to randomize the experimental conditions. The Viking Select IV offers a ready-to-use application for TST, but since it is a clinical instrument and not a research one its software precludes any user customization. Following this there is any possibility to trigger other stimulators and because of this, is not possible to combine the paired-pulse paradigm. We opted then for LabVIEW running a customized application. We asked the software house (Sci-Consulting, St-Sulpice, VD, Switzerland) to modify the current version of the application "EMG Triggering & Acquisition 1.8" to offer more stimulation possibilities thus be able to set up the 6 pulse combinations needed for our study. The channel 1 of the LabVIEW's amplifier was damaged by too high-voltage stimulation or by an electrostatic shock discharge (ESD) therefore to avoid any other damage is important to know that: for the ESD the amplifier is guarantee to support a minimum of 2500 V following the regulation chosen by the factory (MIL-STD-883C method 3015.6); for the long lasting input the maximum tension supported between the two entry ports + (red) and – (black) as well as between these two ports and the grounding system (green) is of 12 V; the grounding system is able to support more than 1200 V. Another important point to mention is that anyone, patients or staff, before touching the electrodes must be connected to a ground to avoid ESDs (we thank the Sci-Consulting for this important information). We run simulation trials to test the stimulation set-up, in particular

to test the correct sequence order, on a circuit simulator build by Mr. Gsponer (see figure 3 below).

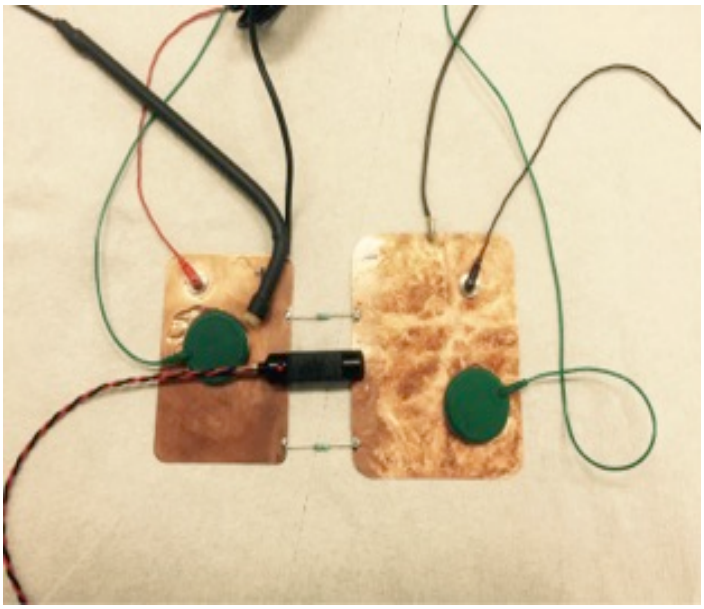

Figure 3

The device is build with two copper plates connected with two resistors of 500 kOhm each. One of the two cables coming from the plates is connected to the – (black) output of the Digitimer's cable. The "wrist" bipolar electrode must be in contact with the two copper plates at the same time. The "Erb's point" monopolar electrode can be placed on any of the plates. The Viking's recording electrodes are placed one on each plate. To reduce the noise two grounds (green) are used.

Viking Select IV

There are two technical challenges raised by Viking. Firstly, the Viking Select IV offers a number of applications for clinical diagnostic purposes, but – for the moment – is limited in offering customized solutions for clinical research. The system, as told in the previous section, doesn't allow end-user customization and therefore we can't perform the expected experimental conditions. Secondly, to trigger the Viking with the LabVIEW at a precise given time is not possible for two reasons: the first reason is that the Viking is not equipped to be triggered with a ms precision and therefore, during another study in our laboratory, was designed an interface box (thanks to Mr. Jaccard) to be able to trigger the Viking with the LabVIEW with a montage to simulate the action of the Viking's pedal, used during clinical exams to begin the stimulation if it is not possible to push the designated button. This interface box, however, "pushes the pedal" with a random delay after the beginning of the programmed experimental sequence on the LabVIEW. The second reason is that the pedal causes the Viking's stimulation to occur not immediately, but few milliseconds after due to the low sampling rate

of input signals. As a result of these two important limitations of the Viking, we decided to use the LabVIEW and other stimulators instead.

Magstim BiStim^2 and 200^2

We had two challenges that we needed to solve: the first challenge was how to pilot the magnetic stimulators to be able to perform the paired-pulse paradigm and the second challenge was how to randomize the experimental conditions.
Since the paired-pulse (PP) paradigm of ICF concerns a focal stimulation of the primary motor cortex (motor hot spot), we can only use a single coil to perform the magnetic stimulation. In order to obtain the paired-pulse stimulation, two stimulators must be connected which is possible with the BiStim^2 and the 200^2. Reading the user's manual and especially testing, we found that the interstimulus interval (ISI) of 10 ms needed for the paired-pulse paradigm must be set up by LabVIEW and not directly on the 200^2 stimulator because otherwise the single-pulse paradigm wouldn't work and the randomization of the experimental conditions would have be impossible to achieve. The LabVIEW set up consists in separating the two Magstim stimulators (BiStim^2 and 200^2) in the "Trigger definition" section and to insert them one after the other with an ISI of 10 ms in the stimulation conditions that need the paired-pulse paradigm. In this way it is possible to pilot the stimulators and to randomize the two magnetic stimulation paradigms with the LabVIEW, thus to solve our two challenges.

Grass stimulators

The Grass S88 stimulator offers a wide array of stimulation possibilities in its two outputs. The technical challenge was to create 3 stimulation conditions; single, double and triple stimulation; with 2 outputs to a single electrode and to be able to randomize these 3 conditions while respecting the sequence order of stimulation. Fortunately we found a solution: we set up the first output to give one stimulus when triggered and the second one to give two stimuli at 3 ms of ISI. We have in any case to maintain the same position of the bipolar electrode for the ulnar nerve stimulation, in order to use a single stimulation electrode, we asked the CHUV biomedical technician Mr. Gsponer to build a customized Y-cable to be able to send the output signals from the two Grass stimulator's outputs to the electrode placed at the wrist. This set-up allows execute all the experimental conditions and at the same time to be able to randomize them.

Digitimer

The following technical challenges of the Digitimer have been solved: firstly, it needs to be reset after switched on otherwise it doesn't work. Secondly, the monopolar electrode's cable color is black instead of being red, as the convention for the positive cathode wants, and thus it doesn't have to be connected to the black output of the Digitimer. We solve the problem simply changing the black output port with the red one. In order to obtain a supra-maximal stimulation of the brachial plexus, higher-voltage stimulation intensity is needed, and the Digitimer provides up to 400 Volts.

Peripheral nerve stimulation

The ulnar nerve is stimulated with round electrodes over the nerve, anode and cathode, both 0.8 cm in diameter, separated by a distance of 2 cm. The cathode is placed 8 cm proximally to the active electrode over the ADM muscle belly, and the anode proximal to the cathode held in place with adhesive tape, but the monopolar electrode (cathode) to stimulate the brachial plexus was not attached to a holding device (the copper-plate electrode (anode) on the back was held in place with adhesive tape as well) and that could be the source of a non regular stimulation; normally, should not be an issue. We took care to guarantee a supramaximal stimulation in all conditions, but varying the pressure of the electrode's tip applied on the skin the consequence was a variable stimulation of the brachial plexus. To avoid this issue and to stimulate the Erb's point every time in the same way, we could for example use adhesive electrodes like the one for the ECG and add a compression on it with a weight. In this way the monopolar electrode would be stable on the skin and the stimulation would be consistent as the wrist stimulation.

Conclusions

The objective of this project was to establish a novel stimulation protocol that combines the paired-pulse stimulation, TST, QuadS and QuintS stimulation techniques to explore whether repMNDs contribute to the intracortical facilitation. We encountered a number of technical challenges and could find solutions to run the protocol. For the TMS we found a method to randomize the single and paired pulse paradigms using the LabVIEW; we found a method to randomize the multiple ulnar nerve stimulations by the mean of the Grass, the customized Y-cable and the LabVIEW; we found a method to stimulates the brachial plexus thanks to the Digitimer and the LabVIEW. The final protocol involves various stimulators and technical devices available in the laboratory and required a number of customized devices (patient's simulator, Y-cable…) and software. The project implied testing and exploring the technical specificities of these devices and a number of issues went beyond the information provided by the manuals and could be solved thanks to the expertise of Mr Jaccard from Neuroswiss, the CHUV biomedical technician Mr Gsponer and Mr Berseth from Sci-Consulting. We're currently running the stimulation protocol and the preliminary data are encouraging. Last, but not least, this work could not have been possible without the collaboration with other member of our laboratory team Dr. Leonardo Caranzano, Eleni Batzianouli and Nathalie Nguissi, and we would like to acknowledge their contribution and we would like to thank them. A very special thanks to my tutor, Dr. David Benninger, for his support and help during the whole development of this thesis, for being always there and available and most especially when I needed his advices during the harder stages. I want to thank all my family for supporting me from beginning to end, going through phases of disappointment and success, during the development of this thesis.

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