Studying cortical involvement in the long latency stretch reflex response using subthreshold TMS

Mark van de Ruit

Supervisors:
Alfred C. Schouten
Carel G.M. Meskers
Jurriaan de Groot

Responsible professor:
Frans C.T. van der Helm

Exam committee:
Alfred C. Schouten
Carel G.M. Meskers
Jurriaan de Groot
Wouter A. Serdijn
Frans C.T. van der Helm

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Preface

Searching for a graduation project started in July 2009 with a visit to Alfred Schouten. He told me about the ongoing projects and at the end of our appointment he mentioned something about a technique to magnetically excite the human brain. That was where my interest for this powerful technique started. I started reading several historical articles on brain stimulation which was summarized in my literature report as follows:

TMS was introduced by Barker et al. (1985), as a result of over 100 years of scientific research on brain stimulation. Already in the late 1800s, researches attempted to stimulate the brain in animals. Fritsch (1870) and Ferrier (1876) stimulated the animal motor cortex and obtained evoked motor responses on contralateral limb muscles. Bartholow in 1874 was the first, reported, who stimulated the exposed cerebral cortex electrically in a subject with cranial fracture. The idea that nerve cells in the brain could also be stimulated magnetically instead of electrically followed about 25 years later when d’Arsonval reported to the Société de Biologie in Paris that when a subject’s head was placed in a strong time varying magnetic field, phosphenes, vertigo, and even syncope were perceived. After this report not much was done with magnetic stimulation. Penfield and Jasper (1954) came up with the famous homunculus, after systematically exploring the human brain with electrical stimulation during surgery. Meanwhile it was also tried to stimulate the human brain electrically through the intact scalp. Gualtierotti and Paterson (1954) applied trains of stimuli similar to those conventionally used in neurosurgery to induce motor responses of a contralateral limb. Problems occurred using this type of stimulation because most of the current went through the scalp instead of through the brain resulting in a very inefficient way of stimulation and tremendous pain for the subject. (Keck et al. (2001), Terao et al. (2002)) Nevertheless in 1980 the first clinically applicable method of transcranial electric stimulation (TES) was introduced by Merton and Morton (1980). They developed a device by which a twitch of contralateral muscles could be induced by stimulating the motor cortex. A few years later, the researchers Cohen and Hallett (1988) were able to reproduce the homunculus of Penfield (1954) making use of TES. The accompanying pain during stimulation limited wide use of TES. Furthermore Barker et al. (1985) came up with transcranial magnetic stimulation (TMS) as an alternative method to stimulate the human brain. The introduction of TMS massively changed the state of neurophysiologic investigation.

Reading about the history of brain stimulation, all its difficulties and trying to assess functioning of the human central nervous system made me so enthusiastic about this topic. A three month internship at the University of Copenhagen in the group of Jens Bo Nielsen confirmed my enthusiasm was valid.

Although the study presented in this thesis didn’t go as smoothly as planned I and people around me learned a lot about TMS and functioning of the central nervous system. I would like to thank my direct supervisors being Alfred Schouten, Carel Meskers and Jurriaan de Groot. Together we had a lot of interesting discussions which really helped me in extensively treating the subject of TMS and stretch reflexes.

Mark van de Ruit,

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Introduction

In this thesis Transcranial Magnetic Stimulation (TMS) is used to study stretch reflexes of the human wrist Flexor Carpi Radialis (FCR) muscle.

In the first part of the thesis a scientific paper is presented in which the involvement of the primary motor cortex in the long latency stretch reflex response is studied by TMS. The second part of this thesis entail several appendices giving a detailed overview of the way the study was performed and problems encountered. These appendices mainly serve as a guide for future students, introducing them into TMS, the used method and related problems.

Appendix A gives a detailed description of the method, including a step by step description of the measurements. In Appendix B the technique of TMS is explained, together with its most important components and considerations in using it. As it was the first time that TMS was used in the Leiden University Medical Center (LUMC) we had to get permission via the medical ethical committee. The report written for the committee is included in Appendix C. Unfortunately during the measurements we encountered a lot of problems. A main problem was a artifact induced in the EMG by TMS. This problem is described in Appendix D. As we included less subjects in the scientific paper as were actually measured a detailed overview of all results obtained is given in Appendix E. Finally Appendix F and G contain a summary of the measurement protocol, description of used data processing files and some Recommendations and Conclusions for future work.
Part I:
Scientific Paper
STUDYING CORTICAL INVOLVEMENT IN THE LONG LATENCY STRETCH REFLEX RESPONSE USING SUBTHRESHOLD TMS

Mark van de Ruit¹, Carel G.M. Meskers², Frans C.T. van der Helm¹, Jurriaan H. de Groot², Alfred C. Schouten¹,³

¹ Laboratory for Neuromuscular Control, Department of Biomechanical Engineering, Delft University of Technology, Mekelweg 2, 2628 CD Delft, The Netherlands

² Lab for Kinematics and Neuromechanics, Department of Rehabilitation Medicine, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands

³ Department of Biomechanical Engineering, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands

Proof and correspondence to:
Mark van de Ruit
Phone: +31 628395960
Email: mlvdrui@hetnet.nl
NOMENCLATURE

Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>AM1</td>
<td>Area under filtered and rectified EMG from 20-50 ms [-]</td>
</tr>
<tr>
<td>AM2</td>
<td>Area under filtered and rectified EMG from 55-100 ms [-]</td>
</tr>
<tr>
<td>Astretch</td>
<td>Amplitude of stretch applied [rad]</td>
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<td>EMGbg</td>
<td>Background EMG from 10-200 ms prior to stretch onset [-]</td>
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<td>EMG_FCR</td>
<td>EMG of the Flexor Carpi Radialis</td>
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<td>T₀</td>
<td>Time of stretch onset [s]</td>
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<td>T_effect</td>
<td>Time of TMS induced changes (T_{TMS}+T_{MEP}) [s]</td>
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<td>T_{MEP}</td>
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<td>T_{TMS}</td>
<td>Time that magnetic pulse is applied [s]</td>
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<td>T_{stretch}</td>
<td>Duration of the stretch applied [s]</td>
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<tr>
<td>V_{stretch}</td>
<td>Velocity of the stretch applied [rad/s]</td>
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Abbreviations

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<th>Abbreviation</th>
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<tr>
<td>CMCT</td>
<td>Central Motor Conduction Time [s]</td>
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<tr>
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<tr>
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<td>Motor Evoked Potential</td>
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<td>Repeated Measurements Analysis Of Variance</td>
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<td>Repetitive Transcranial Magnetic Stimulation</td>
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<tr>
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<td>Silent Period [s]</td>
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ABSTRACT

Assessing mechanisms of peripheral reflex control is important for understanding movement disorders after supraspinal nerve lesions like stroke. In the present study, reflex provocation by ramp and hold rotations (R&H) was combined with Transcranial Magnetic Stimulation (TMS). In four subjects, subthreshold single pulses TMS were applied to the primary motor cortex at carefully timed intervals, while short and long latency EMG responses of the *m. flexor carpi radialis* were elicited by R&H rotations around the wrist joint.

TMS was found to inhibit the long latency response with a maximum inhibition when TMS was calculated to arrive at 45ms after stretch onset in all subjects. Excitation was found at 60 ms in all subjects.

An involvement of the primary motor cortex in peripheral reflex loop operation was demonstrated. This involvement may be either excitatory or inhibitory on the stretch reflex.
INTRODUCTION

Healthy human subjects have the ability to adjust their reflexes according to the environmental conditions and tasks to perform. Central disorders are however often accompanied by changes in reflexes. For example, a lack of reflex adaptation was found in patients suffering from stroke (Meskers et al. 2009).

The involvement of the supra-spinal centers of the central nervous system (CNS) in the ability to adjust the spinal reflexes is not yet fully understood. The CNS consists of the brain and the spinal cord where the motoneurons are located. Motoneurons are responsible for activating a set of muscle fibers, all together forming the muscle, allowing people to activate their muscles and perform complicated movement patterns. Both Transcranial Magnetic Stimulation (TMS) and stretch reflexes, activate a subset of motoneurons of a motoneuron pool. However these subsets are not necessarily the same (Morita et al. 1999) and shown to be dependent on stimulation characteristics (Rossini et al. 1994). Subthreshold TMS does not elicit a direct muscle response but only changes the excitability of a set of motoneurons by depolarizing the membrane. By combining subthreshold Transcranial Magnetic Stimulation (TMS), applied to the primary motor cortex with stretch reflexes it is possible to investigate the involvement of the human cortex in the modulation of stretch reflexes.

After a short muscle stretch, typically two (or even more) electrical activity (EMG) reflex bursts are observed. The M1 response, onset about 25 ms after stretch for the Flexor Carpi Radialis (FCR) muscle, is agreed to be a result from monosynaptic alpha motoneuron activation. The M1 response is known to be velocity dependent and of spinal origin. The origin and the mediating pathways of the M2 response, onset of about 55 ms after stretch onset, is a matter of debate. Pathways involved in a stretch reflex are highlighted in Figure 1. Previous studies found evidence for a transcortical theory as well as the M2 being a repeated spinal stretch reflex M1 response. The transcortical theory has been supported by the notion of dependency of the M2 response to task instruction within a subject group instructed to either let go or resist a stretch perturbation (Crago et al. 1976, Colebatch et al. 1979, Rothwell et al. 1980). Combined with the notion that corticomotoneuronal cells contribute to the M2 response in rhesus monkeys (Cheney and Fetz, 1984), these results used to propose a transcortical pathway as explanation for the M2 response. Varying duration of the applied stretch showed significant M2 changes (Lee and Tatton 1982, Schuurmans et al. 2009). Schuurmans et al (2009) proposed a mechanism in which the initial sensory information synchronizes the alpha motoneurons, and therewith their refractory periods. This is proposed to be an explanation for the stretch duration dependency of the long latency M2 response, and was successfully validated by a model study. Mechanisms involving slower and cutaneous afferents are also suggested for influencing reflex size (Grey et al. 2001, Darton et al. 1985). Nowadays it is generally assumed that the M2 is a compound response (Lourenco et al 2006, Meskers et al 2010).

By TMS (Barker et al. 1985) it is possible to non-invasively stimulate the cortex, with minimal discomfort for the test person. With TMS over the primary motor cortex it is possible to elicit a Motor Evoked Potential (MEP) in a muscle depending on stimulation intensity, relative to the Motor Threshold (MT) and spot to stimulate. During contraction MT is specifically called Active Motor Threshold (AMT) and defined as the intensity eliciting a MEP in 5 out of 10 trials with a minimum of 200 μV peak-to-peak value (Rossini et al. 1994). A MEP is followed by a period of reduced EMG activity, called the silent period (SP). Stimulating just below threshold (subthreshold) is suggested to induce an Excitatory PostSynaptic Potential (EPSP). Reducing the stimulation intensity further is suggested to induce an Inhibitory PostSynaptic Potential (IPSP) (Stuart and Preston 1968, Jankowska et al. 1975). Both an EPSP and IPSP result in a depolarization of the motoneuron membrane,
without exceeding the threshold for generating an action potential. Direct as well as indirect, including synapses, connections exist between the primary motor cortex and alpha-motoneurons (Nielsen et al. 1993). Both are activated by TMS. A MEP is the result of a muscle twitch originating from the most direct pathway between the primary motor cortex and muscle, involving the alpha-motoneuron. Contrary, an EPSP or IPSP result in a de- or hyperpolarization of the alpha motoneuron membrane. The same alpha-motoneuron is involved in the monosynaptic reflex loop. As part of the pathways activated by TMS and a stretch overlap (Figure 1), TMS is considered as a useful technique to investigate involvement of the primary motor cortex in reflex modulation.

Figure 1: Representation of pathways activated by a stretch to the muscle (black) and a TMS (dashed grey) pulse to the primary motor cortex. The monosynaptic pathway known to be source of the M1 stretch reflex is displayed. An afferent transcortical pathway for the reflex is displayed although its purpose is unknown. As well direct as indirect (involving interneurons) pathways to the spinal motoneuron are activated by TMS. The path from the spinal motoneuron up to the muscle fibers is the path both stimuli have in common. Interaction of induced information flow takes place at the spinal motoneuron. The indirect pathways triggered by TMS connect either directly to the motoneuron or to a synapse to the motoneuron (e.g. presynaptic inhibition).
Several authors tried to unravel the different pathways involved in the M2 response by combining reflex and TMS, without closely looking to effects of interference between the reflex and TMS elicited signal. A frequently used method involves comparing the added effect of the two single responses, MEP and reflex, to the combined response. A significant increase in the M2 response was found, when combining MEP and reflex. This effect is not present for M1 indicating existence of different pathways for M1 and M2. (Palmer and Ashby 1992, Petersen et al. 1998, Lewis et al. 2004). Furthermore, task dependent modulation disappears when matching the silent period with the time window that the M2 response occurs (Kimura et al. 2006). This study however stayed inconclusive about the role of the cortex, assigning observed differences to changes in the sensory motor cortex. To our knowledge only one study used subthreshold TMS to investigate stretch reflex organization. Studying this particular study, a suppression of the M3 of the tibialis anterior muscle (equivalent to M2 of the wrist) was found in case TMS was applied 40 ms before M3 onset (van Doornik et al. 2004). As subthreshold TMS does not introduce unpredictable signals interaction at the motoneuron pool it is a potential powerful tool to investigate the role of the primary motor cortex in reflex modulation.

The goal of the present study was to find evidence for a cortical involvement in M2 response of the Flexor Carpi Radialis muscle (FCR) using subthreshold TMS. The relative timing of the TMS and the mechanically induced stretch is our main interest. The results are observed in changed stretch reflex EMG characteristics. Timing subthreshold TMS during ongoing stretch reflexes may shed a light on the cortical contribution to M2 modulation. It is hypothesized that subthreshold TMS timed in and after this coincided refractory state will change motoneuron pool excitability and therefore change the M2 response compared to non TMS trials. Herewith evidence is gained that the primary motor cortex is involved in modulating the M2 response.

**METHODS**

**Experiment**

**Subjects**

Four healthy subjects (mean age 52 ± 7 years in the range 40-70 years, 1 woman) participated in this multi-case study. The subjects had no history of neurological or neuromuscular disorders. Approval for the experiments was given by the Medical Ethics Committee of the Leiden University Medical Center. The subjects gave informed consent prior to the experimental procedures.

**Experimental set-up**

**Manipulator:** The manipulator controlled the angle of the wrist. Hereby the wrist is forced to follow ramp-and-hold (R&H) trajectories (Schou ten et al. 2006). The R&H trajectories are characterized by stretch amplitude ($A_{\text{stretch}}$) and stretch velocity ($V_{\text{stretch}}$). Stretch amplitude is defined as the maximal radius reach in extension direction. Stretch velocity indicates the velocity reached during the ramp phase. Stretch amplitude and stretch velocity together determine stretch duration ($T_{\text{stretch}}$) (1). By applying fast R&H trajectories in extension direction during the task stretch reflexes are evoked in the FCR.

$$T_{\text{stretch}} = \frac{A_{\text{stretch}}}{V_{\text{stretch}}}$$  \[(1)\]
The subject sat on a comfortable, height adjustable, car chair ensuring sufficient back and head support while holding the handle of the manipulator with their right hand (Figure 2). With the wrist in resting position the task was to hold a constant flexion torque of 10% of Maximal Voluntary Torque (MVT) for at least 1 second. This task is equivalent to a ‘let go’ task, in the sense that task performance is optimal when the subject gives way to the perturbations. A percentage of MVT is chosen to ensure a constant background EMG, and relative cortical drive between subjects, during all trials. The lower arm was restrained in an arm support such that the axis of rotation of the wrist and the manipulator coincided. A force transducer in the handle measured the interaction torque. A percentage of the MVT was chosen to ensure a relative equal amount of cortical drive between all subjects. To provide feedback to the subject, the torque was low pass filtered at 1 Hz and displayed on a computer screen in front of the subject as a moving bar in an intuitive horizontal plane (Figure 2).

Transcranial Magnetic Stimulation (TMS): TMS was applied by a Magstim Rapid connected to a figure-of-eight shaped coil with an individual wing diameter of 70 mm. (The Magstim Company, Whitland, Dyfed, UK). Supplied software, ANT ASA 4.7.3 (ANT, Enschede, The Netherlands), together with an optical measurement system (Polaris Spectra, NDI) was used for motion capture of passive markers. Using this system it is possible to stay within 0.5 mm of the motor hot spot (95% Confidence Interval is 0.5 mm, www.ndigital.com)

Figure 2: Representation of the experimental set-up. The task is represented on the monitor in front of the subject who is seated in a comfortable chair with head rest. TMS is applied to the primary motor cortex while...
position perturbations are applied to the handle the subject holds. EMG electrodes are positioned to measure the EMG of the Flexor Capri Radialis (FCR) and Extensor Carpi Radialis (ECR).

Data recording and processing

The angle of the manipulator, the torque at the handle, the EMG of the Flexor Carpi Radialis (EMG FCR), and Extensor Carpi Radialis (EMG ECR) were recorded and sampled at 5 kHz. The EMG was recorded with differential surface electrodes (Delsys Bagnoli System, Delsys Inc., Boston, MA, USA, electrode bar length 10 mm, bar distance 10 mm). Prior to sampling the EMG signals were band pass filtered (20 – 450 Hz). The recorded signals (EMG, angle and torque) were segmented into data blocks, starting 200 ms prior to and ending 300 ms after the onset of each stretch perturbation. The EMG segments were rectified and low pass filtered at 80 Hz (recursive third order Butterworth). Each segment was separately normalized by the mean background EMG from 200 ms to 10 ms prior to onset of the perturbation (2,3).

\[\overline{EMG_{bg}} = \frac{\sum_{k=1}^{n} EMG_{bg,k}}{n} \quad n = \text{number of samples in } t = -200 \text{ to } -10 \text{ ms} \quad (2)\]

\[|EMG_{FCR}| = \frac{EMG_{FCR}}{EMG_{bg}} \quad (3)\]

The normalized segments were averaged over ten repetitions. After normalization, EMG values smaller than one indicate depression with respect to the background EMG and value greater than one indicate excitation.

Two metrics were defined, derived from the rectified and normalized EMG of the FCR (Schuurmans et al. 2009). These metrics were used to quantify the M1 and M2 responses of the reflex. The (dimensionless) magnitude of the M1 response \(A_{M1}\) was defined as the mean amplitude of the normalized EMG in the time window between 20 and 50 ms after stretch onset (4):

\[A_{M1} = \frac{\sum_{k=1}^{n} |EMG_{FCR,k}|}{n} \quad n = \text{number of samples in } t = 20 \text{ to } 50 \text{ ms} \quad (4)\]

Correspondingly the dimensionless magnitude of the M2 response \(A_{M2}\) was determined as the mean value of the normalized EMG between 55 and 100 ms after stretch onset (5).

\[A_{M2} = \frac{\sum_{k=1}^{n} |EMG_{FCR,k}|}{n} \quad n = \text{number of samples } t = 55 \text{ to } 100 \text{ ms} \quad (5)\]

Additionally, one metric was defined representing the time delay until onset of a MEP (T MEP). This time delay was determined by visual inspection of the mean of ten unfiltered EMG MEPs responses.

Protocol

MVT: At the beginning of each experiment, subjects were asked to perform a maximum wrist flexion movement in order to determine their maximum voluntary flexion torque (MVT). Subjects were verbally encouraged to produce maximum torque. Allowing a 30 seconds rest in between the MVT measurement was repeated three times.

Stretch duration effect: The effect of stretch duration (T stretch) on the M2 area (A M2) was constructed for each subject. A series of ten R&H stretch perturbations, all having different stretch durations (T stretch = 12, 15, 20, 30, 40, 50, 60, 70, 80, and 90 ms), with the same ramp velocity (V stretch = 1.5 rad/s) i.e. a different stretch amplitude (A stretch), were applied. Ten
different stretches were applied ten times in semi-random order, in extension direction, effectively stretching the activated FCR. The interval between the perturbations was therefore dependent on the subject although they were encouraged to proceed in one pace. After each 20 perturbations a 30 seconds rest was included. Stretch onset time was defined as \( T_0 \).

**TMS initialization:** In order to ensure proper stimulation of the primary motor cortex, an initialization procedure was performed. Optimal stimulation location was determined from visual inspection of the EMG responses. The site at which suprathreshold stimuli evoked the largest MEP response in a precontracted FCR muscle (10% MVT of wrist flexion) was marked as the ‘motor hot spot’. The active motor threshold (AMT) was determined by reducing stimulation intensity with small steps up to the criterion defined by Rossini et al (1994) was met. TMS was applied to stimulate the primary motor cortex looking for optimal MEPs in the FCR and stimulation intensity to meet MT requirements as defined by Rossini et al. (1994). Time of applying the TMS pulse was defined as \( T_{TMS} \).

**TMS and stretch reflexes:** In combining TMS and stretch reflexes, timing was of main interest. The TMS stimulation intensity was reduced by 2-3% relative to AMT, hence subthreshold stimulation. Stretch reflexes were elicited using a fixed \( T_{\text{stretch}} \). \( T_{\text{stretch}} \) was selected based on the possibility to decrease as well as increase \( A_{M2} \) for each individual subject (Figure 5). For example for Subject 3 \( T_{\text{stretch}} = 30 \) ms was selected. A possible MEP induced by TMS was timed to arrive at the muscle in between the M1 and M2 response of the stretch reflex. As stimulation was subthreshold, no MEP was present and the TMS was assumed to affect the monosynaptic reflex pathway without activating it. Timing of the MEP (\( T_{\text{effect}} \)) was done 35 – 80 ms after stretch onset, for each subject (interval 5 ms). Each timing interval was applied ten times in random order. Neutral trials, without TMS, were randomly applied to check for possible long lasting effects of TMS. Using fixed TMS and reflex characteristics \( T_{\text{effect}} \) is the parameter influencing the observed change of \( A_{M2} \). Actual effect of TMS on the monosynaptic pathway was found earlier as indicated in the example shown in Figure 3. Taking \( T_{\text{effect}} = 45 \) ms, with an average MEP latency \( T_{\text{MEP}} \) of 17 ms (Wasserman et al. 2008), \( T_{\text{TMS}} = T_{\text{effect}} - T_{\text{MEP}} = 28 \) ms after stretch onset (\( T_0 \)). The \( T_{\text{MEP}} \) consists of a central motor conduction time (CMCT), from cortex to motoneuron, and a peripheral motor conduction time (PMCT), from motoneuron to muscle fiber.

\[
T_{\text{MEP}} = CMCT + PMCT
\]

CMCT is identified being about 6-7 ms for upper and lower arm muscles (Wasserman et al. 2008). So the induced EPSP by TMS to the cortex arrives at the motoneuron 6 ms after TMS onset, being 34 ms after stretch onset in this case. Activating also indirect pathways by TMS its effect is suggested to last for about 5 ms (Jankowska et al 1975). The TMS pulse has an effect lasting from TMS onset up to about 39 ms after stretch onset, on the CNS.
Figure 3: Timing sequence of perturbation to evoke a reflex, TMS trigger and the corresponding EMG response. At 0 seconds the perturbation for the reflex is applied (stretch duration = 40 ms). In this example TMS is aimed to arrive at the muscle at 45 ms. With a MEP onset delay of 17 ms TMS is applied at 28 ms (45-17 = 28 ms). The actual TMS effect on the nervous system will be from 28 ms up to 40 ms, hence subthreshold TMS so no MEP, including the conduction delay from cortex to spinal cord and synapse response dynamics. This period is indicated by the dashed window.

Statistical analysis
The effect of stretch duration ($T_{\text{stretch}}$) on the M2 area ($A_{M2}$) was statistically tested per subject using a repeated measurement ANOVA with $T_{\text{stretch}}$ as within subjects factor. The effect of TMS timing ($T_{\text{effect}}$) on $A_{M1}$ and $A_{M2}$ was tested per subject using a linear mixed model with trial number and $T_{\text{stretch}}$ including data without TMS as repeated factors. A compound symmetry covariance model was used. In case of significance, a Bonferroni post-hoc test was applied. SPSS 17.0 was used with an alpha of 0.05.
RESULTS

MVT
Maximum Voluntary Torque for each subject is presented in Table 1.

Effect of Stretch Duration
Figure 4 shows a typical example of the averaged EMG response to one stretch perturbation of a single subject. Perturbation characteristics are $A_{\text{stretch}}=0.12$ rad and $V_{\text{stretch}} = 1.5$ rad/s, making $T_{\text{stretch}} = 80$ ms. The M1 and M2 responses (indicated in gray) are clearly visible for this stretch duration of 80 ms. Figure 4c shows $|\text{EMG}_{\text{FCR}}|$ of ten reflex responses with a clearly visible M1, latency of about 25 ms, and M2, latency about 55 ms after stretch onset, response. The grey areas indicate $A_{M1}$ and $A_{M2}$. The M2 response is followed by relatively low EMG period, a third burst from approximately 150 ms.

Figure 5 shows $A_{M2}$ as a function of the $T_{\text{stretch}}$ for each subject. Stretch duration had a significant effect on $A_{M2}$ for three of the four subjects (Table 1). $A_{M2}$ values higher than one indicate excitation compared to background EMG. Perturbations shorter than 20 ms did not evoke a M2 response. The M2 response leveled off for durations longer than 60 ms.

![Figure 4:](image)

**Figure 4:** Characteristics of the perturbations applied, and a normalized EMG response averaged of ten reflex responses for one subject and single stretch duration. (a) The position, representing a ramp-and-hold perturbation, of the handle in time is shown. (b) The velocity of the handle; showing the constant velocity in the ramp phase, a zero velocity in the hold phase, and a deceleration when the handle goes back to its neutral position. (c) The normalized EMG of ten reflex responses with a clearly visible M1 and M2. The grey areas indicate the area used as the M1 and M2 area.
Table 1: Summary of the results obtained for each single subject in the initialization of the experiment. MVT for each subject is in Nm. The onset of the motor evoked potential (MEP) and M1 reflex response is given in ms, while motor threshold (MT) is in %. T\_stretch is the time used to evoke reflexes in the experiment combining reflexes and TMS. In the final two columns the statistical values are displayed for the effect of T\_stretch on A\_M2.

<table>
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<tr>
<th>Subject</th>
<th>MVT [Nm]</th>
<th>Age [years]</th>
<th>MEP onset [ms]</th>
<th>MT [%]</th>
<th>T\text{_fixed} [ms]</th>
<th>T\text{_stretch effect}</th>
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Figure 5: Effect of stretch duration (T\text{\_stretch}) on the normalized M2 area (A\_M2) for all subjects. A clear increase of M2 area is visible from 20 to about 50 ms for all subjects. All values are displayed as mean ± standard deviation.
**TMS only**

In Figure 6 the average of ten MEPs by TMS is shown for one subject. Average MEP onset over all subjects was $T_{\text{MEP}} = 0.017 \pm 0.002$ ms. Active Motor Threshold (AMT) was determined as being $46\% \pm 10\%$ for all the subjects.

Table 1 summarizes all the subject data obtained during initialization of the TMS combined with reflex experiment; delay of MEP ($T_{\text{MEP}}$), MT and chosen stretch duration for combining TMS with.

**Figure 6:** (a) Shows the normalized EMG response to a suprathreshold TMS pulse (average of ten). A clear MEP is visible from about 20 ms (indicated by the dashed vertical line). (b) The corresponding torque response on the handle of the perturbator, indicating activation of the right muscle, evoking flexion.

The effect of timing ($T_{\text{effect}}$) subthreshold TMS pulses during stretch reflexes in $A_{M1}$ showed a significant effect for one of the four subjects (Table 2). Post hoc analysis indicated significant change of $A_{M1}$ compared to the trials without TMS at the time points of 35 and 40 ms.

Analyzing the effect of timing ($T_{\text{effect}}$) subthreshold TMS pulses during stretch reflexes on $A_{M2}$ showed a significant effect for two of the four subjects (Table 2). The effect of TMS on the M2 response area for all subjects is displayed in Figure 6.

Largest inhibition is found in three of the four subject when $T_{\text{effect}} = 45$ ms. Significant excitation was found in three of the four subjects, being largest at $T_{\text{effect}} = 60$ ms in two of the four subjects.
Table 2: Overview of the results of the linear mixed model analysis of the T_{effect} on AM1 and AM2. The F and P value is given for each subject. (Significant values highlighted)

<table>
<thead>
<tr>
<th>Subject</th>
<th>AM1 effect</th>
<th>AM2 effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>1</td>
<td>0.597</td>
<td>0.813</td>
</tr>
<tr>
<td>2</td>
<td>0.493</td>
<td>0.890</td>
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<tr>
<td>3</td>
<td>14.566</td>
<td>0.000</td>
</tr>
<tr>
<td>4</td>
<td>1.875</td>
<td>0.059</td>
</tr>
</tbody>
</table>

Figure 6: The effect of timing of subthreshold TMS (T_{effect}) on AM1 for all four subjects, represented as a percentage relative to AM1 in reflexes without TMS (indicated in grey). No effect of TMS to the M1 area is observed in three of the four subjects. Only subject three showed a clear effect.

Figure 7: The effect of timing of subthreshold TMS (T_{effect}) on AM1 for all four subjects, represented as a percentage relative AM1 in reflexes without TMS (indicated in grey). A corresponding TMS effect, being inhibition, between all the subjects is visible in the time window of 35-55 ms. In the time window from 55-80 ms differences are observed between subject 1, 2 and subject 3,4.
DISCUSSION

The goal of this multi case study was to find evidence for cortical involvement of the long latency stretch reflex response, i.e. M2, of the Flexor Carpi Radialis (FCR) muscle using subthreshold TMS. It is concluded that the primary motor cortex is involved in the modulation of the long latency stretch reflex response, M2, of the FCR muscle. This involvement may be either excitatory or inhibitory.

TMS timed on the muscle at 45 ms after stretch onset induced largest inhibition while largest excitation was found at 60 ms. To our knowledge this is the first study combining stretch reflexes of the wrist together with subthreshold TMS to be able to identify involvement of the human primary motor cortex in the modulation of the long latency response.

**Multi-case study: Comparability of each subject**

Two subjects (1 and 2) show a comparable W-shape over T effect on $A_{M2}$ (Figure 7). The two other subjects show a different inhibition and excitation pattern over the effect of timing TMS pulses on $A_{M2}$. This mainly holds for the second ‘V’, from 55 to 80 ms. Variance in the measurements can be explained in three different ways. 1. It is shown that different motor units are recruited by TMS as by evoking an H-reflex (Morita et al. 1999) and therefore likely also stretch reflexes. 2. The amount of overlap between these sets of recruited motoneurons determines the effect of TMS. There is evidence that recruitment of motor units by TMS is the same as in voluntary movement (Bawa and Lemon 1993). 3. Measured EMG is dependent on electrode positioning, i.e. what set of motor units is recorded.

The observed variance on the reflex response is mainly explained by variability in performing the task and therewith changed cortical drive. This can be concluded from the fact that also at the reflexes where no TMS was present about the same variance was found (Figure 6 and 7). This makes it favorable to judge every subject on his own and look for changes within the subject. Averaging of a number of subjects will cancel out effects seen in individual subjects and introduce additional variability. The matching variances between no TMS and TMS applied reflexes also make the observation of a significant 20% increase or decrease of $A_{M2}$ stronger.

**Consideration of results**

**Stretch Duration**

A strong effect of stretch duration $T_{\text{stretch}}$ on the M2 area ($A_{M2}$) was found for all subjects. A minimum M2 duration threshold of 20 ms was found, which corresponds well to previously found values (Schuurmans et al. 2009, Meskers et al 2010). $A_{M2}$ was found to level off at a stretch duration of 60 ms, which also corresponds to previous data (Schuurmans et al. 2009).

**TMS effect**

Using suprathreshold TMS pulses clear MEPs were obtained in all the subjects. MEP onset times ($T_{\text{MEP}}$), i.e. 15-21 ms, correspond to values found in literature (Wasserman et al. 2008). Variability in MEP onset times can be explained from subject differences like height and age, but also site of stimulation as shown by Fuhr et al. (1991). The large differences in Active Motor Threshold (AMT) between subjects has been reported repeatedly and may be explained from differences like mental activity during measurements and amount of cortical representation for the FCR muscle (Rossini et al. 1994).

**TMS effect on $A_{M1}$**

The non responsiveness of the M1 response to supra- and and subthreshold TMS shown by other authors (Ashby and Palmer 1992, Petersen et al. 1998, van Doornik et al. 2004), was
confirmed in this study. No significant effect was observed in three of the four subjects. The significant effect (F=14.56, P=0.000) observed in subject 3 is explained from the fact that the subject had no clear M1 response. The low signal to noise ratio makes the results unreliable.

**TMS effect on AM2**

TMS effects on the M2 area will take into account direct as well as indirect pathways activated by TMS (Figure 1). Three mechanisms are proposed to be responsible for the observed M2 area changes:

1. The first mechanism supports the idea of motoneuron pool synchronization triggered by an Ia afferent volley and an subsequent refractory period (Schuurmans et al. 2009). However this theory only explains the first inhibitory ‘V’ (Figure 6). The observed inhibition may be the result of the underlying matched refractory period of a substantial part of the motoneuron pool after a synchronizing M1 response. During the refractory period, neurons are less (or even not) receptive for input.

2. The second possible mechanism is involvement of slower pathways, like the II afferents, or indirect pathways from the cortex involving. These indirect pathways involve several synapses being more sensitive for induced excitability changes by TMS. While it takes longer for the afferent information to arrive in the spinal cord the effects are also visible for a longer time after the TMS pulse in the EMG. Also excitatory and inhibitory synaptic connections within the motoneuron pool may result in a delayed TMS effect observed.

3. Studies supporting this mechanism involve ischaemia of the upper limb, restricting blood flow and as such nerve conductivity. The M1 showed a more rapid and stronger decline than the M2 during ischaemia (Cody et al. 1987). This is explained from a difference in main afferents responsible for both the M1 and M2 response although also property changes of the local tissue may induce a different reflex response. Contrary, Darton (1985) showed that the same conduction velocity exists in the generation of the M1 and M2 component of the first dorsal interosseous, concluding that central processing in the spinal cord is responsible for the M2 delay. However aforementioned author did not propose the possibility of M2 being a delayed M1 response.

4. The third and final mechanism likely to explain observed effects is the involvement of a full transcortical loop. According to this concept, sensory information travels up to the cortex where it is processed. M2 is adapted dependent on excitability of the primary motor cortex. It can be assumed that it takes 20 ms after stretch onset for the sensory information to reach the cortex. Depending on stretch duration, i.e. 40 ms, this information crosses the primary motor cortex between 20 and 60 ms and later. Considering a processing delay of about 10 ms, the earliest point that changes in the EMG may visible is about 45 ms after stretch onset. This delay is comparable to delay of 55 ms found in this study.

The effect of subthreshold TMS on the interval 55-80 ms, is variable between the subjects. Therefore the effects of TMS at these intervals stay inconclusive.

**Integration of signals in the motoneuron pool**

The observed effects of TMS on AM1 and AM2 depend on change induced at the level of the spinal motoneuron by TMS (Figure 1). By taking a fixed stretch duration the same type of sensory input to the motoneuron is assumed every trial with and without TMS. Two mechanisms are suggested to be responsible for observed differences due to TMS. The EPSP induced from the neurons in the primary motor cortex, by subthreshold TMS, can have two different effects. On the one hand it may change the synaptic strength of the monosynaptic pathway while on the other hand it may bring the spinal motoneuron closer to its firing threshold. Increasing the synaptic strength results in enhanced afferent feedback i.e. position
and velocity information from the Ia afferent has a larger effect on the motoneuron pool. This study does not allow for a further distinction between these two mechanisms.

**Methodological considerations**

*Transcranial Magnetic Stimulation*

The effect of Transcranial Magnetic Stimulation, and specifically the orientation of the coil, on the primary motor cortex is not exactly known. Difficulties in repositioning the coil introduces variability in the results; where misalignment of the angle is likely more important than the distance. Optimal positioning of the coil was marked with the neuronavigation system, making it possible to reproduce the optimal spot within ± 2.5 mm and 3°. The effects of misalignment are likely to be minor compared to the natural variability in MEP size.

MEP onset was determined from visual inspection of an average of ten clear MEPs. Likely this introduces some variability in estimated MEP onset and therefore the effect observed when combining TMS with stretch reflexes. The variance in estimated MEP onset was proven not to be more than 1 ms between three experienced users of TMS (own observations). Determining AMT was done following guidelines set by Rossini et al. (1994). During determining AMT stimuli were applied each five seconds, being shown to prevent any facilitatory or inhibitory influence on the subsequent stimulation (Rothwell et al. 1999).

*The advantages of subthreshold TMS*

The strength of using subthreshold TMS is that it does not directly activate the motoneuron at the spinal level and only changes its excitability. Suprathreshold TMS has been used to investigate the existence of transcortical pathways in the long latency M2 response; the combined response of TMS and stretch is larger than the linear summation of the individual effects, which is regarded as proof for the transcortical pathways (Palmer and Ashby 1992, Petersen et al. 1998, Lewis et al. 2004). However doubts rise about the location of observed facilitation. Literature suggests that the effect originates from increased cortical excitability due to TMS, while non-linear summation at the spinal motoneuron is not considered.

*Stretch reflexes versus H-reflexes*

Stretch reflexes instead of H-reflexes were used because of their higher relevance resembling normal system functioning. The different nature of the reflex, mechanically instead of electrically, makes the afferent pathways activated differently, changing synapse responses. A significant higher sensitivity to presynaptic inhibition was shown for the H-reflex as compared to the stretch reflex (Morita et al. 1998). This was proposed to be a consequence of the different Ia afferent input, being a single high frequent burst for evoking a H-reflex compared to a tonic short lasting input in case of a stretch reflex.
**Conclusion**

This study demonstrates cortical involvement in peripheral reflex generation by application of subthreshold TMS. Furthermore it is shown that timing of the TMS pulse relative to stretch onset is very important for the effect observed, which can be either excitatory or inhibitory. The largest inhibition was found when TMS was calculated to arrive at 45 ms on the muscle in all subjects. Excitation of the M2 response was found at 60 ms. Three mechanisms are proposed as being responsible for the observed effects: 1: Motoneuron pool synchronization (Schuurmans et al. 2009), 2: Involvement of slower afferents, 3: Involvement of a complete transcortical loop. Further research has to be done to distinguish between proposed mechanisms.

**Future work**

This study showed that cortical modulation of the stretch reflex of the human wrist flexor muscle is possible by subthreshold TMS. However, no exact mechanism could be attributed to observed inhibition and excitation of the M2 response. Three mechanisms were proposed all being likely to have a role in reflex modulation. Because of the complexity and unknown effects of magnetic stimulation a lot of uncertainties about the origin of M2 modulation remain. Due to equipment failure only four reliable data sets could be collected. By repeating the experiment using a different fixed stretch duration, at which minimum and maximum M2 area is observed, combined with the intervals of maximum inhibition and facilitation with TMS enhanced inhibition and facilitation could be observed. As we reported, recruitment of different motor units by TMS and stretch reflexes is an important factor in observed differences between subjects. Therefore an interesting experiment would be to use high density EMG to quantify differences in activate motor units by suprathreshold TMS and stretch reflexes. Additionally the effects of overlapping motor units by combining TMS and stretch reflexes could be quantified. Another experimental paradigm is repetitive TMS (rTMS), which influences cortical excitability for a longer period of time. As such TMS does not directly interfere with the stretch reflex measurements, and stretch reflexes can be investigated before and after the application of rTMS.

**Acknowledgments**

The authors would like to thank the Department of Neurology in name of Prof. Dr. van Dijk and Prof. Dr. van Hilten for making available the TMS equipment used in this study. Further the openness to learn in the group of Jens Bo Nielsen at the University of Copenhagen and extra support during this study by especially SS Geertsen and MJ Grey was really helpful.
REFERENCES


Part II:
Appendices
Appendix A: Methodology explained

In this Appendix a detailed description is given how we came to the study goal and about the development of the measurement protocol. Finally a step by step description is given of how the measurements were performed.

A.1 Study goal explained

All the measurements were performed at the Kinetics and Neuromechanics laboratory at the department of rehabilitation of the Leiden University Medical Center. As no experience existed in using Transcranial Magnetic Stimulation (TMS) first a three months internship was done at a well respected institute regarding the use of TMS. The neuromuscular control group of Jens Bo Nielsen in Copenhagen has extended experience in using all the types of TMS to study functioning of human control system. They approach the problems from a more physiological point of view having more knowledge in this field then the field of signal processing and control theory. Nevertheless it helped the current authors to develop their own ideas on the use of TMS in studying stretch reflex functioning.

As the internship, together with the literature study preceding, really made clear what the dos and don’ts of TMS are (Appendix B), some clear ideas existed of the way to go. Based on the recent publication of Zuur et al. (2009) studying the stretch reflex modulation of the tibialis anterior muscle by rTMS, the initial idea we started with was studying transcortical pathways in reflex modulation of the Flexor Carpi Radialis (FCR) wrist muscle. Two different methods of using TMS to study transcortical pathways are available:

- Repetitive TMS (rTMS): either exciting or inhibiting, hence long lasting!
- Single pulse TMS: sub (below) – or suprathreshold, exciting or inhibiting

(for details about TMS see Appendix B)

Due to the complicated nature of rTMS, involving a lot of difficult stimulation parameters and higher safety risk, we decided in an early stage not to use this technique. Two possibilities remained: sub – or suprathreshold TMS. The benefits of suprathreshold TMS is that a direct muscle response is visible, called a Motor Evoked Potential (MEP), making it easier to use it in an experimental procedure. On the other hand you introduce a new signal in the central nervous system. As it also crosses the motoneuron pool, it is difficult to predict its behavior with respect to for instance the reflex afferent signal. Subthreshold TMS does also evoke a signal in the central nervous system but this signal, either and EPSP or IPSP (Stuart and Preston 1968, Jankowska et al. 1975), will terminate at the level of the motoneuron pool were it changes its responsiveness. Therefore it is difficult to exactly identify the type of signal you are introducing. Nonetheless we decided to use subthreshold TMS because of it’s better predictable behavior. Suprathreshold TMS is often used to study transcortical involvement but we had from the beginning some doubts about the method (As discussed in the scientific paper).

Besides the choice for technique of TMS we had to choose if we aimed for studying H-reflexes or stretch reflexes. H-reflexes are more commonly used combined with TMS. However it was shown that significant differences exist between the effect of TMS on H-reflexes and stretch reflexes (Morita et al. 1998), likely cause by the different nature of afferent signals induced. As at the Leiden University Medical Center they have a lot of experience with evoking stretch reflexes with a standardized method (Schouten et al. 2006), and because its higher relevance in daily life, we choose to use stretch reflexes.
Earlier work from Schuurmans et al. 2009 showed velocity dependency of the M1 response and amplitude dependency of the M2 while Meskers et al. (2009) showing lack of reflex adaptation in patients after stroke. We decided to study the possibility the cortex involved on the modulation of the human stretch reflex as observed in the wrist muscle by subthreshold TMS.

### A.2 Development of experimental set-up

Development of the experimental set-up started with analyzing the available equipment at the Leiden University Medical Center. The protocol as used by Schuurmans et al. (2009) was still available and usable after some changes. The robot to apply position perturbations to the reflex, the pols perturbator (PoPe), is also available. A brand new MagStim Rapid² TMS device just came available at the department of ‘Klinische Neuro Fysiologie’ (KNF). Details and steps of development of the main components of the set-up are described below:

*The pols perturbator (PoPe)*

The PoPe is displayed in Figure A1. The PoPe consists of a servo motor controlling the handle. The servo motor is driven by a simulink model from Matlab (Mathworks). Angle (position), velocity and torque applied to the handle are fed back. Previous research indicated that the current model was not able to achieve low enough amplitudes with reasonable velocity, and therefore we had to develop a new model. This model had to include also an extra input and output trigger to be able to synchronize TMS and reflex EMG data. The new build model consists of a simple PD controller with a feed forward loop. It is displayed in Figure A2.

The P and D values were tuned by trial and error comparing the output to the input being a step response. We aimed to have as less overshoot as possible and getting to a steady state, the requested amplitude, as fast as possible.

*Figure A1: The Pols Perturbator (PoPe)*

*Figure A2: The simulink model used to stir the PoPe. It contains a simple PD controller with a feedforward loop*
TMS device is required to be a step-like signal. It is triggered on the side or top of this step response depending on the settings applied. Some tests were performed to check for possible delay between triggering the TMS device to stimulate and the actual stimulation, which was possible to check for by looking at the incoming signal from the TMS device. No delay was found between the trigger and actual TMS pulse (Figure A3).

**Figure A3:** Proof that no delay is present between input and output trigger of TMS

**TMS device**

From the department of KNF we were allowed to use their just bought MagStim Rapid² device. This device is displayed in Figure A4. It is equipped with a figure-of-8 air film coil and the ANT neuronavigation system. This MagStim system is mainly used for rTMS as the coil is protected for heating by air flowing around the copper wires of the coil. Nonetheless it is also useful for applying single pulses as we aimed to do. The MagStim device consists of a special input-output interface to which an external input trigger can be connected and an output could be collected to use in data processing. These connections are used to synchronize with the EMG data and automatically trigger a TMS pulse with respect to perturbation onset evoking reflexes. Settings for this input and output signals could be adjusted. The figure-of-8 coil is connected to a special designed stand making it easy to move around on the head. To the coil a set of markers is attached making it possible to monitor the position of the coil with respect to the head. (See Figure A5). Therefore the subject involved also needs to wear a set on markers on his head which is done by a head strap. Using specifically designed software of ANT, ASA 4.7.3, it is possible to visualize coil position with respect to the head on a MRI scan. Because of the limited financial possibilities during this study we choose to use a generic MRI scan for all subjects. From a MRI a so called ‘head model’ could be created using the software. I followed some instruction sessions with somebody from
the company of ANT to learn how to do this. The head model is used to calculate the magnetic field you apply to the head, which is also displayed on the monitor. However, during initialization of each subject the head model could still be slightly changed by adjusting the contours of the head. This is done by a special pointer, also instrumented with markers. Coil configuration is very important, but was done by the suppliers because no suitable equipment was available at the time I performed my measurements. When stimulating, all stimuli are recorded and displayed being reproducible when needed. Only requirement is that the markers on the head of the subject always stay in position. When these markers are moved all positions recorded for the stimuli are useless.

A few notes have to be added about this MagStim device:
In most papers treating reflexes and TMS a MagStim 200 stimulator is used. This stimulator has slightly different characteristics from the one used in this study. The maximal magnetic field induced is a bit lower in the Rapid compared to the MagStim 200. Additionally the use of the air film coil is not beneficial. This coil is especially designed for use during rTMS. Therefore the copper wires are less tight wounded in order to make the air cooling more efficient. This, however, also reduces the outgoing magnetic field.

Other equipment
Besides the main equipment being the PoPe and the TMS stimulator some other equipment was used. For measuring EMG signals of the flexor and extensor carpi radialis we used a Delsys Bagnoli 4 channel system having an intern band pass filter of 20-450 Hz. The EMG was recorded with differential surface electrodes (Delsys Bagnoli System, Delsys Inc., Boston, MA, USA, electrode bar length 10 mm, bar distance 10 mm). Because the experiments were planned to last about 2.5-3 hours and people were asked to sit as still as possible comfortable seating is very important. Because the pope is positioned high no proper chair with sufficient back and head support was available. Therefore I decided to build my own one. To make it height adjustable I bought a hair dressers chair but only used the supporting structure, being height adjustable. I also bought an old car chair which I mounted on top of the hair dressers chair base. This made a comfortable chair height adjustable and probably useful for other applications.

PoPe and TMS measurement protocols
As various new components are included and online data processing is needed, development of some new matlab code was required. It was decided to start with initialization of the TMS, meaning finding the best spot to stimulate, the motor threshold and MEP latency. Online data processing was needed to be able to quickly observe the result of a TMS pulse. The flexor and extensor EMG as well as the torque applied to the handle were displayed. In the first session applying TMS pulses was done by hand to quickly scan the motor cortex. This was done in as well a resting as active state of the muscle. In a resting state it is easier to find the right motor spot because a very low background EMG exists. With activation at a level of 10% MVT the MEP is more difficult to distinguish from the background EMG although it should be recognizable based on the silent period. The torque on the handle is also very useful to
analyze during applying of TMS. In the following session TMS was triggered automatically when the subject held a 10% MVT force for at least 1 second. The screen showed to the subject is in Figure A6. This was used to find the motor threshold (MT). TMS was followed by a session of reflex testing in order to keep the possibility to exclude the subject before the main experiment started. After these important steps to identify suitability of the subject two long sessions started. The first being to determine the effect of increasing stretch amplitude on M2 size and the second to determine the TMS effect on the M2 size applied at different time points. These sessions did include direct data processing at the end to show the results. For this data processing special files were written. Based on the results of the first long session a fixed stretch duration was chosen for the final session combining reflexes and TMS.

Figure A6: The feedback screen as showed to the subject. Applied force displayed in red, the target is the blue window.

Total Set Up
The set up with all its components is displayed in Figure A7.

Figure A7: The total set-up during measurement.
A.3 Pilot Studies

Perturbation characteristics
Because Schuurmans et al. (2009) showed the effect of stretch velocity on the M1 response and stretch amplitude on the M2 effect we had to choose a fixed stretch velocity resulting in clear M2 responses while a clear M1 remained. The same experiments were used to test the protocol and data processing of the obtained EMG data. In total I performed six pilot tests on friends and fellow students to determine the velocity and amplitude effect on reflex characteristics and working of the written protocols (the reflex parts). I varied between the stretch velocities 1 and 1.5 rad/s and amplitudes from 0.0015 rad to 0.15 rad. In the first tests I discovered that the PoPe was not able to reach the prescribed velocity with amplitudes smaller than 0.03 rad (meaning duration smaller than 20 ms). This was the reason to implement a new model including feed forward control. Implementing this model resulted in the possibility to reduce the duration up to 12 ms while still reaching the velocity. From the five subjects it was concluded that a more clear duration effect on M2 size was observed with a velocity of 1.5 rad/s. Therefore we used 1.5 rad/s in the main experiments. Because we were interested in variance of the stretch amplitude effect on M2 size one subject came in three times were we did the same experiment. It turned out that this effect is reasonable reproducible over the different experiments.
For pilot results see Appendix E

TMS trials
Because the TMS device available in Leiden was slightly different from the one I was used to during my internship, I had to get used to handling it. Main differences are the coil, the way of positioning and the system to localize coil and subject with respect to each other in space. We are not fully satisfied with the current way of positing of the coil, with a large arm. This way of positioning is sometimes very frustrating because not enough degrees of freedom are available to correctly position the coil. To practice the positioning of the coil we borrowed a dummy used for MRI imaging. This was very useful because we were able to find way to work with the limitations present. In TMS testing on humans the main goal was to find the good spot and get an idea about motor threshold. This is where we found out that distinguish a MEP with 10% MVT background level is difficult and therefore we decided to include TMS during a resting state of the muscle. Also the written programs to steer the TMS device were frequently tested.

A.4 Detailed description of an experiment

Each subject coming in for the experiment was treated following a strict protocol to ensure safety and the same conditions for each of them. Before every single subject all the equipment was checked for malfunctioning and possible small deficits. Each experiment was planned to last for three hours. People were awarded with 30 euro’s when completing the study. At all times they were allowed to stop during the experiment. We rejected people during the experiment if they lacked clear MEPs or reflex responses. Inclusion and exclusion criteria were defined following the guidelines presented next:

Inclusion criteria: age between 40-70 years (to be matched with future stroke study population).
Exclusion criteria: cardiac pacemakers; any metal implant within the brain; any medical history of general and local neurological disorders, especially epilepsy. All exclusion criteria
will be systematically checked by using a questionnaire (see Appendix C). Any history of orthopedic problems with the upper extremities; current medication that may influence nervous function. Considering the wide variety in possible medication, we will check for eligibility per case (responsible physician: Dr C.G.M. Meskers). The following main equipment was used: (Further explanation Appendix A.2)

*To evoke reflexes:*

Pols Pertubator (abbreviated by: PoPe), developed by TU Delft (Schouten et al. 2006).

*Transcranial Magnetic Stimulation:*

Magstim Rapid² stimulator with the Magstim Air Film Coil (figure-of-8 70mm). Visor neuronavigation to keep stimulation position (ANT, Enschede, The Netherlands).

*To record EMG:*

Delsys Bagnoli System, 4 channels, Delsys Inc.

Below the full protocol is described divided in the different phases:

**Setting up the measurement set-up (Checklist before subject entrance)**

To start with, the height adjustable comfortable car chair is positioned in front of the PoPe. The base of a hair-dressers chair is fixed below the car chair to make it height adjustable in an easy and fast way for each subject. After getting the chair in position the Magstim stimulator is positioned near the chair. Connect the coil the coil and then plug the device in. Because the Magstim stimulator is automatically triggered from an in Matlab designed script it should be connected to a data acquisition box. The same box is used for data acquisition of the PoPe. A BNC cable should be placed between the ‘trigger in’ of the Magstim stimulator and the DACH7 of the DAQ box while another cable should connect the ADCH8 and the ‘trigger out’. When using the Neuronavigation system, the stand containing the computer and camera’s should be positioned in such a way that it is easy to control as well as that the camera’s can be directed towards the subject’s head. Next step is to connect a BNC cable from a special supplied box towards the ‘trigger out’ of the Magstim stimulator. This is to match a stimulus to the corresponding position of stimulation. To end with, positioning of the two feedback monitors is important. The one used for subject feedback should be positioned in front of the subject, in such a way that the subject doesn’t have to turn or bend its head to get a clear view on the screen when comfortably seated in the chair. The other screen, used for feedback to the experiment leader, should be positioned in a way that it is easy to analyze first results accurate and quickly. It might be clear that positioning of both screens should be adapted to the demands of the subjects and users.

**Checklist before start**

- Chair is in position
- Position Magstim Stimulator and connect coil. Plug it in.
- Connect trigger in and out cables to DAQ box
- Position Neuronavigation stand and turn computer on
- Connect trigger out to Neuronavigation system
- Position subject feedback and experiment leader monitor
- Turn PoPe computer on.
After subject entrance

Because a questionnaire and further information is supplied before the experiment it is expected that all the subjects have some prior knowledge about the experiments before they come in. Then explain the experiment verbally and answer possible questions from the subject extensively. When the subject is satisfied and knows what to expect, the informed consent should be signed. After completing this part it is important to first make the subject feel comfortable with the equipment which is used and make sure that he/she is comfortable in the position which has to be hold during the experiment. Adjust chair and monitor positions when needed, also make sure the chair is not too high or too low. When the subject feels comfortable it is time for placing the EMG electrodes and fix the arm into the PoPe. Before placing the EMG electrodes on the flexor and extensor of the wrist clean and scrub the skin in order to remove dead skin cells and dirt possibly affecting the recording. To make sure the electrodes stay in position use a pressure band over the lower arm. After placing the electrodes it is time to fix the lower arm in the PoPe. Ask the subject to grasp the handle and hold it while you fix the lower arm using clamps at wrist level with form adjustable foam between skin and clamp. Fixate the clamps as tight as possible as long as the subject thinks he is able to hold it for three hours. Making sure the subject feels well with his current position it is time to start the neuronavigation session. Place the head strap with the markers on the forehead of the subject and make sure that the markers are visible in the center of the space observable in the NTI Tool Tracker. When the cameras and the subject are positioned in the right way (Figure A8), you have to start the ASA 4.7.3 program. Start a neuronavigation session and load a ‘standard’ MRI. Following all the instructions will guide you through all the steps resulting in different views of the MRI image. In these views the coil and calculated electric field of stimulation is visible when the coil is positioned near the head. This finishes the preparations before measuring.

Checklist preparations with subject

- Verbally explain the protocol
- Answer questions
- Sign informed consent
- Adjust chair and monitor positions according to subject’s needs
- Clean the skin
- Place EMG electrodes
- Fix the lower arm
- Place ‘head’ markers
- Initialize neuronavigation

The measurement protocol

All the measurements are stirred and analyzed from a self written Matlab script. It is divided in a section in which the handle of the pope is mechanically fixed and a section in which the handle is controlled to evoke reflexes in the wrist. The first part is with the handle fixed. This
is to be able to do a maximum voluntary torque (MVT) measurement and to initialize the Transcranial Magnetic Stimulation (TMS). TMS initialization contained searching for the optimal spot for stimulation and the right intensity to stimulate on. Start the script by typing ‘PoPe’ in the command window and follow the instructions on the screen. When the MVT measurement starts, ask the subject to do a maximum flexion movement of the wrist (Figure A9). Repeat this measurement three times. Then start the Magstim stimulator and choose the single pulse mode. Set the stimulation intensity to 50% in order to find the best spot to stimulate on and see a clear motor evoked potential (MEP) in the wrist flexor EMG. Increase the intensity by steps of 5% when no MEPs are identified. Ask your subject to relax and hold their head as fixed as possible without sitting convulsive. Start the next protocol and ask the subject to fully relax the wrist and hand muscle and rest with the hand opened against the handle. Place the coil on the side of the subjects head and stimulate by hand. This ‘by hand’ triggered stimulation is to be able to quickly find the best spot to stimulate in rest. When a spot is found with a large flexor MEP and a small extensor response, ask the subject to do a contraction up to the blue bar in the middle of the feedback screen. Check if you are still at the best position and if you are satisfied stop the current session by clicking stop. Mark the best point of stimulation in the ASA 4.7.3 software by selecting ‘Reproduce stimuli’ and then click the corresponding stimulus. Now it is possible to navigate back to the best spot to stimulate on.

By reducing the stimulation intensity step by step you are now able to find the motor threshold, represented by 5 MEPs out of 10 responses with a peak to peak amplitude of 200 μV in active state (Rossini et al. 1994). Being ready with this part the next part is to identify the MEP onset delay. Ten clear MEPs are evoked by setting the stimulation intensity to 70% and positioning the coil in the right spot. Having done this the handle is loosened and given in control to the central controller. Now it is able to move and thereby evoke reflexes in the flexor. First a test set is supplied to make the subject feel confident with the movements of the handle. After the test session, the next session is to determine the stretch reflex characteristics. Using one velocity and two amplitudes (a small and large one) are supplied ten times and the average response is plotted directly after the session. The final session containing only reflexes is to determine the stretch duration dependent recruitment curve of the M2 size. This session takes a while and has a short break every 20 disturbances. The plot of the stretch duration versus M2 size is directly supplied afterwards. The stretch duration were it is possible to enhance as well as suppress the M2 area was used in the final experiment. Supply the characteristics asked for and prepare the subject for the session containing both TMS and reflexes. Before starting this final session, turn off the PoPe again. To check if still the same position is ok to stimulate on. If fone, set the stimulation intensity 2-3% below motor threshold and position it in the right spot again. Ask the subject again to move as less as possible in order to make your measurements more accurate. Tell the subject that it is not for safety reasons that he/she has to sit as still as possible because this may cause that the subject sits not relaxed.
Checklist during measurements
- Neuronavigation is initialized and working
- MVT measurement (repeat three times!) → Check EMG response!
- ‘Hand’ triggered TMS to find ‘hot spot’
- Automatic triggered TMS to find motor threshold
- Identify MEP characteristics (f.e. onset)
- Run test session of reflexes
- Identify stretch reflex characteristics
- Stretch duration effect
- Check TMS positioning
- Supply characteristics of reflexes
- Perform experiment TMS and stretch reflexes

References:


Appendix B: TMS for dummies

Transcranial Magnetic Stimulation (TMS) was used to study stretch reflexes of the human wrist and this appendix serves to introduce the reader into the basics of TMS which every user has to know.

TMS was introduced by Barker et al. (1985). It was mainly presented as the method to non-invasively and painlessly excite the human nervous system at various levels. TMS developed since that time to a commonly used technique in research, for studying functioning of the neuromuscular system, as well as in clinics to mainly treat psychiatric patients. For instance people suffering of depression.

B.1 The physics and equipment behind TMS

B.1.1 Electromagnetism in TMS

The technique of TMS is mainly based on the fundamental principles of electromagnetic induction: an electric current in the stimulation coil results in a magnetic field perpendicular to it, while a changing magnetic field induces a flow of electric current in nearby conductors, in our case the human brain. Basically, by placing a wire on the scalp and passing a high and rapidly changing current through it, a magnetic field is produced which penetrates the scalp almost without any losses.

It is known that when two loops of wire are placed close together, a changing primary current in loop 1 produced a changing magnetic field, which generates an electric field, which in turn induces a secondary current of opposite direction in loop 2 (Figure B1).

This secondary current induced in the nearby conductor, in the TMS case brain, is commonly called an eddy current. These eddy currents penetrate the membranes of the neurons, resulting in action potentials or excitatory (or inhibitory) postsynaptic potentials.

B.1.2 The TMS circuit and pulse waveforms

The (eddy) current induced in the brain by the magnetic pulse itself creates a magnetic field that tends to cancel the applied field. From this it can be concluded that when the current is able to flow freely in the circuit, an oscillatory magnetic field is induced which rises from zero to its maximum and then falls and reverses its direction. Important to notice is that due to losses the magnetic field gradually damps out with time. The same holds for the magnetic field in the brain.

The circuit through which the primary current flows to generate the magnetic field can be decomposed to only three simple single elements, namely: a power capacitor, the inductance of the stimulation coil, and a switch to open and close the circuit, Figure B2. These components together form the magnetic stimulator of the brain. Depending on some more specific elements in
the magnetic stimulator circuit two different kinds of TMS pulse waveforms are defined: mono – and biphasic pulses, Figure B3. When a biphasic TMS pulse begins, all the energy is stored in a charged capacitor, Figure B4. The moment the capacitor discharges and current starts to flow, all energy is transferred from the capacitor to the coil. Because we deal with an oscillatory magnetic field, we can state that when the current is zero the capacitor is fully charged and all the energy is still there, while when the current is maximum all the energy is in the inductor (the stimulation coil). The duration of a full cycle, from charged capacitor to fully recharged capacitor, is the resonant period of the inductor-capacitor circuit (also called LC circuit) defined by:

$$T = 2\pi\sqrt{LC}$$  \hspace{1cm} (4)

During the oscillatory behavior until all energy is used or dissipated, the current constantly changes direction. In a monophasic circuit, the first quarter of the waveform of the voltage is exactly the same as in the biphasic case, Figure B5. But after this first quarter, the built up current is dissipated slowly rather than being allowed to recharge the capacitor. This is achieved by providing an extra switch or diode to the biphasic circuit by which the coil current is prevented from flowing in the reverse direction. This avoids the system to generate the oscillatory behavior as observed in the biphasic circuit. The pulse duration for monophasic pulses is usually in the order of 600 µs. Monophasic pulses are most widely used in TMS studies.

**Figure B3:** There exist two types of pulse waveforms: Monophasic (left) and Biphasic (right). From Wasserman et al. (2008)

**Figure B4:** The coil current and induced voltage corresponding to a biphasic pulse waveform. From Wasserman et al. (2008)

**Figure B5:** The coil current and induced voltage corresponding to a monophasic pulse waveform. From Wasserman et al. (2008)

**B.1.3 Types of TMS stimulation coils and their design considerations**

The coil is the most important part of the TMS circuit. The coil mainly determines the surface area which will be stimulated, also expressed as the focality of a coil. It is desirable to limit stimulation only to the target neurons, because unknown side effects could occur when stimulating to many other neurons.
The simplest TMS coil, and also the first which has been used, forms a simple circle, Figure B6. A changing current in this simple circular form will induce a circular current flow of opposite direction in the brain tissue. A second commonly used coil design is the so called ‘figure-8’ or ‘butterfly’ coil, Figure B6. This coil can be easily imagined as two circular coils placed side-by-side.

Figure B6: The influence on the cortex of either a round coil (left) and a figure-8 coil (right). From Wasserman et al. (2008)

The figure-8 coil is most used in clinical practice. There are some other special coils developed. An example is the H coil developed by Roth et al. (2002). The coil has the shape of a hatband. It was designed to reduce the field at the cortical surface while augmenting it at depth. The H coil is most promising for deep brain stimulation but still a lot is unknown about this coil type. Another special coil is the double cone coil, Figure B7, consisting of two round coils, like the figure-8 coil. Main advantages and disadvantages of the different coils are summed in Table B1. (Wasserman et al. (2008), Chen et al. (2008), Roth et al. (2002.)) Currently used stimulators and coils usually produce a magnetic field of 1.5 to 2.0 T along the winding of the coil. The limited stimulation depth is due to the rapid fall off of the magnetic field.

<table>
<thead>
<tr>
<th>Coil type</th>
<th>Characteristics</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round</td>
<td>O.D = 8 - 15 cm</td>
<td>Good penetration</td>
<td>Lack of focality</td>
</tr>
<tr>
<td></td>
<td>Stimulation depth = 1.5 – 2.0 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Figure-8</td>
<td>O.D = 7 - 10 cm</td>
<td>Maximum electric field below junction</td>
<td>Lack of penetration depth</td>
</tr>
<tr>
<td></td>
<td>Stimulation depth = 1.0-2.0 cm</td>
<td>Very focal stimulation over small area</td>
<td>Size of area stimulated difficult to determine</td>
</tr>
<tr>
<td>Double cone</td>
<td>O.D. = 9-12 cm</td>
<td>Possible to stimulate deeper parts of brain</td>
<td>Difficult field of stimulation</td>
</tr>
<tr>
<td></td>
<td>Two round coils at angle 90-100 degrees</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stimulation depth = 3.0-4.0 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Hatband shaped</td>
<td>Reduced surface field</td>
<td>No increase in electric field at certain depth</td>
</tr>
</tbody>
</table>

Table B1: Overview of the most used and discussed coil types together with some characteristics and (dis)advantages. (O.D. = outside diameter). No more information available about H coil.
field with distance from the coil: with a typical round coil, the field strength is already half at a distance of 4-5 cm. This results mainly in activation of the cerebral cortex or the subcortical white matter, which is just below the scalp. (Hess et al. (1987), Epstein et al. (1990), Wasserman et al. (2008)).

B.2 The TMS protocols
In research basically three types of TMS pulse protocols are used, these involve single pulse TMS, paired pulse TMS and repetitive TMS (rTMS). All will be discussed in this section. (Wasserman et al. (2008), Kobayashi et al. (2007))

B.2.1 Single pulse TMS
Single pulse TMS is the most direct form of TMS. Only a single TMS pulse is delivered to a certain level of the motor system, most often the brain, by which information is gained about the functioning of the system and the stimulated region. Depending on the exact site of stimulation the excitability and conductivity along all the pathways of the motor system are examined.

Figure B7: Corticospinal tract stimulation using a double coin coil. From Chen et al. (2008)

B.2.2 Paired pulse TMS
The paired pulse TMS protocol describes the application of two stimuli, with the same coil, with a certain interstimulus interval in between (typically from 1 to 200 ms). From these two stimuli the first is called the conditioning stimulus (CS), while the second is the test stimulus (TS). As is quite straightforward the measurements are taken after the test pulse, making the first pulse the conditioning stimulus. The stimuli can be applied on a subthreshold and suprathreshold level and depending on the level of the conditioning and test stimulus a specific effect is observed. With the paired pulse protocol it is possible to study inhibitory and facilitory interactions in the cortex and examine the functional integrity of intracortical neuronal structures.

B.2.3 Repetitive TMS (rTMS)
Finally repetitive TMS (rTMS) is refered to as a protocol in which trains of TMS pulses of the same intensity are applied to a single brain part at a given frequency. This frequency can range from about 1 Hz to 20 Hz, usually applied for 20-30 minutes depending on the frequency. Up to a stimulation frequency of 1 Hz the rTMS protocol is called low frequency while all other frequencies are categorized as high frequency rTMS. The higher the frequency of stimulation and the greater the intensity, the more disruption of function of the brain during the pulse train. The most clear difference between rTMS and the other types of TMS is that the modulation of cortical excitability may last longer than the duration of the pulses itself. Depending on the exact parameters of the process rTMS has a facitlor or inhibitory effect. This is the only technique already used in clinics as a therapy and mainly in psychology. (for review: Fitzgerald et al. (2006)
B.3 TMS measures and influencing parameters

The three discussed TMS protocols all have their own measures although some are used in every protocol. The most important measure from which almost all other measures are derived is the motor evoked potential (MEP). In this the basic measures and parameters for single pulse TMS are discussed as those are of importance in the current study. (Wasserman et al. (2008), Chen et al. (2008), Kobayashi et al. (2007), Terao et al. (2002))

B.3.1 General measures: The motor evoked potential (MEP) and motor threshold (MT)

Transcranial magnetic stimulation of the brain induces muscle responses, measured with EMG, which are termed motor evoked potentials (MEPs). MEPs are used in TMS to study the state of corticospinal conduction in healthy as well as in patients with a disease to the nervous system. Parameters involved with the MEP are the latency, the amplitude, duration and area of the response and the stimulation thresholds. All the involved parameters and observations on the MEP, with corresponding conclusions, will be discussed below in a logical order while they will also be, when necessary, related to a specific protocol.

To evoke a MEP a certain stimulation threshold should be exceeded to activate the mechanisms in the brain finally leading to a signal toward the peripheral target muscle. This threshold is called the motor threshold (MT). It is defined as ‘the lowest TMS intensity capable of eliciting small MEPs, usually defined as more than 50 µV in peak to peak amplitude in muscles at rest and 200 µV in active muscles in at least five out of 10 trials’ (Rossini et al. (1994)). From this definition it may be clear there is a difference in motor threshold between active and resting muscles. Motor threshold is lower in the voluntarily contracting muscle (active motor threshold, AMT) compared to a resting muscle (resting motor threshold, RMT). The motor threshold most likely is dependent on the excitability of the elements which are activated by TMS. These elements involve the cortico-cortical axons and their excitatory synaptic contacts with the corticospinal neurons, and also the excitability of the motor neurons in the spinal cord, neuromuscular junction and muscle. Main driver of the process of excitation is depolarization of a neuronal membrane leading to an action potential. In addition to the membrane excitability, the motor threshold should reflect the activities of neural inputs that may affect membrane excitability, that is, tonic inhibitory and excitatory drives onto the cortical output neurons, and also the efficacy of a chain of synapses from presynaptic cortical neurons to muscles.

As the threshold is exceeded action potential(s) propagate along the corticospinal tract towards the motor neurons which need sufficient input to start firing to the target muscle. This process takes time which is called the latency of the MEP. It is expresses by the central motor conduction time (CMCT) which is an estimate of the conduction time between the motor cortex of the brain and the spinal motor neurons. The CMCT is calculated by subtracting the latency from spinal motor neuron to muscle from the motor cortex to muscle latency (Kobayashi et al. (2007)). It is measured while the target muscle is active giving the shortest latency from brain to muscle. This is due to the fact that when the muscle is active the spinal motor neuron pool is more close to its threshold which causes an earlier discharge from descending brain signals.

The MEP, its MT and CMCT depend on the targeted muscle and are likely affected by inter subject variations including age, height and gender. Significant differences in the CMCT are reported for height (Rossini et al. (1987)). The CMCT is larger for more distal muscles and does not depend on the side of stimulation.
Depending on the muscle which is aimed for the exact spot of magnetic stimulation is very important as well as the direction of the electrical field at that spot. If the magnetic pulse is given the forthcoming MEP is also influenced by the state of excitability of the corticospinal pathway. As mentioned before MT is lower in a contracted muscle. This also shortens the CMCT and increases the MEP size. This is not the only way the MEP size is shown to be influenced (facilitated or inhibited). Some examples:

- Use of the right conditioning stimulus (paired pulse protocol, further discussed in next section)
- Afferent input (tendon, cutaneous, muscle)
- Muscle stretch
- Thinking about a movement or a contraction of the target muscle
- Speech

However all these maneuvers influence MEP size, a voluntary background contraction is by far the most effective. Due to the high variability a broad range of normal values is available.

### B.3.2 Single Pulse TMS measure: Silent period

If a subject is instructed to maintain muscle contraction of a targeted muscle and a single suprathreshold TMS pulse is applied to the part of the cortex representing that specific muscle, the EMG activity will be suppressed for a certain time after the MEP, Figure B8. This period until the voluntarily EMG activity returns is called the silent period (SP). The silent period is thought to be caused by some cortical inhibitory mechanisms of the motor cortex and the first 50 ms seem also to depend on spinal inhibitory mechanisms (e.g. Renshaw inhibition and the refractory period of a cell). The duration of the silent period is related to the stimulation intensity but not the MEP size which precedes the SP or the level of EMG voluntarily background activity (contraction strength) (Inghilleri et al. (1993)). Furthermore, there is not much interhemispheric difference in SP. It is believed that the SP is related to the strength of the corticospinal projection of a muscle: hand muscles have a much longer SP than proximal arm and leg muscles (Ziemann et al. (1993)). Main drawback of the SP measure is the high interindividual variability.

Be aware that in some literature the silent period is indicated by cSP which does not indicate contralateral silent period but cortical silent period. SP is usually referred to as contralateral silent period while also an ipsilateral SP exists (iSP). (Fuhr et al. (1992), Chen et al. (1999))

### B.4 Concluding remarks on TMS

In this section some important issues considering TMS not discussed until now will be presented shortly.

#### B.4.1 Safety

TMS can be compared to magnetic resonance imaging (MRI), both using magnetism as a driving force to change properties of the body. Like MRI single and paired pulse TMS have some contraindications. These contraindications mainly involve having intracranial ferromagnetic material such as aneurysm clips or other implants. This also holds for a cardiac...
pacemaker although it is very unlikely to be damaged by TMS. People suffering of epilepsy may be considered having an extra contraindication; nevertheless the risk of inducing seizure is very low. Although the risk of inducing seizure is low a history of seizure, subjects who use medication that might increase the risk of seizure and subjects which have a family history of epilepsy are excluded. Subjects involved in experiments of therapy using TMS often (10-20%) complain about headache due to muscle tension after the treatment, which is one of the most common reported complaints after TMS.

It may be clear that the main safety concern is the risk of inducing seizure. The risk is low, 1 in 1000 studies, and mainly present in rTMS. During rTMS subjects are advised to wear earplugs in order to avoid ringing of the ears or even transient hearing loss. It is extremely important that even when the safety guidelines are followed people who perform the experiment are aware that induction of seizure is always possible, and the laboratory should be set up in such a way that seizure is recognized and can be treated as soon as possible. (Wasserman et al. (1998), Kobayashi et al. (2007), Wasserman et al. (2008))

B.4.2 Current direction and pulse waveform

Depending on the current direction on the coil the current direction in the conductor is opposite as was explained in the physics part. It has proven to be very important in which direction the current flows in the brain looking onto measures like MEP and MT. For single pulse TMS, biphasic pulses yield a lower MT than monophasic pulses making it plausible that the second and third quarter cycles contribute essentially to the net effect of stimulation. MEP latency depends also on the current direction and the pulse waveform. When a monophasic pulse is applied posteriorly oriented pulses have longer latencies compared to the latency when a biphasic waveform is used and the anteriorly directed current has longer latency. It was also reported that when the silent period was studied it could be concluded that inhibitory interneurons are best activated by posteriorly oriented pulses. An optimal MEP amplitude and the shortest latency was found with monophasic and biphasic pulses flowing anteriorly in the brain and approximately perpendicular to the presumed location of the central sulcus, Figure B9.

(For more information: Literature study: Supraspinal modulation of the human stretch reflex by transcranial magnetic stimulation (TMS) by Mark van de Ruit, January 2010)

References


Appendix C: Medical Ethical Committee Report

Before we were able to perform the experiments we had to write a report to the medical ethical committee to get permission. This committee judges if research done in the Leiden University Medical Hospital is not demanding for the people involved, and if possible hazards are acceptable. Therefore I wrote the report present in the next part of Appendix. Additionally the letter supplied to the subject (in Dutch) and the questionnaires are included. Because of the complexness of the study questions come from the ethical committee but late November we finally got permission to go.
Investigating changes in the human stretch reflex characteristics with Transcranial Magnetic Stimulation applied in the refractory period between the short and long latency reflex response

Date: 23-11-2010 P10-178

Version number: 2.0

Applicants:
- Mark van de Ruit *
- Dr. ir. A.C. Schouten*
- Dr. ir. J.H. de Groot#
- Dr. C.G.M. Meskers# (responsible physician)

Experiment leader:
- Mark van de Ruit*

Medical intervention and supervision during experiment:
- Dr. C.G.M. Meskers# (responsible physician)

Independent physician
- Drs. J.M. van der Krogt

Participating institutes:
*Dept. of Mechanical Engineering, TU Delft
#Dept. of Rehabilitation Medicine, LUMC

Contact address

<table>
<thead>
<tr>
<th>Mark van de Ruit</th>
<th>Dr C.G.M. Meskers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Department of Neuromuscular Control, TU Delft, the Netherlands</td>
<td>Department of Rehabilitation Medicine</td>
</tr>
<tr>
<td>Contact: <a href="mailto:mlvdruit@hetnet.nl">mlvdruit@hetnet.nl</a>, mobile phone +31-628395960</td>
<td>Laboratory for kinematics and neuromechanics, B0-Q</td>
</tr>
<tr>
<td></td>
<td>LUMC</td>
</tr>
<tr>
<td></td>
<td>Albinusdreef 2, 2333 ZA Leiden</td>
</tr>
<tr>
<td></td>
<td>Phone +31-71-5263457</td>
</tr>
<tr>
<td></td>
<td>Fax +31-71-5266697</td>
</tr>
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Summary

Humans have the ability to adjust their reflexes according to the environmental conditions and task to perform. Modulation of reflexes is paramount for optimal movement in daily life. In central neurological diseases like stroke, inability to modulate reflexes may be the cause for the observed movement disorders. Therefore it is of importance to understand if and how the central nervous system (CNS) modulates peripheral reflexes.

Peripheral reflex activity can be assessed by stretch reflexes of the m. flexor carpi radialis evoked by a wrist manipulandum and measured by EMG (electromyography). EMG results in typical short (M1) and long latency (M2) reflex bursts. CNS activity can be evoked by Transcranial Magnetic Stimulation (TMS). The present proposal comprises the combination of aforementioned techniques to study reflex modulation by the CNS of peripheral reflexes. The influence of careful timed TMS pulses on the contralateral sensorimotor cortex of healthy subjects on EMG activity evoked by motorized stretches of the m. flexor carpi radialis is investigated.

N=15 healthy subjects will be included to participate in the present experiment. Total experimental time will be 3 hours. Subjects are asked to exert low flexion torques around the wrist, while ramp-and-hold stretches with a fixed velocity of 1.5 rad/s and various stretch durations (30-70 ms) are imposed by a wrist manipulandum. Simultaneous and variable timed sub threshold single pulse TMS is imposed. Burden and risks for the subjects is minimal, because of small perturbation amplitude and subthreshold TMS single pulses. Subjects with any history of epilepsy will be excluded.

Primary outcome parameter is the amplitude of the M2 response as assessed by EMG activity. Dependents are characteristics of ramp-and-hold stretches and timed TMS pulses. Repeated measures Analysis of Variance will be applied for statistical analysis. Statistical significant differences of 30% in normalized M2 amplitude will confirm CNS modulation of peripheral reflex activity.
Samenvatting

De mens is in staat zijn reflexen continue aan te passen aan de omstandigheden en taken. Reflexmodulatie is zeer belangrijk voor optimaal bewegen in het dagelijks leven. In centraal neurologische aandoeningen zoals CVA is het onvermogen om reflexen te moduleren waarschijnlijk de oorzaak van de geobserveerde bewegingsstoornissen. Het is daarom van klinisch belang te begrijpen of en hoe het centraal zenuwstelsel (CZS) reflexen moduleert. Perifere reflexactiviteit kan worden bepaald door rekreflexen van de m. flexor carpi radialis opgewekt door een polsmanipulator en gemeten met behulp van electromyografie (EMG). EMG geeft typische short latency (M1) and long latency (M2) responses. Activiteit van het CZS kan worden gemanipuleerd met behulp van Trans Craniele Magnetostimulatie.

Het voorgestelde onderzoek behelst het combineren van bovenstaande technieken om de reflexmodulatie door het CZS te kunnen bestuderen. De beïnvloeding van reflexactiviteit door zorgvuldig getimede TMS pulsen bepaalt de moduleerbaarheid van reflexen door het CZS.

N=10 gezonde proefpersonen zullen worden geïncludeerd in het onderzoek. Totale experimenttijd zal 3 uur bedragen. Gedurende het experiment zal aan de proefpersonen worden gevraagd een flexiekoppel te leveren rond de pols door het aanspannen van de flexoren van de onderarm. Ramp-and-hold perturbaties met een vaste snelheid (1.5 rad/s) en variabele rekduren (30-70ms) worden toegevoerd door een polsmanipulator. Simultaan worden subthreshold TMS single pulsen gegeven over de contralaterale sensorimotor cortex. Belasting en risico zullen minimaal zijn. Proefpersonen bekend met epilepsie worden uitgesloten van deelname.

Primaire uitkomstparameter is de amplitude van de M2 response zoals vastgesteld met behulp van EMG. Determinanten zijn de rekduren van de ramp-and-hold stretches en getimede TMS pulsen. Statistische analyse wordt verricht met behulp van repeated measurements ANOVA. Een statistisch significante verandering van 30% van de genormaliseerde M2 amplitude zal worden gezien als een bevestiging van de beïnvloedbaarheid van de perifere reflex door het CZS.
Background

Introduction
Humans have the ability to adjust their reflexes according to the environmental conditions and task to perform. The nature of this ability to adjust the reflex is not well understood. The human stretch reflex response, for most of the upper arm muscles, consists of two responses usually labeled by M1 and M2, or short and long latency response. Origin of these responses mainly lies in the proprioceptive feedback from muscle spindle and golgi tendon organ while also tactile feedback is proven to have a role in reflex size adaptation. Lack of reflex adaption is hypothesized to play a key role in movement disorders after Central Nervous System damage like stroke (Meskers et al 2009). It is therefore important to study the possible reflex adaptability by the CNS. To be able to do this, it is required to:

1) Be able to evoke reflexes in a standardized way and to quantify stretch responses
2) Be able to elicit activity via the CNS in a non-invasive way, in order to study the influence on stretch reflexes.

Human stretch reflexes and its quantified assessment

Reflexes are very important for human beings to protect themselves against harmful situations. Stretch reflexes have an essential role in the maintenance of muscle tone and posture. The most well known stretch reflex is the knee jerk which is a simple stretch reflex initiated by tapping the tendon of the relaxed quadriceps femoris muscle.

The electrical activity of the muscle during the reflex can be measured using EMG (ElectroMyoGraphy). This will give a pattern with two or three different responses, depending in the muscle under investigation. The tibialis anterior muscle in the lower leg will give three responses labeled by M1, M2 and M3 while the flexor carpi radialis muscle of the wrist will give two responses, labeled M1 and M2. It is important to notice that M2 of the wrist notice is not the same as M2 of the leg muscle. The responses are in some cases also labeled by the: Short, Medium and Long latency response. The pathways and parts of the central nervous system mediating these different responses are already subject of discussion for over 50 years since Hammond showed that the M2 response had a too long latency to fit into the view of the whole reflex response originating from a monosynaptic pathway as was proposed by Lidell and Sherrington in 1924. Recently Schuurmans et al (2009) proposed a mechanism in which the M1 response synchronizes the motoneurons, and therewith their refractory periods, as an explanation for the stretch duration dependency of the long latency M2 response. Nowadays it is generally assumed that the M2 response is a compound response (Lourenco et al 2006, Meskers et al 2010). A transcortical or supraspinal mediated component is hypothesized (Lewis et al 2006).

![Figure 1: A clear pattern of a M1 and M2 response for the human wrist. From Lewis et al. (2005)](image-url)
Eliciting activity of the CNS in a non-invasive way: Transcranial Magnetic Stimulation in humans

The technique of TMS (Barker et al., 1985) is mainly based on the fundamental principles of electromagnetic induction: an electric current in the stimulation coil results in a magnetic field perpendicular to it, while a changing magnetic field induces a flow of electric current in nearby conductors, in our case the human brain. Basically, by placing a wire on the scalp and passing a high and rapidly changing current through it, a magnetic field is produced which penetrates the scalp almost without any losses.

It is known that when two loops of wire are placed close together, a changing primary current in loop 1 produced a changing magnetic field, which generates an electric field, which in turn induces a secondary current of opposite direction in loop 2 (Figure 2).

This secondary current induced in the nearby conductor, in the TMS case brain, is commonly called an eddy current. These eddy currents penetrate the membranes of the neurons, resulting in action potentials or excitatory (or inhibitory) postsynaptic potentials.

The stimulation coil is the most important part because it determines the characteristics of the area of the brain you are actually stimulating. There are three different stimulation coils which are most commonly used namely: the round coil, figure-8 coil and the double cone coil. These different coils all have their own stimulation characteristics and choice depends on which part of the brain or nervous system you are about to stimulate. We will to use the figure-8 coil mainly for reasons of focality.

Types of Transcranial Magnetic Stimulation

In research basically three types of TMS pulse protocols are used, these involve single pulse TMS, paired pulse TMS and repetitive TMS (rTMS). (Wasserman et al. (2008), Kobayashi et al. (2007)). Single pulse TMS is the most simple and safe form of TMS. Only a single TMS pulse is delivered to a certain level of the motor system, most often the brain, by which information is gained about the functioning of the system and the stimulated region. Depending on the exact site of stimulation the excitability and conductivity along all the pathways of the motor system are examined and changed. Single pulse TMS is the only form of TMS used in this project.

TMS Measures

Transcranial magnetic stimulation over the primary motor cortex (M1) of the brain induces muscle responses, measured with EMG, which are termed motor evoked potentials (MEPs). MEPs are used in TMS to study the state of corticospinal conduction in healthy as well as in patients with a disease to the nervous system. Parameters involved with the MEP are the latency, the amplitude, duration and area of the response and the stimulation thresholds.

To evoke a MEP a certain stimulation threshold should be exceeded to activate the mechanisms in the brain finally leading to a signal toward the peripheral target muscle. This threshold is called the motor threshold (MT). It is defined as ‘the lowest TMS intensity capable of eliciting small MEPs, usually defined as more than 50 µV in peak to peak
amplitude in muscles at rest and 200 µV in active muscles in at least five out of 10 trials’ (Rossini et al., 1994). When a MEP is observed after a magnetic stimulus the stimulation intensity is suprathreshold. When subthreshold stimulation intensities are used sometimes a MEP may be present but usually no MEP is observed. We use only subthreshold pulses in the present study.

**Combining stretch reflexes with TMS: towards understanding the role of the human brain in the modulation of stretch reflexes**

By combining TMS, applied to the primary motor cortex, with stretch reflexes knowledge is gained about the role of the human brain in the modulation of stretch reflexes. TMS is able to depress as well as excite the central nervous system, at different levels, depending on stimulation intensity. While it is believed that the signals generated by inducing a fast stretch to a muscle cross some of this influenced parts of the CNS a changed reflex is expected. TMS is able to generate a MEP via the same spinal pathway as in which the reflex gets to its observed potential. It is assumed that these signals add together in a non linear way, by which supraspinal modulation would be possible. By decreasing the synaptic time constants the timing gets proportional more critical. The relative timing of the TMS and the mechanically induced stretch is our main interest.

The results are changed stretch reflex EMG characteristics. Addressing the sources of these changes is very valuable for understanding changes observed in reflexes of patients after for example stroke. Since the source and mediating parts of the CNS are largely known for the M1 short latency response, we will focus on changes of the M2 long latency response by timing the TMS in the refractory period between the short and long latency response. While the duration of the applied stretch is shown to have a large influence on M2 size, a fixed stretch duration is used. Timing TMS in this period is believed to largely influence the M2 long latency response characteristics.

**Goal**

The goal of the experiment is to investigate the involvement of the CNS in peripheral reflexes modulation. Peripheral reflexes will be evoked mechanically and visualized by EMG. CNS activity will be evoked by TMS.

**Hypothesis**

Adaptation of the stretch reflex responses is possible by applying subthreshold TMS (not evoking a motor evoked potential) over the human motor cortex timed to arrive at the motoneuron pool during the stretch reflex response. This shows an important modulating role of the human brain on the motoneuron excitability directly observed in the reflex response sizes. Subthreshold TMS just after the short latency peak has reached his peak value will increase the long latency response size and makes it occur earlier in time. The later TMS is applied in between M1 and M2, the larger the M2 response and even an M3 response becomes more likely when stretch duration is long enough.

Note that only in case of a positive outcome we are able to conclude that supraspinal modulation of the stretch reflex is possible. When changes are not present we can neither validate nor falsify our hypothesis.
General protocol

The protocol exists of an initialization phase in which characteristics of the reflex and some settings for applying the TMS are identified. In this part of the experiment subjects are checked for being suited for performing the whole experiment. The stretches applied are position perturbations. Perturbations are applied when the subject maintains a target torque, which is a percentage of the maximal voluntary contraction (MVC), for a few seconds. After every perturbation the subject is able to relax again. The main experiment is a combination of stretches and TMS pulses applied together at different intervals. The experiment will last about 3 hours. This is including the informed consent, instruction and breaks. The total time that TMS is used during the experiment will be about 1 hour.

Evoking stretch reflexes by using the PoPe (Pols Pertubator)

We will evoke stretch reflexes in the flexor carpii radialis (FCR) muscle. We will use a wrist manipulandum. The subject is seated in front of the manipulandum with his hand positioned around the handle after positioning the EMG electrodes on the FCR muscle. The lower arm is restrained in an arm support such that the axis of rotation of the wrist and the manipulator coincide. The stretch reflexes are evoked by applying small position perturbations to the handle of the manipulandum. With the wrist in its neutral position (0° flexion) the subject is instructed to maintain a constant flexion torque of 10% of the MVC. This task is equivalent to a ‘let go’ task, in the sense that task performance is optimal when the subject gives way to the perturbations. The applied torque by the subject is shown on the monitor in front of the subject. The experimental protocol to evoke stretch reflexes in the human wrist by using the PoPe has been previously approved by the Medical Ethics Committee (Commissie Medische Ethiek of the LUMC, e.g. P07-229)

The setup is stationed in the Laboratory for kinematics and neuromechanics (Bewegingsanalyse-lab) at the Department of Rehabilitation of the LUMC. The programming of the manipulator will be performed by two engineers (ir. Alfred Schouten and Mark van de Ruit). Mark van de Ruit will conduct the experiments.

Applying TMS to modulate the human stretch reflex

We will stimulate the part of the motor cortex representing the wrist muscle. We approximately know the location of the representation of this muscle on the motor cortex according to the well known homunculus but it still is important to search for the optimal location to stimulate. This is in order to reduce the possibility of stimulating neuronal tissue negatively influencing the results. While looking for this so called ‘hotspot’ the subject is asked to do the same contraction as he did before and he will do during the main experiment (10% of MVC). Besides the fact that the contraction is used during the main part of the experiment, searching for the hotspot during slight contraction of the muscle lowers threshold. This means the stimulation intensity needed to see clear Motor Evoked Potentials (MEPs) is lower. When stimulating subthreshold an excitatory postsynaptic potential or inhibitory postsynaptic potential may be induced at a motoneuron level in the spinal cord. We will make sure that at the intensity we stimulate no MEP is evoked and therefore we assume that there are no motor units activated. The motor threshold is defined as ‘the lowest TMS intensity capable of eliciting small MEPs. Below motor threshold, no EMG activity is detectable and it is assumed that no motor units are being activated. After finding the ‘hotspot’ searching for the motor threshold (MT) is the next step. During the search for the threshold the subject still has to hold the contraction although some rest will be allowed in between finding the hotspot and searching for the threshold.
To be sure to induce the effects of TMS in the right time window the latency of the MEP should be determined for each individual subject. This is easily done from the data obtained after finding the hotspot and searching for the threshold. All this data analysis is done online and directly during the experiment and will not take any extra time. Rest time of the subject is used to quickly analyze the results. To prevent for accumulating and long lasting effects due to repetitive single pulses an interstimulus interval of at least 5 seconds is used. Next to trials with combined mechanical and TMS stimuli, we will insert trials with only mechanical stimuli to control for possible long term effects of TMS. We will time the TMS to be in the refractory time window between M1 and M2 to conclude about possible modulating effects originating from the human brain.

The mechanically applied stretch to the wrist starts when the subject holds a prescribed time 10% of his/her MVC. The TMS starts later based on predetermined characteristics of the reflex response for each person. Based on the delay of the MEP the interval between M1 and the TMS is determined. TMS is hereby triggered automatically (sample frequency = 5000 Hz), based on this interval. The standard deviation of the delay of the MEP is an important measure for the accuracy of the interval. Expected delay is approximately 15 ms, with an expected standard deviation in the order of tenths of milliseconds.

Study group: sample size justification and inclusion-exclusion criteria.

We will consider the M2 normalized amplitude as primary continuous response variable from matched pairs of study subjects. Prior data indicate that the difference in the response of matched pairs is normally distributed with standard deviation 0.5 (normalized amplitude, Meskers et al. 2010). If we consider a true difference in the mean response of matched pairs of 30% as a function of TMS as relevant, we will need to study minimally 6 pairs of subjects to be able to reject the null hypothesis that this response difference is zero with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. As we found previously that about 20% of the subjects does not show sufficient signal response, we may have to exclude about n=2 subjects after the first set of stretch experiments, before we start the TMS experiments. Thus we will consider n=15 subjects as a safe margin. Inclusion criteria: age between 40-70 years (to be matched with future stroke study population). Exclusion criteria: cardiac pacemakers; any metal implant within the brain; any medical history of general and local neurological disorders, especially epilepsy. All exclusion criteria will be systematically checked by using a questionnaire (see appendix). Any history of orthopedic problems with the upper extremities; current medication that may influence nervous function. Considering the wide variety in possible medication, we will check for eligibility per case (responsible physician: Dr C.G.M. Meskers).

Recruitment & reward of the subjects

Subjects will be recruited by advertisement in the weekly university journals of Leiden (“Mare”) and TU Delft (“Delta”). The subjects will be granted a reward of €30 for their cooperation.

Burden and risk

Application of single pulse TMS has a number of contraindications, i.e. intracranial ferromagnetic material such as aneurysm clips or other implants and a cardiac pacemaker although it is very unlikely to be damaged by TMS. People suffering of epilepsy may be considered having an extra contraindication; nevertheless the risk of inducing seizure is very low. Although the risk of inducing seizure is low a history of seizure, subjects who use
medication that might increase the risk of seizure and subjects which have a family history of epilepsy are excluded. Contra-indications are checked by application of a dedicated questionnaire.

Subjects involved in experiments of therapy using TMS often (10-20%) complain about headache due to muscle tension after the treatment, which is one of the most common reported complaints after TMS. During all the experiments, a physician will be present. Single pulse TMS is considered as a safe and useful tool for investigating the human nervous system. (Wasserman et al. (1998), Kobayashi et al. (2007), Wasserman et al. (2008)

The risks of the reflex measurements are very small. The displacements of the handle are only several centimeters and the motor is safeguarded against larger displacements. The motor and other moving or fragile parts are sufficiently insulated. Prior to participation of the research the researcher will assess the subject’s ability to perform the research.

**Scientific and clinical value.**

Movement disorders of patients after stroke may be explained by lack of reflex adaptation. The current lack of understanding of the functioning of the brain in reflex adaptation makes it difficult to develop the right treatment for patients. With more knowledge about the influence of the brain on the reflex characteristics, specific treatments methods can be developed to regain the right reflex characteristics.
References


Deelnemer informatiebrief betreffende het onderzoek naar de modulatie van the rek reflex van de pols met behulp van Transcranial Magnetic Stimulation.

Datum 22-6-2010, Versie 2.0

Geachte mevrouw/meneer,

U bent gevraagd mee te werken aan een onderzoek dat de rol van de hersenen bestudeert in veranderingen van rek reflex van de pols. In deze brief vindt u uitgebreide informatie over het onderzoek. Mocht u nog vragen hebben, dan kunt u terecht bij de personen die onderaan de brief zijn vermeld.

Achtergrond van het onderzoek
Door reflexen kunnen wij automatisch reageren op onverwachte situaties van buitenaf, zodat we bijvoorbeeld onszelf kunnen beschermen tegen gevaarlijke prikkel of verstoringen. Reflexen dienen ook om verschillende spieren goed te laten samenwerken, zodat twee spieren niet gelijktijdig het tegenovergestelde doen. Het is waarschijnlijk dat reflexen automatisch aangepast worden aan de omstandigheden via het centrale zenuwstelsel (CZS). Deze regeling kan verstoord raken bij schade aan het centrale zenuwstelsel bijvoorbeeld na een beroerte. Een verstoorde regeling kan leiden tot bewegingsstoornissen, zoals spasticiteit. Om dit beter te begrijpen, is het belangrijk te weten hoe en wat de invloed is van het CZS op reflexen.

Transcranial Magnetic Stimulation (TMS) is een goede en veilige manier om delen van het centrale zenuwstelsel te stimuleren. Een voorbeeld hier van is het stimuleren van een deel van de hersenen zodat een bepaalde spier samentrekt. Voordelen van deze techniek zijn dat deze niet invasief is (van buiten het lichaam), geen pijn veroorzaakt en geen vervelend gevoel veroorzaakt.

Doel van het onderzoek
Het onderzoek waar wij uw medewerking voor vragen heeft tot doel te testen of en hoe reflexen worden aangepast door het CZS. Daarvoor moeten we de hersenen gecontroleerd signalen laten sturen die we zelf opwekken zodat we zijn invloed op de rek reflexen kunnen bestuderen.

Wat houdt deelname aan het onderzoek in?
Om rek reflexen op the kunnen weken in de pols zullen de pols spieren snel gerekst moeten worden door de pols te buigen. Het onderzoek wordt uitgevoerd met een apparaat dat aan de Technische Universiteit Delft is ontwikkeld. Het apparaat bestaat uit een elektromotor waaraan een hendel is bevestigd. De motor is verbonden met een computer waarmee de positie van de hendel wordt gestuurd. Uw hand zal worden bevestigd aan de hendel terwijl uw onderarm ondersteund zal worden. De buiging van de pols zal dus automatisch gestuurd worden, u hoeft er niks voor te doen. Spieractiviteit wordt gemeten door middel van plakelektroden die op de onderarm worden gelakt. Van het opnemen van de spieractiviteit merkt u niets. Het onderzoek zal bestaan uit meerdere fases: het bepalen van uw reflexen, het vertrouwd raken met TMS en de juiste positie zoeken om de TMS toe te passen, en tot slot de combinatie van beiden. Om reflexen te kunnen bestuderen, moeten ze goed opgewekt kunnen worden. Bij sommige personen is dit niet goed mogelijk. Van te voren is niet in te schatten wanneer dit het geval is. Als dit zo is, zal het experiment afgebroken worden. U ontvangt dan de helft van de totale vergoeding. Rustpauzes kunnen op verzoek overal worden ingelas.
Het experiment zal in totaal ongeveer 3 uur in beslag nemen waarvan u in totaal ongeveer voor 45 minuten te maken zal hebben met TMS. In totaal zult u ook ongeveer 1 uur een gevraagde taak uitvoeren.

Het onderzoek vindt plaats in het Laboratorium voor Bewegingsanalyse van de afdeling Revalidatie op de begane grond van het LUMC.

**Transcranial Magnetic Stimulation**

Transcranial Magnetic Stimulation (TMS) is een techniek waarmee je van buiten het lichaam hersenweefsel zonder pijnklachten kan stimuleren. Veel processen in het menselijk lichaam worden geregeld door middel van elektrische signalen tussen vele soorten gespecialiseerde cellen. De hersenen zijn een soort centrale computer van al deze processen en bevatten onmiljoenen gespecialiseerde cellen die ook met elkaar communiceren door elektrische signalen. Met behulp van het TMS apparaat (figuur 1) zijn we in staat het deel van de computer dat beweging aanstuurt ‘aan’ te zetten. Door het inschakelen van een klein deel van de centrale computer (de hersenen) kunnen we een beweging opwekken in bijvoorbeeld de duim. Doordat de hersenen de goede eigenschappen hebben om elektrische signalen te geleiden is het mogelijk een deel in te schakelen met behulp van het TMS apparaat dat via magnetisme elektrische stroompjes opwekt. Elke deel van het lichaam kan vanuit een specifiek deel van de hersenen in beweging gezet worden, en dus ook door dat specifieke gebied van buiten in te schakelen met het TMS apparaat. Dit maakt het mogelijk om specifiek een bepaalde spier aan te sturen door het gebruik van TMS op het gebied van de hersenen van waar uit die spier wordt aangestuurd.

![Figuur 1: Een MagStim TMS stimulator](image)

**Risico’s**

De risico's van de metingen zijn klein. De beweging van de pols is slechts enkele centimeters en de motor is beveiligd tegen grotere bewegingen. De motor en andere bewegende en kwetsbare onderdelen zijn voldoende afgeschermd. Vóór deelname aan het onderzoek wordt door de onderzoeker een inschatting gemaakt of u in staat bent het onderzoek te voltooien door het invullen van een korte vragenlijst. Om te kunnen deelnemen, moet u gezond zijn en geen voorgeschreven medicatie gebruiken.

Transcranial Magnetic Stimulation wordt als volledig veilig beschouwd en in het bijzonder de vorm die wij zullen gebruiken gedurende het experiment. Het is vergelijkbaar met het krijgen van een MRI scan in de zin van het magnetisme, al is de dosis met TMS veel lager en de blootstelling korter. Sommige mensen kunnen lichte hoofdpijnklachten overhouden aan de TMS of even wat vreemd horen door het ‘click’ geluid dat te horen is als er gestimuleerd wordt. Het is ook mogelijk dat u een wat vreemd aanvoelende tintelend gevoel heeft op de hoofdhuid tijdens de stimulatie.
Vrijwillige deelname
Uw medewerking aan dit onderzoek is vrijwillig. Als u toestemming geeft aan dit onderzoek mee te doen heeft u te allen tijde (ook tijdens het onderzoek) de vrijheid om op die beslissing terug te komen. U hoeft hiervoor geen verklaring te geven. Als u bereid bent aan het onderzoek mee te doen, zal ter plkke een toestemmingsformulier worden voorgelegd. Deelname aan het experiment zal worden beloond met een vergoeding van 30 euro (in cadeaubonnen).

Vertrouwelijkheid gegevens en betekenis van het onderzoek
We zullen vertrouwelijk met uw gegevens omgaan. Wij zullen er voor zorgen dat niet-bevoegde buitenstaanders geen inzage hebben in uw gegevens. Wanneer het onderzoek gepubliceerd wordt, zal informatie niet op u terug te herleiden zijn.

Onafhankelijke arts
Als onafhankelijke arts zal optreden mw. drs, J.M. van der Krogt, afdeling Revalidatigeneeskunde, LUMC, telefoon 071-5263457

Samenvatting
Meedoen aan dit onderzoek geschiedt volledig vrijwillig. Het staat u geheel vrij om wel of niet mee te doen. Samengevat betekent het dat als u deelneemt:

- u bereid bent mee te werken aan het rek reflex onderzoek waarin metingen aan de pols worden gedaan en TMS toegepast wordt op de hersenen.
- u akkoord gaat met het gebruik van uw gegevens ten behoeve van het onderzoek;
- u zich realiseert dat u geen onderzoeksuitslag voor uzelf kunt verwachten.

Namens de onderzoekers, bij voorbaat zeer hartelijk dank voor uw eventuele medewerking.

Mark van de Ruit, onderzoeker; dr. C.G.M. Meskers, arts
Afdeling Revalidatigeneeskunde en afdeling Neurologie LUMC, tel 071-5264357
Wetenschappelijk onderzoek: Rek reflex modulatie van de pols m.b.v. TMS
Tijdens het experiment zullen verstoringen op de pols toegepast worden door een special ontwikkeld apparaat. In enkele fases van het experiment zal ook TMS stimulatie op de hersenen gebruikt worden om het effect op de reflex respons te bestuderen. De reflex respons kan gemeten worden door het meten van de spier activiteit met behulp van oppervlakte electrode op de onderarm. De proefpersoon zal zitten voor het apparaat dat de verstoringen aanbrengt. Tevens zal er een computer scherm staan waarop terugkoppeling wordt gegeven over de geleverde kracht op de hendel van het apparaat. Hem/haar zal gevraagd worden om een constante maar kleine kracht uit te oefenen tijdens de meeste van de fases van het experiment. Als de TMS gebruikt wordt zal de proefpersoon ook gevraagd worden zo stil mogelijk te zitten om er zeker van te zijn dat de juiste locatie van de hersenen gestimuleerd wordt.

Voor het starten van het experiment zal de proefpersoon gevraagd worden een vragenformulier in te vullen om zeker te zijn dat hij/zij geschikt is voor het ondergaan van TMS. Daar boven op wordt, zowel voor als na het experiment, een calibratie meting gedaan waarin de maximale kracht van de proefpersoon bepaald wordt.

Ik verklaar hierbij, op voor mij duidelijke wijze, mondeling en schriftelijk, te zijn ingelicht over de aard, doel, risico’s en belasting van het onderzoek. Mijn vragen zijn naar tevredenheid beantwoord. Ik heb te allen tijde, dus ook tijdens het experiment, de vrijheid om op deze beslissing terug te komen, zonder dat ik daarvoor een verklaring hoeft te geven.

Hierbij stem ik WEL/NIET* in met deelname aan dit onderzoek. (*doorhalen wat niet van toepassing is)

Indien ik instem, betekent dat het volgende:
- Ik ga akkoord met deelname aan het experiment
- Ik verklaar naar behoren te zijn geïnformeerd over het experiment en eventuele gevolgen.
- Ik ga akkoord met het onder code opslaan van mijn gegevens t.b.v. het onderzoek.
- Ik realiseer me dat ik meedoe aan wetenschappelijk onderzoek en ik geen onderzoeksuitslag voor mijzelf kan verwachten.

Handtekening deelnemer: ___________________ Handtekening onderzoeker: ___________________

Naam: ____________________________ Naam: ____________________________
Leiden, datum: _______________ Leiden, datum: _______________
Datum 22-6-2010, Version 1.0

Transcranial Magnetic Stimulation (TMS) Volwassenen veiligheids screening

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Beantwoord de volgende vragen:

Heeft u ooit:
- Enige bijwerkingen gehad van TMS?  Yes No
- Een epileptische aanval gehad? Yes No
- Een electroencephalogram (EEG) als onderdeel van een medische procedure gehad? Yes No
- Een beroerte gehad? Yes No
- Serieuze schade aan het hoofd gehad? (inclusief operatie)? Yes No

Heeft u enig metal in u hoofd (buiten de mond) zoals operatie clips, of deeltjes door lassen of andere metaalbewerking? Yes No

Heeft u enige geimplanteerde apparatuur zoals een pacemaker, medicijn pompen, of intracardiac lines? Yes No

Heeft u vaak (ten minste 1 keer per maand) last van ernstige hoofdpijn? Yes No

Heeft u ooit een ziekte gehad die schade aan de hersenen heft veroorzaakt? Yes No

Heeft u ooit last gehad van ernstige hart problemen? Yes No

Gebruikt u voorgeschreven medicatie? Yes No

Heeft u recent veel alcohol tot zich genomen? Yes No

Als u een vrouw bent met de leeftijd dat u een kind kan dragen,

Bent u zwanger of zou u zwanger kunnen zijn? Yes No

Heeft iemand in u directe familie last van epilepsie? Yes No

Heeft u ooit andere hersen gerelateerde medische behandelingen ondergaan? Yes No

Wilt u verdure informative over TMS en de daarbij mogelijke risico’s? Yes No

Als u ja heeft geantwoord op één van de bovenstaande vragen, kunt u hier eventuele details kwijt (gebruikt achterzijde indien nodig)

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

HANDTEKENING: _______________ GETUIGE: _______________

DATUM: _______________
Appendix D: EMG/TMS artefact problems

After two subjects were successfully measured having a MT value far below 50% stimulator output at the third subject some problems occurred. It turned out that threshold for this subject was above 50%. This resulted in a large artefact in the EMG making it impossible to identify a MEP. This appendix describes the problem and the problem solving process.

D.1 EMG artefact

When trying to determine the Resting Motor Threshold (RMT) for evoking a MEP, meaning that the muscle is fully relaxed, we discovered that large artefacts where present in the EMG signal while triggering. It is displayed in Figure D1.

Due to the large time window in which this artefact exists it is impossible to clearly identify a MEP plus silent period. After some literature research and getting in contact with some more experienced TMS users we discovered the problem had to do with an impulse response to the high pass filter of the Delsys EMG system. In literature it is advised to use a 1 Hz – 2 kHz filtering (Rossini et al. 1994). Switching to an old ‘basic’ EMG system it was immediately clear that the long decaying artefact was gone although still a large short one existed. Although the artefact was a lot shorter above 55% stimulator output again a disturbing artefact was present and it was still impossible to determine resting motor threshold. Problems also rose in measuring reflex responses. It seemed that due to the high noise level we were unable to get clear reflex responses and stretch duration effect, probably being result of normalizing to background EMG which included more noise while measuring with this system.

Comparing to literature the artefact amplitude with the basic EMG system still is a main factor of concern. As it is known that electronics nearby could also disturb the EMG signal measured we removed all electronics. This had a small effect on the ‘artefact threshold’. As we need intensities above 55% to get a MEP in resting state we are unable to determine motor threshold and find the best spot to stimulate.

While using the Delsys system with a 10% MVT force applied by the test subject we were sometimes able to clearly get a MEP but when reducing stimulation intensity it was impossible to distinguish MEPs from background EMG. MEP identification was mainly done on basis of the force signal although in this way we are unable to determine MEP onset which is very important in the rest of the experiment. It was therefore that we included the
measurement with the muscle in resting state, to be more sure about the right spot to stimulate on.

D.2 Solving the problem
A few possibilities existed to get rid of the artefact:

- Change coil: We can try to borrow another coil and use it. I was able to get one from people I met during my internship. We would then get the bended figure of 8 coil which fits more closely to the head. The current film coil makes use of a thin air film between the copper wires to cool them during long TMS sessions to prevent overheating. As a consequence the distance between the copper wires is increased and the magnetic field less strong. This forces us to use higher stimulation intensities than strictly necessary when using a slight bended figure-8 coil.

- Change coil orientation: Coil orientation is very important for threshold. It has to do with the induced current direction with respect to fiber orientation of axons. Most effective way of stimulation would be with the induced currents in posterior-anterior direction. I tried it a few time but it is very difficult with the current coil because of the lack of possibility to move freely.

- Other EMG system: We tested the TMSi system and we saw fewer artefacts. Nonetheless the signal was floating and movement artefacts from the wires were clearly present. Besides that, a problem could be synchronization of the data although that seems to be solvable with the available AUX ports.

- We also can get back to the original situation in which we use the Delsys system without trying to determine RMT. In the first 2 subjects two showed the same pattern with a stimulation intensity of 35% without being sure if that is just below AMT. In the case that we are far below AMT we induce IPSPs only on a cortical level giving more evidence for the existence of a transcortical pathway. We then purely activate a lot of inhibitory circuits on a central level without having any influence on the spinal motoneuron pool. If we actually were just below threshold then we just were lucky but then there is some evidence for supraspinal influences because of the nice excitatory-inhibitory influences on M2.

Concluding: Solving the problem is difficult but we could not imagine that we are not able to get clear MEPs without a large artefact. There were so many groups which encountered this problems and apparently were able to solve it, so why can’t we? All the proposed solutions were however not suitable or difficult to arrange and therefore we first decided to change EMG system, one with a lower high pass filter. This EMG system seemed to result in less sensitivity for the artefacts but stretch reflex responses came out worse. This was likely due to increased background noise, and while the EMG is normalized to background this resulted in strange reflex responses.

After getting back to the Delsys EMG system and keep on trying to remove the artefact we quickly discovered that the problem of an impulse response to the high pass filter was present again. The conclusion is that we should implement another EMG system are change the internal filter characteristics of our delsys system. There was, unfortunately, no time to do this. Partly because our MagStim TMS device was broken.

References:
Appendix E: Detailed results

In order to test and set up the measurement protocol some pilot experiments were performed. The results of these pilot experiments are discussed in this appendix. Additionally detailed results of the full measurement protocol are included.

E.1 Pilot experiments
To test the programs written for this study and get used to the measurement protocol some pilot experiments were performed. Separately the stretch reflexes and TMS were tested.

Stretch reflex testing
Before doing the main experiment practicing of positioning the electrodes and determine the stretch characteristics was required. Therefore 6 subjects were asked to come in to perform a short test on. As Schuurmans et al. (2009) reported that at short stretch duration he was not able to reach the applied stretch velocity we had to check minimal stretch duration possible. While he also showed that the M1 response depends on stretch velocity we had to choose one single stretch velocity inducing a clear M1 response. (Detailed description in Appendix A.3).

(Results on following pages)

Conclusions
The six subjects tested did all show the expected behavior and not much difference was observed between a velocity of 1 and 1.5 rad/s. It would be better to test all subjects with both velocities and compare M1 areas. However this was too demanding for the subjects which already had difficulties completing one session. We therefore cannot reproduce the results of Schuurmans et al. (2009) showed also enough data is obtained to make it reliable.

The main reason to not choose higher speeds than 1.5 rad/s is that it does not influence M2 area where we are interested in. Using higher velocities would make the stretch amplitudes uncomfortable high to reach sufficient duration. A larger M1 than obtained with 1.5 rad/s is also not necessary. At the end we decided to use 1.5 rad/s during the main experiment with as main reason that a slightly higher M1 is present, likely being result of more motoneurons recruited.
E.1.1 Subject 1

Used stretch velocity = 1.5 rad/s
Stretch durations: 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ms
E.1.2 Subject 2
Used stretch velocity = 1 rad/s
Stretch durations: 15, 30, 45, 60, 75, 90, 105, 120, 135 and 150 ms
E.1.3 Subject 3

Used stretch velocity = 1.5 rad/s
Stretch durations: 5, 7.5, 10, 20, 40, 50, 60, 70, 80 and 90 ms

Stretch Duration effect on M2 area

Stretch Duration: 5 ms

Stretch Duration: 7.5 ms

Stretch Duration: 10 ms

Stretch Duration: 20 ms

Stretch Duration: 40 ms

Stretch Duration: 50 ms

Stretch Duration: 60 ms

Stretch Duration: 70 ms

Stretch Duration: 80 ms

Stretch Duration: 90 ms
E.1.4 Subject 4

Used stretch velocity = 1 rad/s
Stretch durations: 11.25, 15, 30, 45, 60, 75, 90, 105, 120 and 135 ms

Stretch Duration effect on M2 area
E.1.5 Subject 5

Used stretch velocity = 1.5 rad/s
Stretch durations: 7.5, 10, 20, 30, 40, 50, 60, 70, 80 and 90 ms

Stretch Duration effect on M2 area

Stretch Duration: 7.5 ms
Stretch Duration: 10 ms
Stretch Duration: 20 ms
Stretch Duration: 30 ms
Stretch Duration: 40 ms
Stretch Duration: 50 ms
Stretch Duration: 60 ms
Stretch Duration: 70 ms
Stretch Duration: 80 ms
Stretch Duration: 90 ms
E.1.6 Subject 6

Used stretch velocity = 1.5 rad/s
Stretch durations: 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ms

Stretch Duration effect on M2 area

Stretch Duration: 10 ms
Stretch Duration: 20 ms
Stretch Duration: 30 ms
Stretch Duration: 40 ms
Stretch Duration: 50 ms
Stretch Duration: 60 ms
Stretch Duration: 70 ms
Stretch Duration: 80 ms
Stretch Duration: 90 ms
Stretch Duration: 100 ms
TMS testing
The pilots with TMS had as main goal to get used to using the new device, coil handling and the neuronavigation system. I visited several training sessions to get used to using the supplied software. As we decided to use a general MRI scan the head model generation had only to be performed once. Again several subjects were asked to come in. It became clear soon that repositioning the coil to a marked spot was not that straightforward as one might think. This also has to do with the limitation in moving the coil due to a badly designed coil stand. Therefore we borrowed a dummy body, used for calibrating a MRI system, to practice positioning of the coil. It allowed us to get more feeling for how the positioning worked and what all the markers on the screen mean. Results of the pilot tests are not really interesting to show. The most difficult is distinguishing a MEP from background EMG as shown in the figure below.

![Raw EMG trial1](image-url)

Search for the MEP….(red line = trigger from Magstim)
E.2 Detailed results overview

On the following pages all the results of the main experiment are shown. The first four subjects presented are the ones used in the scientific paper. All other were not included due to problems encountered during measuring. Description of obtained results is done in between all the figures.

Subject five to nine are not included in the scientific paper but however presented, possibly being useful in future research.

Due to late adjustments on the paper there was no time to change all the plots presented below. Therefore a translation of used axis description to paper used symbols

\[ A_{M2} \quad = \quad \text{Normalized M2 [-]} \]
\[ T_{Stretch} \quad = \quad \text{Stretch Duration [s]} \]
\[ T_{effect} \quad = \quad \text{Time of TMS arrival [s]} \]
### E.2.1 Subject 1

Age = 43
Gender = M
Arm= right

**Notes:**
No clear distinguishable M1 and M2 of the reflex response

Average of 10 MEPs for FCR: (first MEPref was wrong!)
MEP onset = 0.021 s
MT = 37%

![MEP graph](image)

![Force graph](image)

Check of stretch reflex responses (average of 10 trials):

![Angle graph](image)

![Velocity graph](image)

![Average EMG graph](image)
Stretch Reflex Check
Velocity: 1.5 rad/s / Amplitude: 0.12 rad / 80 ms

Amplitude: 0.12 rad

Velocity: 1.5 rad/s

Average EMG:

Stretch duration vs Normalized M2 Amplitude and EMG responses (next page):

Stretch Duration effect on M2

Normalized M2

Stretch duration [s]
Used stretch duration for further analysis: 45 ms

TMS influence on M2 amplitude (relative to M2 amplitude without TMS at 45 ms stretch duration):
TMS on: 35 ms

TMS on: 40 ms

TMS on: 45 ms

TMS on: 50 ms

TMS on: 55 ms

TMS on: 60 ms

TMS on: 65 ms

TMS on: 70 ms

TMS on: 75 ms

TMS on: 80 ms

Grey line is the average of 10 responses without TMS; Black lines with TMS

Background EMG during experiment combining reflexes and TMS
Values over the ten different trials measured in percentage relative to no TMS trials. No trend is visible which possibly induced by fatigue. (Time = 0 is No TMS)
E.2.2 Subject 2

Age = 53
Gender = M
Arm= right

Notes:
Subject had very clear MEPs but a high motor threshold which was difficult to find. This may indicate wrong position, although no side effects were observed. Had severe difficulties with getting coil back in place, possibly badly influencing the results (Accuracy ± 10 mm / 5°). Reflexes showed no M1 in first instance while at the final experiment in some cases a M1 showed up.

Average of 10 MEPs for FCR:
MEP onset = 0.017 s
MT = 55%

![Graphs of MEP and Force](image-url)

Check of stretch reflex responses (average of 10 trials):

![Graphs of Angle, Velocity, and Average EMG](image-url)
Stretch Reflex Check
Velocity: 1.5 rad/s / Amplitude: 0.12 rad / 80 ms

Stretch duration vs Normalized M2 Amplitude and EMG responses (next page):

Stretch Duration effect on M2
Used stretch duration for further analysis: 35 ms

TMS influence on M2 area

TMS influence on M2 amplitude (relative to M2 amplitude without TMS at 35 ms stretch duration):
Grey line is the average of 10 responses without TMS; Black lines with TMS

Background EMG during experiment combining reflexes and TMS
Values over the ten different trials measured in percentage relative to no TMS trials. No trend is visible which possibly induced by fatigue. (Time = 0 is No TMS)
E.2.3 Subject 3

Age = 51
Gender = F
Arm= right

Notes:
Very clear reflex response and MEPs. Nice threshold value.
Different stretch durations with reflex check compared to earlier subjects!

Average of 10 MEPs for FCR:
MEP onset = 0.017 s
MT = 36%

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Check of stretch reflex responses (average of 10 trials):

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Stretch Reflex Check
Velocity: 1.5 rad/s / Amplitude: 0.12 rad / 80 ms

Amplitude: 0.12 rad

Stretch duration vs Normalized M2 Amplitude and EMG responses (next page):

Stretch Duration effect on M2

Normalized M2 [-]

Stretch duration [s]
Used stretch duration for further analysis: 30 ms

TMS influence on M2 amplitude (relative to M2 amplitude without TMS at 30 ms stretch duration):
Grey line is the average of 10 responses without TMS; Black lines with TMS

Background EMG during experiment combining reflexes and TMS
Values over the ten different trials measured in percentage relative to no TMS trials. No trend is visible which possibly induced by fatigue. (Time = 0 is No TMS)
E.2.4 Subject 4

Age = 59
Gender = M
Arm= right

Notes:
Difficult to find good spot. Subject didn’t feel the responses although they were clearly visible. ECR electrode was ‘crushed’ between the skin and fixation of the elbow.

Average of 10 MEPs for FCR:
MEP onset = 0.015 s
MT = 55%

![Graph of MEP (avg of ten responses)](image)

![Graph of Force (avg of ten responses)](image)

Check of stretch reflex responses (average of 10 trials):

![Graph of Amplitude and Velocity](image)
Stretch Reflex Check
Velocity: 1.5 rad/s / Amplitude: 0.12 rad / 80 ms
Amplitude: 0.12 rad

Velocity [rad/s]

Velocity: 1.5 rad/s

Average EMG [-]

Stretch duration vs Normalized M2 Amplitude and EMG responses (next page):

Stretch Duration effect on M2

Normalized M2 [-]

Stretch duration [s]
Used stretch duration for further analysis: 35 ms

TMS influence on M2 amplitude (relative to M2 amplitude without TMS at 35 ms stretch duration):
Grey line is the average of 10 responses without TMS; Black lines with TMS

Background EMG during experiment combining reflexes and TMS
Values over the ten different trials measured in percentage relative to no TMS trials. No trend is visible which possibly induced by fatigue. (Time = 0 is No TMS)
E.2.5 Subject 5

Age = 52
Gender = M
Arm = right

Notes:
No clear MEPs found, subject didn’t feel any effect of TMS being applied to the motor cortex. Experiment was stopped after studying the stretch duration effect.

Not performed:
Average of 10 MEPs for FCR:
MEP onset =
MT =

Check of stretch reflex responses (average of 10 trials):
Stretch Duration effect on M2

Used stretch duration for further analysis: 45 ms
Not performed: TMS influence on M2 amplitude
**E.2.6 Subject 6**

Age = 58  
Gender = M  
Arm= right

Notes:  
No MEPs found, nonetheless experiment was completed. Subject was not aware of any effect of the TMS on the arm, wrist or thumb.

Average of 10 MEPs for FCR:  
MEP onset = 0.015  
MT = 40%

[EMG and Force plots]

Check of stretch reflex responses (average of 10 trials):

**Stretch Reflex Check**  
Velocity: 1.5 rad/s / Amplitude: 0.0675 rad / 45 ms  
Amplitude: 0.0675 rad  
Velocity: 1.5 rad/s  
Average EMG: [inputs]
Stretch Reflex Check
Velocity: 1.5 rad/s / Amplitude: 0.12 rad / 80 ms

Stretch duration vs Normalized M2 Amplitude and EMG responses (next page):

Stretch Duration effect on M2
Used stretch duration for further analysis: 30 ms

TMS influence on M2 amplitude (relative to M2 amplitude without TMS at 30 ms stretch duration):

![Graph showing TMS influence on M2 area over time of TMS arrival.](image)

Normalized M2 area varies with time of TMS arrival, showing peak influence at specific intervals.
Grey line is the average of 10 responses without TMS; Black lines with TMS
Values over the ten different trials measured in percentage relative to no TMS trials. No trend is visible which possibly induced by fatigue. (Time = 0 is No TMS)
E.2.7 Subject 7

Age = 52
Gender = F
Arm= right

Notes: Second test with slight changed protocol. Decided to first search for resting motor threshold to be more sure about stimulation spot and then decrease stimulation intensity to find AMT. Big artifact appeared in EMG while measuring in resting state, discovered in subject which came in before. We thought that we were able to solve it by removing the high pass filter and make use of the old EMG system. Nevertheless the artifact appeared again and we had to estimate MEP onset and threshold depending on force responses.

Average of 10 MEPs for FCR:
MEP onset = 0.015
MT = 63%

![Graph of MEP and EMG](image1)

![Graph of Force and Time](image2)

Check of stretch reflex responses (average of 10 trials):

![Graph of Angle and Velocity](image3)
Stretch Reflex Check
Velocity: 1.5 rad/s / Amplitude: 0.12 rad / 80 ms

Stretch duration vs Normalized M2 Amplitude and EMG responses (next page):

Stretch Duration effect on M2

Normalized M2 [\text{\textbf{\text{-}}}] vs Stretch duration [\text{\textbf{\text{s}}}]
Used stretch duration for further analysis: 35 ms
TMS influence on M2 area (relative to M2 amplitude without TMS at 35 ms stretch duration):

![Graph showing TMS influence on M2 area](image-url)
Grey line is the average of 10 responses without TMS; Black lines with TMS

Background EMG during experiment combining reflexes and TMS

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Values over the ten different trials measured in percentage relative to no TMS trials. No trend is visible which possibly induced by fatigue. (Time = 0 is No TMS)
E.2.8 Subject 8

Age = 46
Gender = F
Arm = right

Notes:

Average of 10 MEPs for FCR:
MEP onset = 0.015 s
MT = 52%

Check of stretch reflex responses (average of 10 trials):
Stretch Reflex Check
Velocity: 1.5 rad/s / Amplitude: 0.12 rad / 80 ms

Stretch duration vs Normalized M2 Amplitude and EMG responses (next page):
Used stretch duration for further analysis: 45 ms

TMS influence on M2 amplitude (relative to M2 amplitude without TMS at 45 ms stretch duration):
Grey line is the average of 10 responses without TMS; Black lines with TMS

Background EMG during experiment combining reflexes and TMS
Values over the ten different trials measured in percentage relative to no TMS trials. No trend is visible which possibly induced by fatigue. (Time = 0 is No TMS)
E.2.9 Subject 9

Age = 58
Gender = F
Arm= right

Notes: Experiment lasted for almost 4 hours due to artifact handling. Finally performed experiment at 50% SO to be sure that no artifact was present. MT determined on force data! Also experienced difficulties with getting a clear M2 stretch duration effect. Switched back to delsys EMG system 20-450 Hz! Choice of fixed stretch duration was based on the 20 reflexes not the 100!

Average of 10 MEPs for FCR:
MEP onset = 0.015 s
MT = 50%

![MEP Graph](image)

![Force Graph](image)

Check of stretch reflex responses (average of 10 trials):

![Angle Graph](image)

![Velocity Graph](image)

![Average EMG Graph](image)
Stretch Reflex Check

Velocity: 1.5 rad/s / Amplitude: 0.12 rad / 80 ms

Stretch duration vs Normalized M2 Amplitude and EMG responses (next page):

Stretch Duration effect on M2
Used stretch duration for further analysis: 45 ms

TMS influence on M2 area (relative to M2 amplitude without TMS at 45 ms stretch duration):

![TMS influence on M2 area graph](image)
Grey line is the average of 10 responses without TMS; Black lines with TMS

**Background level**

**Background EMG during experiment combining reflexes and TMS**
Values over the ten different trials measured in percentage relative to no TMS trials. No trend is visible which possibly induced by fatigue. (Time = 0 is No TMS)
Appendix F: Summary of measurement protocol

In this appendix a short overview of the measurement protocol is given. Additionally the data processing files used are explained.

F.1 Protocol checklist

Checklist before start
- Chair is in position
- Position Magstim Stimulator and connect coil. Plug it in.
- Connect trigger in and out cables to DAQ box
- Position Neuronavigation stand and turn computer on
- Connect trigger out to Neuronavigation system
- Position subject feedback and experiment leader monitor
- Turn PoPe computer on.

Checklist preparations with subject
- Verbally explain the protocol
- Answer questions
- Sign informed consent
- Adjust chair and monitor positions according to subject’s needs
- Clean and scrub the skin
- Place EMG electrodes
- Fix the lower arm
- Place ‘head’ markers
- Initialize neuronavigation

Checklist during measurements
- Neuronavigation is initialized and working
- MVC measurement (repeat three times!) \(\rightarrow\) Check EMG response!
- ‘Hand’ triggered TMS to find ‘hot spot’ and RMT
- Automatic triggered TMS to find active motor threshold (AMT)
- Identify MEP characteristics (f.e. onset)
- Run test session of reflexes
- Identify stretch reflex characteristics
- Stretch duration effect
- Check TMS positioning
- Supply characteristics of reflexes
- Perform main session

For detailed description see Appendix A.2

F.2 Data processing details

For future students interested in the obtained data and data analysis scripts a separate CD is supplied. This CD contains all the data, analyzing scripts and a detailed description of which files were used in every step of analysis.
Appendix G: Recommendations and Future Work

Besides studying the effect of TMS on the stretch reflex of the human flexor wrist muscle this study also had as a goal to set up measurements with TMS and learn how to use it. We succeeded in both although we experienced a lot of problems; learning a lot about TMS and the human nervous system. In this Appendix I will write about my experiences during this study and do some recommendations considering the current method and about using TMS in general. Finally I will focus on the possibilities for future work in the lab.

G.1 Recommendations in using TMS

As TMS is a powerful method in exciting human tissue care has to be taken in using it. One should be aware of the dos and don’ts with TMS. Also extensive research is needed about inclusion and exclusion criteria during an experiment with TMS. Everyone needs a period of practicing with a more experienced TMS user to know how to handle subjects from which most of them are not directly enthusiastic about this method. A broad literature study and internship for practical experience is therefore recommended. Experiencing TMS yourself is also worth it, as it enhances understanding of your subjects. I experienced myself how valuable such an internship is. Besides subject handling and theoretical knowledge about TMS also equipment handling is very important. Stimuli above 80% can be unpleasant to your subject. It is advised to play first with it on a dummy or someone who knows more about TMS. Because of its risks the user should be sure about his capabilities in handling the TMS coil, software and complicated questions of the subject.

G.2 Recommendations for the current study

During the current study we tackled a lot of problems which are described in other appendices. When using currently used protocol it is advised to extensively test it including pilots mimicking real measurements. Making a more intuitive Graphical User Interface (GUI) showing MEP, reflex responses and possibilities to look back in the results will speed up the experiments.

As the onset of the MEP is determined by visual inspection it introduces uncertainty in the timing of TMS. An automatic algorithm for determining onset may be an advantage in that a measure could be given to the uncertainty in timing the TMS pulses.

Considering the results it would be worth to check for the possibility to repeat the obtained results several times in the same test person. This increases the reliability of the observed effects which are quite uncertain due to the high variance observed in the results. The same holds for the stretch duration effect. We performed the stretch duration test several times and although it was repeatable over the measurements care should be taken with choosing the fixed stretch duration during applying TMS.

An important assumption during this study was constant background EMG over all trials because of the steady task to perform for the subjects every trial. Therefore we rejected none of the trials. We confirmed that in almost 90% of the trials background EMG was constant but rejecting trials with bad background EMG may improve the results.

The most important step may be to do the experiment with a fixed timing of reflex and TMS, taking the time points at which the largest inhibition and excitation is observed. If we combine these timing intervals with a stretch inducing either minimal or maximal M2 size we would expect a larger excitation and larger inhibition compared to the fixed stretch duration chosen in this study.
G.3 Future work

After a lot of research to the origin of the long latency stretch reflex response it should be concluded that it is not mediated by a single spinal or corticospinal pathway but that both these and many other pathways are involved. The question now rises how the cortex has influence on the reflex; is it by changing the synaptic strength, lowering activation threshold of a motoneuron or a complete other way. As Stienen et al. (2006) showed that there exists a balance between synaptic strength and effect of mechanisms like presynaptic inhibition a first step may be to use rTMS to check for these effects. If TMS is able to change the synaptic strength this should be visible in the reflex responses for a longer time after TMS is applied. Care should be taken in interpreting the results of such an experiment as it is not directly straightforward that due to en enhance effect of presynaptic inhibition the reflex response in inhibited. Many other pathways may play an important role. Before doing such a study one should be sure about the effects induced by a used type of rTMS, being dependent on many stimulation parameters. As an addition to the current study one could focus on the observed inhibition pattern in between 35-55 ms as obtained in the current study. A higher time resolution may support current results or show other interesting effects likely representing change of state of the motoneuron pool. A first and important step in checking the currently developed protocol is to use suprathreshold TMS instead of subthreshold to check earlier obtained results (e.g. Lewis et al. 2004).

References:
