Assessing cortical involvement in stretch reflex response using subthreshold TMS

Master of Science Thesis

For the degree of Master of Science in Biomedical Engineering at Delft University of Technology

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Preface

Bearing in mind the saying ‘choose a thesis topic you really enjoy studying’, I visited Alfred Schouten in February 2011. We discussed some interesting possibilities, but I remember a guy walking in and delivering his Master’s thesis. Suddenly, another possibility was brought up; stimulating the human brain with transcranial magnetic stimulation (TMS) and studying its effect on stretch reflexes. Being familiar with TMS from previous courses at Utrecht University, my interest was triggered.

That guy walking in was Mark van de Ruit, and I had the privilege to repeat and elaborate on his research. During Mark’s work at Leiden University Medical Centre (LUMC), problems with electromyography (EMG) did hinder measurements. New EMG equipment was already scheduled and arrived before I started. It offered new possibilities, as high density electrode surfaces could be recorded. These surfaces provide spatial information of muscle activity, besides temporal information measured with conventional EMG. To get knowledge about this EMG technique, my graduation project started with a literature survey about the use of high density EMG (HD-EMG). The combination of HD-EMG with TMS and stretch reflexes was the specific topic. Several data analysis techniques already existed, but the muscle activation of TMS and stretch reflexes proved to be not suitable for single motor unit analysis. However, using the spatial component of the data, activation patterns could still be extracted. Also, using HD-EMG data, every electrode configuration could be constructed afterwards. This gave the opportunity of repeating Mark’s experiment by reconstructing a bipolar bar electrode configuration while generating more data for a broader perspective on muscle activation.

Before I could set up my own experiment, experience had to be gained with conducting research in general and the use of TMS in particular. I was warmly welcomed at the University of Birmingham (UK) by Michael Grey, Jonny Mathias and Mark van de Ruit, now pursuing his PhD over there. With their help, my enthusiasm about conducting research grew and I returned to Delft and Leiden with knowledge about the nuts and bolts of TMS.

The research conducted at the LUMC in the past months resulted in the manuscript of which this is the preface. I would like to thank Michael Grey and especially Mark van de Ruit for the time in Birmingham and all help afterwards. Mark provided an excellent base to build my own research on. Also, thanks to Prof. Dr. Van Hilten and Gijs van Velzen (Dept. of Neurology, LUMC) for allowing me to use their TMS equipment. Of course, without supervisors no thesis, so thank you Jurriaan, Carel and Alfred for our discussions and your ability to dig through my earlier attempts to write down the research. And last but not least, no day at the office without a 4 o’clock Cup-a-Soup break, with everyone to share the latest troubles and small research victories. It was a blast!

Thijs Perenboom

January 2013
Part I

Scientific paper
Assessing cortical involvement in stretch reflex response using subthreshold TMS

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Abstract In movement control cortical signals are integrated with afferent feedback from reflexes. Disturbed integration is suggested to underlie many movement disorders. Cortical and afferent signals can integrate in the spinal cord and at supraspinal centres, though the exact location and mechanism of integration are unknown yet. The goal of this study is to assess the cortical involvement during the stretch reflex response. Mechanically induced stretches of the muscle flexor carpi radialis were combined with subthreshold transcranial magnetic stimulation (TMS, 97\% of active motor threshold) at interstimulus intervals ranging from 35 to 80 ms. Muscle response was measured using high-density electromyography (EMG), providing additional spatial muscle activation patterns. Magnitude of resulting EMG reflex activity, i.e. short (M1, 20-50 ms) and long (M2, 55-100 ms) latency reflex responses were compared to stretch-only trials. Subthreshold TMS was found to significantly increase the stretch evoked EMG response (p < 0.001) when TMS pulses were timed to arrive at the muscle in the time window of the M2 response. Absence of facilitation of the spinally mediated M1 response indicates that integration of cortical and afferent feedback signals in M2 occurs at a supraspinal level. Spatial muscle activation patterns of suprathreshold TMS were consistent over trials, while spatial patterns due to stretch reflexes were less consistent. Spatial patterns of combined trials are therefore not conclusive about the mechanism of integration.
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>$A_{M1}$</td>
<td>Response size of M1</td>
</tr>
<tr>
<td>$A_{M2}$</td>
<td>Response size of M2</td>
</tr>
<tr>
<td>$A_{stretch}$</td>
<td>Amplitude of stretch perturbation [rad]</td>
</tr>
<tr>
<td>CMAP</td>
<td>Compound muscle action potential</td>
</tr>
<tr>
<td>CMCT</td>
<td>Central motor conduction time [s]</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography [-]</td>
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<tr>
<td>EPSP</td>
<td>Excitatory postsynaptic potential</td>
</tr>
<tr>
<td>FCR</td>
<td>Flexor carpi radialis</td>
</tr>
<tr>
<td>HD-EMG</td>
<td>High density electromyography</td>
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<tr>
<td>IPSP</td>
<td>Inhibitory postsynaptic potential</td>
</tr>
<tr>
<td>ISI</td>
<td>Interstimulus interval</td>
</tr>
<tr>
<td>M1</td>
<td>Short latency stretch reflex response component (20-50 ms)</td>
</tr>
<tr>
<td>M2</td>
<td>Long latency stretch reflex response component (55-100 ms)</td>
</tr>
<tr>
<td>MEP</td>
<td>Motor evoked potential</td>
</tr>
<tr>
<td>MT</td>
<td>Active Motor threshold [% maximum stimulator output]</td>
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<tr>
<td>MVT</td>
<td>Maximum voluntary torque</td>
</tr>
<tr>
<td>PMCT</td>
<td>Peripheral motor conduction time [s]</td>
</tr>
<tr>
<td>RMS</td>
<td>Root mean square</td>
</tr>
<tr>
<td>subTMS</td>
<td>Subthreshold transcranial magnetic stimulation</td>
</tr>
<tr>
<td>TES</td>
<td>Transcranial electric stimulation</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial magnetic stimulation</td>
</tr>
<tr>
<td>$T_{MEP}$</td>
<td>Time subTMS pulse is aimed to arrive at muscle [s]</td>
</tr>
<tr>
<td>$T_{peak}$</td>
<td>Time of maximum response size [s]</td>
</tr>
<tr>
<td>$T_{stretch}$</td>
<td>Duration of stretch perturbation [s]</td>
</tr>
<tr>
<td>$V_{stretch}$</td>
<td>Velocity of stretch perturbation [rad/s]</td>
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</table>
Introduction

Human movement is controlled by the central nervous system through voluntary action within the motor cortex and reflexive actions. Reflex adaptation to changing environment and task is at the core of daily life activities. Disorders like Parkinson’s Disease show disturbed reflexes (Jankovic, 2008) and a lack of reflex adaptation was found in patients after stroke (Meskers et al., 2009). Understanding mechanisms of reflex control provides insight into movement disorders following supraspinal nerve lesions.

Stretching upper limb muscles induces a reflex response which shows two bursts of activity in the electromyogram (EMG). The first, short latency, response component (M1) is mediated by a monosynaptic spinal reflex loop via the muscle spindles and Ia afferents. For the second, long latency, response peak (M2), there is no consensus about the pathways involved. Slower type II afferents appear to be involved (Grey et al., 2001), as well as cutaneous afferents (Corden et al., 2000). The notion that task instruction affects M2 response (Rothwell et al., 1980) combined with ipsilateral motor response after cortical stimulation in participants with congenital mirror movements (Capaday et al., 1991) led to the idea of the existence of a transcortical pathway in long latency reflex modulation. Thus, M2 response is not ascribed to be caused by a single mechanism, but rather is considered a compound response (Lourenco et al., 2006, Schuurmans et al., 2009, Meskers et al., 2010).

Cortical signals and afferent reflexive feedback can integrate in the spinal cord and at supraspinal centres. The exact location and mechanism of integration are unknown yet. Output of the spinal motoneuron pool is adjusted depending on the input. Subpopulations of activated motoneurons depend on stimulation type, TMS activates other motoneurons than radial nerve stimulation (Morita et al., 1999). Stretch reflexes (Calancie and Bawa, 1985) and magnetic stimulation (Bawa and Lemon, 1993) recruit motor units (MUs) in the same order as happens with voluntary activation. From single motor unit recordings there is evidence of overlap in targeted motoneurons by TMS and stretch reflexes (Palmer and Ashby, 1992). However, to what extent cortical and reflexive feedback signals converge onto the same subpopulations of motoneurons is unclear and spatial recordings of the muscle might provide new insights.

With transcranial magnetic stimulation (TMS) the motor cortex can be stimulated to provide controlled cortical activation, opposed to uncontrollable voluntary activation. TMS evokes a so called motor evoked potential (MEP) in a targeted muscle, when stimulation intensity is above active motor threshold (MT). TMS also provides the opportunity to interfere with stretch reflexes to study cortical involvement in reflex control. A non-linearly facilitated M2, but not M1, response was found when combining TMS with stretch perturbation compared to applying both stimulations separately (Palmer and Ashby, 1992, Petersen et al., 1998, Lewis et al., 2004, Pruszynski et al., 2011), indicating a supraspinal effect in the stretch reflex. However, these studies used suprathreshold TMS, and observed increase in response size might therefore contain not only a cortical component but includes effects of afferent feedback pathways as well. To distinguish between the contribution of these pathways and the cortical component is not possible, as contributions integrate in the spinal cord. Increased cortical excitability due to the stretch reflex could also lead to an increased MEP size, which might cause the facilitation of M2 response without a transcortical loop (Van Doornik et al., 2004).

Magnetic stimulation below motor threshold does not elicit a direct response in a resting muscle, but might alter excitability of spinal motoneurons (Petersen et al., 2003). On a cortical level, subthreshold TMS (subTMS) is observed to activate inhibitory and excitatory circuits depending on stimulation intensity (Kujirai et al., 1993). Just below threshold an excitatory postsynaptic potential (EPSP) is elicited, while an inhibitory potential (IPSP) is induced at
lower intensities (Ziemann et al., 1996). Inhibition of the long latency reflex component of the leg muscle tibialis anterior was achieved with subTMS at 90% motor threshold (Van Doornik et al., 2004). This provided proof of a transcortical pathway as no descending corticospinal volleys were present in the spinal cord (Di Lazzaro et al., 1998) and thus the inhibitory action was due to interaction at a cortical level.

Muscle response measured with conventional surface EMG provides temporal but not spatial information about the stretch reflex and magnetic stimulation. Using high density EMG (HD-EMG) also spatial data is recorded and with the recorded data different electrode configurations can be constructed (Zwarts and Stegeman, 2003, Farina et al., 2004). The compound muscle action potential (CMAP) generates a specific spatiotemporal ‘fingerprint’ (Van Dijk et al., 2009) allowing comparison of the effect of different stimulations on the muscle. Generated sequences of amplitude plots (so called CMAP movies) provide information about spread and timing of activation of the muscle. Consistency of muscle activation patterns between trials and stimulation types is used to study recruitment patterns within the muscle during stretch reflex, TMS and combined input.

The goal of this study is to assess the cortical involvement during the stretch reflex response. Main objective was quantification of the change in stretch reflex response of the forearm muscle flexor carpi radialis (FCR) as function of the relative timing of subthreshold TMS (97% MT). A temporal TMS effect profile shows cortical involvement, and especially its lag time, during the stretch reflex. By choosing a fixed stretch profile, equal afferent input is assumed and subTMS might inhibit or facilitate the stretch reflex. Muscle response was recorded with high density EMG and used to quantify the effect of subTMS on short and long latency response components of the reflex. It is hypothesized that motoneuron pool excitability is affected by subTMS and thereby influences M2 response size compared to reflexes applied without cortical stimulation. It is expected that the muscle is activated by stretch reflexes and TMS either similar, with an overlap in motoneurons, or spatially different.

Methods

Participants

Twelve healthy participants (mean age 49±13 years, range 23-65, 4 women) participated in this experiment. Participants had no history of neurological or neuromuscular disorders, nor any contraindications to TMS as checked by a questionnaire (based on Keel et al., 2001). Participants gave written informed consent prior to the experiment. This study obtained approval of the Medical Ethics Committee of the Leiden University Medical Centre.

Experimental setup

Manipulator

Ramp-and-hold perturbations were applied by a mechanic wrist angle manipulator (Schouten et al., 2006). Angular perturbations determine stretch amplitude ($A_{\text{stretch}}$, maximum extension) and stretch velocity ($V_{\text{stretch}}$, maximum ramp velocity) of the muscle, causing a stretch with a defined duration ($T_{\text{stretch}}$). Perturbation of the wrist was in extension direction, evoking stretch reflexes in flexor carpi radialis (FCR) muscle. Torque around the wrist was measured by a force transducer in the handle. Visual feedback about torque level (2 Hz low pass filtered) was provided to participants via a screen. Participants were seated in a height adjustable chair with head support, their right arm fixed in elbow and wrist supports.
Transcranial Magnetic Stimulation (TMS)

Stimuli to the motor cortex were delivered via a Magstim Rapid² system (Magstim Co, Whitland, UK) with a figure-8 coil (70 mm individual wing diameter). Coil placement was tangentially to the scalp with the handle pointing backwards at an angle of approximately 45° from the mid sagittal axis of the head. Relative coil position was monitored with an optical measurement system (Polaris Spectra, NDI) using passive markers on a head band and coil combined with neuronavigation software (ANT ASA 4.7.3, ANT, Enschede, NL).

Data acquisition

High density monopolar muscle EMG activity of the FCR was recorded using a taped on 8x8 electrodes flexible surface (inter-electrode distance 4 mm, 4x8 electrodes recorded), placed longitudinal parallel to muscle fibers (Figure 1), connected to a Porti7 system (TMSi, Enschede, NL). Angle of the wrist manipulator and torque at the handle were recorded at a second Porti7 system. All data was sampled at 2000 Hz, recordings from 200 ms prior to and 500 ms after stretch onset or TMS pulse for TMS initialization. Prior to sampling the 32 EMG channels were low-pass filtered at 540 Hz in the Porti7 system. Bipolar bar electrode configuration was constructed from monopolar HD-EMG data by taking the mean of three consecutive electrodes for one bar (Figure 1) to mimic conventional surface EMG.

Protocol

Maximum voluntary torque

Before and after the experiment the maximum voluntary torque (MVT) of the wrist was recorded three times with 10 seconds breaks. Participants were verbally encouraged to maximize their performance. During the experiment, 10% of the maximum filtered (moving average, window of 100 ms) before MVT was used as pre-contraction level.

TMS initialization

First, the TMS hotspot was determined by stimulating the motor cortex and visually inspecting
the motor evoked potential (MEP) peak-to-peak value. Second, the motor threshold (MT) was defined by gradually reducing stimulation intensity until 5 out of 10 stimuli elicited a visually detectable MEP in the EMG (Rossini et al. 1994). Third, MEP onset latency was determined at stimulator output of 130% MT from mean-subtracted EMG (mean of 10 trials). Onset latency was defined by first point in time where EMG response exceeded three times standard deviation of background EMG (180-20 ms before stimulation). MEP rise time was defined as time between MEP onset and maximum peak. In case of very high motor threshold (> 80% maximum stimulator output), the experiment was aborted and the participant was excluded from the study as no MEP onset latency could be determined at 130% MT.

**Stretch reflex testing**

Stretch duration is a key factor in the M2 response size (Lewis et al., 2005). Ramp-and-hold stretches with two different durations (40 and 80 ms) were randomly applied 10 times each with a stretch velocity of 1.5 rad/s. In case of absence of M2 response or adaptability of $A_{M2}$ due to stretch duration, the experiment was aborted.

**TMS & stretch reflexes**

A fixed stretch duration was used to equalize sensory input to the spinal cord. Stretch reflex perturbations ($T_{stretch}$: 40 ms, $V_{stretch}$: 1.5 rad/s) and subthreshold (97% MT) magnetic stimuli were merged at ten different relative intervals ($T_{MEP}$). TMS pulses were timed to arrive at the FCR within a range from 35 to 80 ms after stretch onset with 5 ms intervals. Time of application of the TMS pulse was adjusted for motor conduction times by subtracting MEP onset latency from $T_{MEP}$. The interstimulus interval (ISI) between TMS and stretch perturbation, combined with central (CMCT) and peripheral (PMCT) motor conduction times, together form $T_{MEP}$ (visual overview in Figure 2).

Single stimulus trials, TMS-only or stretch-only, were applied to create a baseline reflex response and check motor threshold level, respectively. Each condition was repeated ten times, resulting in 120 trials (10x10 ISIs at 97% MT + 10x stretch-only + 10x subTMS-only). The trials were presented randomly in sets of 20 stimulations with breaks of one minute in between.

![Figure 2](image-url)

**Figure 2** – Overview of timing within the experiment, timeline is not scaled. In this particular example, TMS pulse is timed to arrive at the muscle at $T_{MEP}$ of 60 ms. Time of stimulation of the motor cortex is adjusted for MEP onset latency of 17 ms (consisting of estimated central (7 ms) plus peripheral (10 ms) motor conduction times). CMCT: central motor conduction time, PMCT: peripheral motor conduction time, ISI: interstimulus interval, Act: activation.
Extra trials were carried out at the individual intervals of largest inhibitory and facilitatory
effect of subTMS on stretch reflex, determined from calculated outcome parameters of combined
trials. Ten pulses of subTMS at 90% MT were applied to check for inhibitory effects at maximum
inhibition interval. Interval with largest facilitation was used for 10 stimuli at 110% MT to study
non-linear facilitation as found in previous studies.

Data processing

EMG data of stretch test and combined trials was high-pass filtered (20 Hz, recursive third order
Butterworth) to remove movement artefacts, rectified and low-pass filtered (200 Hz, third order
Butterworth). All data processing was done in Matlab (Version R2007B, The Mathworks Inc,
Natick, USA). Recordings were normalized per trial to background activity (180-20 ms before
stimulus onset). The mean of 10 normalized trials was used for each trial type in calculation of
outcome parameters to decrease variance of measurements.

Outcome parameters were the short ($A_{M1}$) and long latency ($A_{M2}$) EMG response. $A_{M1}$
and $A_{M2}$ were calculated by taking the mean of the 40% highest samples in the respective
time windows (20-50 ms for M1, 55-100 ms for M2) of the filtered and normalized mean EMG record-
ings. By using the 40% samples with highest amplitude, interference of lower-than-background
activity (i.e. silent period) on effect size within aforementioned time windows was avoided.
Maximum peak height and time of occurrence ($T_{peak}$) were determined from the averaged pro-
cessed EMG traces over all participants.

HD-EMG processing

Muscle activation patterns were summarized in root mean square (RMS) plots of parallel bipolar
EMG. Consistency in muscle activation between trials was checked by comparing the mean
activity of 10 trials with each single trial, for individual participants. HD-EMG data was used to
create root mean square (RMS) values of parallel bipolar EMG activity in time windows of M1,
M2 and MEP. Muscle activation was summarized in matrices of 7x4 data points. Correlation
coefficients between trial matrices and mean activity matrices were calculated according to
equation 1, where $A$ and $B$ are compared matrices and $\overline{A}$ and $\overline{B}$ the respective averages of
matrix elements.

$$r = \frac{\sum_{i=1}^{7} \sum_{j=1}^{4} (A_{ij} - \overline{A})(B_{ij} - \overline{B})}{\sqrt{\left(\sum_{i=1}^{7} \sum_{j=1}^{4} (A_{ij} - \overline{A})^2\right) \left(\sum_{i=1}^{7} \sum_{j=1}^{4} (B_{ij} - \overline{B})^2\right)}}$$

(1)

To check for influence of placement of EMG electrodes, data processing of constructed
bipolar data (i.e. calculation of outcome parameters) was repeated for different bipolar con-
structions. Both interelectrode distances and location of bars on the muscle with fixed distance
were compared.

Statistical analysis

Effect of subTMS on $A_{M1}$ and $A_{M2}$ was tested using a linear mixed model with compound
symmetry covariance matrix and $T_{MEP}$ as factor (alpha = .05, SPSS version 20). Bonferroni
post-hoc testing compared 10 $T_{MEP}$ combinations and the 90% subTMS condition with baseline
(stretch-only) trials. Stimulation at 110% MT was omitted from statistical testing due to its
high variance compared to subTMS-reflex trials. To check effect size and separate responders and non-responders, a statistical analysis was carried out per participant.

SubTMS-only trials were tested on presence of an MEP by comparing RMS values of background EMG activity (180-20 ms before stimulus) with MEP window (5-45 ms) RMS values using a paired-samples T-test. Difference between MVT before and after experiment was tested with a paired-samples T-test.

Results

One participant’s motor threshold was too high (exceeding 80% of stimulator output) and therefore the experiment was aborted. Eleven participants are included in the data analysis.

General data overview

An overview of baseline characteristics of study participants is given in table 1. MVT before (mean: 11.9 Nm) and after (mean: 12.6 Nm) the experiment differed not significantly (t(10) = -1.605, p = .140). MT ranged from 37 to 63% of stimulator output with a mean of 48%. SubTMS was applied at an intensity of 96% of individual MT. MEP onset ranged from range 16 to 21 ms, with mean of 17.5 ms, MEP rise time had a mean of 4.9 ms (±0.9 ms SD). An example of MEP onset determination (Figure 3) and stretch reflex test (Figure 4) is given for participant 5.

Table 1 – Baseline characteristics of study participants who completed study. MVTb / MVTa: maximum voluntary torque before / after experiment [Nm], MT: motor threshold [% max. stimulator output], M.O.: motor evoked potential onset [ms]. * This value deviates from mean±SD of age given in Methods section, as one participant did not complete study.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Age [yr]</th>
<th>Gender</th>
<th>MVTb [Nm]</th>
<th>MVTa [Nm]</th>
<th>MT [%]</th>
<th>M.O. [ms]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>M</td>
<td>16.4</td>
<td>16.2</td>
<td>37</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>M</td>
<td>13.8</td>
<td>16.6</td>
<td>46</td>
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<td>53</td>
<td>F</td>
<td>6.1</td>
<td>5.9</td>
<td>37</td>
<td>17</td>
</tr>
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<td>5</td>
<td>58</td>
<td>F</td>
<td>5.6</td>
<td>5.5</td>
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<td>47</td>
<td>M</td>
<td>11.6</td>
<td>10.5</td>
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<td>8</td>
<td>62</td>
<td>M</td>
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<td>M</td>
<td>17.2</td>
<td>17.4</td>
<td>56</td>
<td>19</td>
</tr>
<tr>
<td>11</td>
<td>58</td>
<td>F</td>
<td>6.0</td>
<td>6.9</td>
<td>57</td>
<td>16</td>
</tr>
</tbody>
</table>

Mean±SD  48±13*  11.9±4.2  12.6±4.6  48±9  17.5±1.5

Effect of TMS timing on stretch reflex components

Electromyographic recordings (typical example in Figure 5) did not show a significant effect of TMS on the subTMS-only trials (t(10) = -1.103, p = .296), while the stretch-only trials showed a distinguishable short and long latency reflex component. Combining stretch and TMS pulse at different intervals showed an increase in activity in the M2 time window.

The overall effect of subTMS on stretch reflexes is summarized in Figure 6. Effect of differ-
ent intervals is plotted against the baseline condition (stretch-only trials). No effect of subTMS on baseline $A_{M1}$ is visible, statistical analysis shows that $T_{MEP}$ influences $A_{M1}$ ($F = 2.558$, $p = .005$). Bonferroni post-hoc analysis indicated that for $A_{M1}$ a significant effect is present between $T_{MEP}$ of 35 ms and 50 ms, but no effect with respect to baseline $A_{M1}$. Results for $A_{M2}$ did show an increase at $T_{MEP}$ of 60 ms and higher ($F = 7.669$, $p < .001$). Post-hoc analysis of $A_{M2}$ showed a significant difference between baseline $A_{M2}$ and $T_{MEP}$ at 60, 75 and 80 ms. Results of an analysis per participant are presented in table 2. Responders (showing a significant effect on M2) were separated from non-responders and statistical testing was repeated for responders only. Results were not affected by this separation (responders only: $F = 5.376$, $p < .001$), so pooled data of all participants was used in all analyses. The time of highest peak within M1 and M2 response ($T_{peak}$) is plotted against $T_{MEP}$ in Figure 7, as is maximum peak height. Between 50-80 ms a linear relationship is present for M2 response, where $T_{peak}$ is 5 ms after $T_{MEP}$.

**High Density EMG**

An example of a muscle activation RMS plot of suprathreshold TMS is shown in Figure 8. Suprathreshold TMS resulted in a consistent activation pattern over trials (mean correlation between individual trials and mean ($r = 0.74 ± 0.12$). Stretch-only reflexes are less consistent for both M1 ($r = 0.54 ± 0.19$) and M2 ($r = 0.40 ± 0.10$) time windows. Consistency of M1 response is not affected by subTMS, but increases slightly for M2 response with $T_{MEP}$ of 60 to 80 ms ($r = 0.55 ± 0.14$).

Muscle activation patterns of stretch trials were compared to suprathreshold MEP activation. M1 window activity had a correlation of $r = 0.30 ± 0.25$ with suprathreshold stimulation, independent of $T_{MEP}$. Activity within M2 time window had a low correlation for stretch-only trials ($r = 0.12 ± 0.11$) and for combined trials with $T_{MEP}$ of 35 to 55 ms ($r = 0.18 ± 0.04$), and higher correlation for $T_{MEP}$ of 60 to 80 ms ($r = 0.34 ± 0.04$).

Outcome parameters did not depend on the bar electrode configuration, showing comparable results for both different locations on the muscle and interelectrode distances.

**Figure 3** – Typical mean of 10 MEP’s at 130% of MT for participant 5. MEP onset indicated with red dot at 19 ms. MEP onset is the (rounded to ms) time instance where EMG is larger than 3 times background EMG (180-20 ms before stimulus, indicated with dashed lines).

**Figure 4** – Typical stretch reflex test for participant 5, mean of 10 stretches at durations of 40 (dashed line, light grey shading) and 80 (solid line, dark grey shading) ms, where shading indicates windows of M1 (20-50 ms) and M2 (55-100 ms) response.
Figure 5 – Normalized rectified EMG recordings averaged over all participants ($n = 11$). Combined trials (thick line) for 10 intervals ($T_{MEP}$, indicated by red dot) are plotted with averaged stretch-only perturbation (thin line in each plot). Dark grey shaded area between lines indicates increase in response after subTMS pulse. Light grey shaded areas indicate M1 (20-50 ms) and M2 (55-100 ms) time windows.
Table 2 – Results of individual statistical analysis for effect of TMS on M1 and M2. Significant results (p < 0.05) of linear mixed model are printed in boldface.

<table>
<thead>
<tr>
<th>Participant</th>
<th>M1 effect</th>
<th>M2 effect</th>
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<td></td>
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<td>p</td>
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<tr>
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<td>.578</td>
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</table>

Figure 6 – Overall effect (n = 11) of subTMS on short (AM1) and long (AM2) latency components of stretch reflex. Grey line is baseline condition, taken as 100%, shaded area indicates ± standard error of baseline. For 10 combined trials (T MEP from 35-80 ms) mean ± s.e.m. are plotted. 90% MT TMS (red) and 110% MT TMS (blue) conditions show mean ± s.e.m. in y-direction and mean ± s.d. in x-direction.
Figure 7 – Top: Timing of the maximum peak response of M1 and M2 as a function of TMS timing ($T_{MEP}$) over all participants ($n = 11$). Note the linear relationship in range 50 to 80 ms for M2 but not M1 response. Bottom: Maximum peak height in normalized EMG for M1 and M2.

Figure 8 – RMS plot of suprathreshold MEP for one participant. RMS values calculated from parallel bipolar EMG (mean of 10 trials). Averaged correlation of all trials with their corresponding mean was $r = 0.76$. 
Discussion

The effect of subthreshold TMS on stretch reflexes of the muscle flexor carpi radialis at different intervals after stretch onset was quantified. An increase, compared to stretch-only trials, in the long latency reflex component (M2) was found when magnetic stimuli were timed to arrive at the muscle in the time slot of the long latency response. This demonstrates involvement of supraspinal modulation in the long latency reflex component.

Effect on M1

Reflexes in combination with subTMS did not result in significant differences in $A_{M1}$ from baseline stretch-only $A_{M1}$. This is in line with existing literature, where no effect of suprathreshold (Day et al., 1991, Palmer and Ashby, 1992, Petersen et al., 1998, Lewis et al., 2004) or subthreshold (Van Doornik et al., 2004) magnetic stimulation on short latency response is reported.

SubTMS pulses were timed to arrive at the muscle from 35 ms after stretch onset onwards. This coincides with maximum activity within M1 window. The absence of an effect underlines the spinal nature of M1.

Effect on M2

SubTMS facilitated the M2 response significantly when timed to arrive at the muscle in the 55-80 ms time window after stretch onset. No MEPs were present in subTMS-only trials, so combination of stretch reflex and cortical stimulation caused this facilitation. This is in accordance with previous observations, stating that just below threshold TMS facilitates cortical activity compared to inhibiting lower intensity stimulation (Kujirai et al., 1993, Ziemann et al., 1996). No change of M2 response was found for stimulation in the 35-50 ms time window, indicating an instantaneous effect. This is supported by the linear relation between $T_{MEP}$ and response peak time of 5 ms. As confirmed by the MEP rise time found in current study, 5 ms is the depolarization time of motoneurons due to TMS, from MEP onset to maximum EMG activity. A facilitating effect of subTMS on long latency response was found before (Day et al., 1991), but no consistent decrease in stimulation intensity was used in that study. Current experiment used a consistent individual stimulation intensity per participant. A supralinear response when suprathreshold TMS was combined with stretch reflexes has been reported for muscles tibialis anterior (Petersen et al., 1998), posterior deltoid (Pruszynski et al., 2011) and flexor carpi radialis (Lewis et al., 2004). This supralinearity suggests that the long latency stretch reflex is at least partially transcortical. Suprathreshold TMS, however, might affect afferent feedback at a spinal level and facilitate the reflex response without a cortical pathway. Current observation of facilitation of M2 response by subTMS makes cortical involvement more plausible.

Effect of 90% MT subthreshold and suprathreshold TMS

Application of subthreshold TMS at 90% MT did not show an inhibiting or facilitating effect on $A_{M1}$ and $A_{M2}$. The timing of low subthreshold stimuli was only in the M1 response time window and, as already argued, there is no proof of cortical influence. Suprathreshold stimuli (110% MT) interfered significantly with $A_{M2}$, the induced MEP overruled the stretch reflex response. Response size was larger than the combined response of subthreshold TMS and stretch reflex, indicating that a fraction of entire motoneuron pool was excited during combined trials.
Spatial data

With HD-EMG spatial and temporal data about muscle activation was recorded. Muscle activation patterns are a summation of motor unit activity, summarized in RMS plots for time windows of M1, M2 and MEP. Suprathreshold TMS is consistent in activation of the muscle, whereas both M1 and M2 components of the stretch reflex show a large variation in activation patterns between trials. The increase in correlation of M2 response with suprathreshold TMS muscle activation for $T_{MEP}$ of 60 to 80 ms indicates an increasing overlap in targeted motoneurons. However, the inconsistency in stretch reflex response hinders a conclusion from these results.

Integration of cortical and reflexive signals

The integration of cortical and reflexive input led to the observed facilitated M2 response. Two levels of integration of magnetic and reflexive input are possible, cortical and spinal. Two possible integration mechanisms are spatial, stimulating different motoneurons, and temporal, facilitating already active motoneurons.

First and most likely, the integration takes place at a supraspinal, possibly cortical, level. Lag time between stretch onset and facilitation by subTMS is consistent with the lag time when signals travel over a cortical pathway. About 20 ms is necessary for sensory information to reach the motor cortex from the muscle. With stretch duration of 40 ms, information is processed within the cortex 20 to 60 ms after stretch onset. Taking into account a cortical processing time of about 10 ms and time to send information back to the muscle of 20 ms, the first possible instant of altered muscle activity is 50 ms. Maximum peak time is already affected from 50 ms after stretch onset, a change in $A_{M2}$ is observed after 55-60 ms. Absence of an effect of subTMS on M1 adds to the evidence of supraspinal pathways in the long latency stretch reflex. As M1 has a monosynaptic origin, no facilitation above the spinal cord is induced. Without facilitation from stretch reflex, subTMS was not able to induce an effect in the muscle. However, present study cannot conclude if the supraspinal level of reflex pathways is transcortical. Previous studies provided proof that facilitation occurs at a cortical level, based on transcranial electric stimulation (TES). TES is thought to stimulate axons of corticospinal neurons directly, whereas TMS activates the same neurons indirectly or near the cell soma (Day et al., 1989). Comparing effect of TMS and TES on stretch reflexes, it is reported that TES did not cause facilitation of stretch reflexes compared to TMS (Day et al., 1991, Petersen et al., 1998) or inhibited reflexes (Petersen et al., 2001, Zuur et al., 2010).

Second, cortical signals induced by subTMS, not strong enough to evoke an MEP, reach the motoneuron pool. There, integration of cortical signals and afferent input results in an action potential along motoneurons towards the muscle, leading to the observed response. The monosynaptic Ia afferent pathway is involved in M1 and M2 response (Schuurmans et al., 2009). As M1 is not affected by subTMS, it is unlikely that Ia afferents contribute to M2 facilitation. Slower type II afferents are multisynaptic and connect indirectly to alpha motoneurons. Interaction with cortical signals might occur at these synapses, by strengthening input to excitatory interneurons or by inhibition of inhibiting interneurons. However, the observed effect of subTMS is instant while slower pathways would provide more dispersed facilitation with a longer latency. Spinal integration requires involvement of reciprocal interneurons, which are not a likely candidate for reflex modulation by TMS. Studies showing inhibition of stretch reflexes or voluntary activity by TMS observed suppression but not facilitation of the antagonist muscle (Davey et al., 1994, Zuur et al., 2010). This response opposes involvement of reciprocal interneurons in cortically induced inhibition, and most probably also facilitation.
Two possible mechanisms of integration of cortical and afferent signals are proposed. First, cortically induced activity could recruit additional motoneurons and thereby facilitate the M2 response. As suprathreshold TMS shows a consistent spatial muscle activation pattern, it is expected that additional motoneuron recruitment would result in consistent, but different from stretch-only, activation patterns as well. Physiologically, this spatial mechanism is comparable to separate afferent and transcortical pathways.

Second, subTMS strongly facilitates M2 response by increasing the firing rate of motoneurons activated by the stretch reflex within the narrow time window of cortically induced activity. Muscle activation patterns would be comparable to stretch-only trials, but with a larger amplitude. Physiologically, this temporal mechanism is comparable to cortical and afferent feedback pathways connecting to the same motoneurons.

From the spatial muscle activation patterns, conclusions cannot be drawn about the integration mechanism of cortical and reflexive signals due to the inconsistent response to stretch reflexes. Therefore, mechanism of integration remains unclear.

**Methodological considerations**

*Choice of fixed stretch duration*

Previous studies have shown the relation between stretch duration and long latency response for different muscles (Lee and Tatton, 1982, Lewis et al., 2004, Schuurmans et al., 2009, Meskers et al., 2010). For current experiment, a fixed stretch duration of 40 ms was used. With this duration M2 response is of average size, therefore an inhibiting or facilitating effect due to the experiment could be shown. A confirming pilot experiment was carried out to test whether a comparable duration dependency curve was found for the stretch velocity of 1.5 rad/s and the FCR muscle measured in this study (results in Appendix B).

*Transcranial magnetic stimulation*

Previous studies show variable spinal activation depending on intensity of magnetic stimulation. A decrease of 5% stimulator output below motor threshold did not show any descending volleys along the spinal cord (Di Lazzaro et al., 1998). This result was used as proof of a transcortical pathway as at 92% motor threshold stimulation (similar to 3% decrease in stimulator output) an inhibition was present (Van Doornik et al., 2004). Magnetic stimulation in current study was performed at 96% of MT (2% decrease in stimulator output) which, combined with reflexive input, resulted in facilitated stretch reflex responses.

Stimulation intensities relative to motor threshold differ minimally between these studies, but resulting effect on muscle activity differs. Motor threshold is not a single boundary where muscle activation occurs when stimulating above this threshold and no activity is present once below, but rather an overlapping area with less or more activation (Mills et al. 1997). Besides, threshold varies with attention, and despite care taken to ensure levelled attention, motor threshold might be shifted during the experiment. The definition of motor threshold used in present study (Rossini et al., 1994) means that at MT approximately 5 out of 10 stimulations resulted in a small motor effect visible in the EMG. Lowering stimulation intensity to 96% MT does not guarantee absence of motor evoked potentials. However, the effect of subTMS on stretch reflex response is consistent over participants and all trials were randomized. If results were caused by change in threshold, resulting in more MEPs, than the effect would not only be on M2 response or reproducible over participants.
High density EMG

A surface of electrodes over the muscle was used to provide a more detailed, spatial, view of muscle activity compared to a bar electrode configuration. Reflexes and magnetic stimulation both induce bursts of muscle activation, not separable into single motor unit activity from the EMG. Spatial RMS data showed regions of activity but due to the size of the surface, just a small part of the muscle was monitored. After creating bipolar signals and interpolating the data not much data points were left. Though with the observed inconsistency in muscle activation patterns within participants, the size of electrode surface is not restrictive. Derivation of outcome parameters with different bipolar configurations did not result in deviating results. For the measurement of the effect of subTMS on the stretch reflex, electrode placement was thus not a limiting factor.

Conclusion

Involvement of the primary motor cortex in the stretch reflex was demonstrated for the human wrist flexor muscle. Absence of facilitation of the spinally mediated M1 response shows that integration of cortical and reflexive feedback signals in M2 happens at a supraspinal, probably cortical, level. Spatial muscle activation patterns of suprathreshold TMS were consistent over trials, while muscle activation due to stretch reflexes was less consistent. Spatial patterns of combined trials are therefore not conclusive about the mechanism of integration.

Future work

Results from current study do not allow a clear distinction in involved pathways in the stretch reflex response. Combining application of subTMS and stretch reflexes with feedback pathway blockers (e.g. Tizanidine) allows for a comparison of the cortical involvement without afferent input. Involvement of blocked pathway in facilitation of cortical activation could be confirmed or rejected.

A larger range of interstimulus intervals could affirm the spinal origin of (earlier) M1 response. SubTMS timed to arrive at the muscle in the refractory period of M2 and after the response would give a wider TMS effect profile, showing when cortical and reflexive feedback do and do not interact.

Repeating this experiment in diseased instead of healthy participants could show cortical involvement, or lack thereof, in combination with affected reflexes, for example in Parkinson’s Disease or stroke patients.
References


Part II

Appendices
Appendix A

Experiment Setup and Protocol

Current experiment builds on the experimental work of Mark van de Ruit. His work, including set up and protocol used for his experiment, are extensively described in his Master’s thesis. However, the use of a new (high density) EMG system and the addition of extra protocols to the experiment requires an update. This appendix describes the new setup and protocol, although not as detailed as done before. For this detailed information the reader is referred to Mark van de Ruit’s work.

A.1 Setup

During the experiment, an interplay between three computers, wrist perturbator and TMS device existed. All these devices had to communicate with one another, leading to a BNC-cable infrastructure between them.

A.1.1 Transcranial magnetic stimulation

For application of magnetic stimuli, the MagStim Rapid system (borrowed from Dept. of Clinical Neurophysiology, LUMC) was used. A conventional figure-of-8 coil was used, compared to the air cooled coil used before. This conventional coil belonged to an old MagStim 200 but could be attached to the current system via a conversion plug. Using the calibration board of the air cooled coil, the conventional coil was calibrated for the Visor neuronavigation system. During the experiment, the coil was placed in a special – but not limited to TMS – stand (see figure A.3).

A.1.2 Wrist perturbator

Stretching of the wrist was done with the wrist perturbator PoPe (from Pols Perturbator) (Schouten et al., 2006). The PoPe consists of a handle with a force transducer inside, and the handle is controlled by a servo motor. This motor is controlled by a Simulink model in Matlab. The already existing Simulink model, which was adapted to reach low amplitudes with high velocity, was extended for this study. An extra trigger was added to control TMS and EMG system separately. Besides, force, position and velocity of the handle were exported via the PoPe output deck to record with the EMG system (see section A.1.3). An overview of the used output ports is given in section A.1.4.

During pilot studies, the force feedback as given on the screen and as measured with the EMG system did not match. Eventually, the problem appeared to be present in the Simulink
model, where measured force had a 0.1 Hz low pass filter. This resulted in a feedback force which was not at all realistic and comparable to the real exerted force. The existing filter was replaced by a 2 Hz low pass filter, solving the problem but making the task slightly more difficult for participants. The updated Simulink model is shown in figure A.1 and is available on the data DVD.

During pilot studies, feedback was given about user-friendliness of the feedback screen. The color scheme as used before could lead to difficulties for color blind participants and exhausted the eyes. Therefore a new color scheme was developed that presented a lower burden and had a higher visibility.

![Updated Simulink model including new force filter (top left), EMG and TMS triggers (bottom left, output at DAC_07 and DAC_08) and output of force (top right, DAC_03). Output of position and velocity are in block Baumuller Servo.](image)

**Figure A.1**

A.1.3 Electromyography

During his project, Mark van de Ruit ran into one big problem with the EMG system used; a large artefact of the TMS pulse in the recording which hindered analysis of the data. Sometimes, stretch reflexive signals were completely distorted due to this artefact. Data analysis was therefore severely hindered and just four out of nine subjects could be included in the final paper.

A completely new EMG system just arrived at the Department of Rehabilitation Medicine, the Porti 7 system manufactured by TMSi (Enschede, NL). A total of 32 (monopolar) channels can be recorded simultaneously. Using special electrode surfaces, up to 64 signals could be
measured with two coupled EMG-Porti’s. When surface recordings are made with multiple electrodes, this is known as high density EMG (HD-EMG) (Zwarts and Stegeman, 2003, Merletti et al., 2008). More info about HD-EMG can be found in the literature review written before this experimental thesis.

To record 64 channels, two Porti sets have to be linked together using a Synfi device, sending all signals at once to the computer via USB. TMS artefacts had no effect on the signals as an actively shielded 64-channel cable was used between electrode surface and Porti’s. Besides the EMG-Porti, an AUX-Porti was available to record signals other than EMG. Both Porti’s and the Synfi connecting them are shown in figure A.2.

The Porti system as used in Leiden has a maximum sampling rate of 2000 Hz, compared to 5000 Hz sampling at the PoPe ssytem. To avoid any problems in data alignment and missed samples, relevant signals were sent from PoPe to an AUX-Porti. Force, position and TMS trigger were recorded as they provide valuable data for processing of signals (determination of stretch duration, interval between stretch onset and TMS pulse). As back-up, all signals from the PoPe were stored at the PoPe PC as well.

With the AUX-Porti connected to the Synfi, no longer 64 channels could be recorded at once. Hence, 32 channels of the surface were recorded. As the Flexor Carpi Radialis muscle is not as broad as the 8x8 channels configuration of the surface, it was no problem to record 4x8 channels in the direction of muscle fibers.

![Figure A.2](image) – Top: Synfi connected input from both Porti’s via glass fiber (orange cables). Middle: EMG-Porti with 32 monopolar channels, ground (green plug) and trigger input (grey). Bottom: AUX-Porti with three inputs.

Real-time read out of the EMG signals is necessary to provide direct feedback of the magnetic stimuli. Therefore, the Porti system had to be linked to Matlab (Mathworks). Luckily, some work was already done by Matthieu Urvoi (Universit de Nantes, France) and Edwin van Asseldonk (University of Twente, NL). Mr. Urvoi had written drivers that have access to the buffer of the Porti’s and were also able of changing recording parameters. Mr. Van Asseldonk build upon this work by creating a real-time read out script. For the drivers to be working, the Porti’s could only be connected to a Windows XP computer. Unfortunately, the PoPe PC has
Windows 7 installed on it and thus a separate computer had to be used. On the other hand, running both PoPe and EMG recording in one script would have caused different challenges.

However, both pieces of code had some drawbacks. No trigger input was used to start measurements, and it was not possible to record data before such a trigger (for instance for background EMG). A ‘running buffer’ was programmed in Matlab, which allowed search for triggers and also to save data before and after this trigger (as long as the buffer size was not exceeded). Besides, especially for TMS hot spot search, a peak-to-peak value is given. Also a common reference subtraction is programmed as the Synfi was used to record data. This code is available in realTimeTMSiTMS.m and timerTMSiTMS.m.

A different version was constructed that waits for a trigger to start recording of EMG signals until the recording is manually stopped at the Porti PC. MVC start and end recordings, of which the duration varies per participant, are recorded with this code (realTimeTMSiMVC.m and timerTMSiMVC.m).

A.1.4 Infrastructure overview

In the current Matlab coding, PoPe output channels and Porti input channels are defined for specific signals. These are listed below per channel, it is important to change this if coding is changed. There is no particular reason why AUX-Porti channels 11-13 were used. PoPe output channels are specified in Simulink Model and chosen because of their availability. Input to AUX-Porti’s with special BNC-AUX cables, trigger of AUX-Porti is not used.

- TMS out
  - Cable to EMG-Porti Trigger in (at TMS init protocols)
  - Cable to Visor-ANT box

- TMS in
  - Cable from PoPe DACH 7 (via splitter)

- PoPe output deck
  - DACH 3: Force output, cable to AUX-Porti channel 11
  - DACH 4: Position output, cable to AUX-Porti channel 12
  - DACH 5: Velocity output, not recorded in this experiment but it is possible
  - DACH 6: Cable to EMG-Porti Trigger in (at stretch protocols)
  - DACH 7: Short cable, splitted to ‘TMS in’ and AUX-Porti channel 13 (TMS trigger)

Note that no signal is sent from TMS out to Pope deck. This was (and is) present in Mark van de Ruit’s code as real-time read out of the (Delsys) EMG was done on the PoPe PC. In the new situation, this was handled at the Porti PC so this line was no longer necessary.

A.1.5 Overall setup

The setup as used during measurements is shown in figure A.3. Participant’s feedback screen is placed in front of him. Neuronavigation is positioned to have both headband and coil in view while not hindering the participant. During the TMS-stretch protocol, the experiment ran automatically with TMS coil placed in the stand. The only effort of the researcher was to check EMG recordings and placement of the coil.
Figure A.3 – Overall setup during experiment, with TMS device in front, feedback screen in the middle and neuronavigation to the left. Coil is positioned using a special stand. Not on photograph is the Porti System and computer, positioned to the right of this figure. Insert: HD-EMG surface placed on the clamped arm.

A.2 Protocol

In total, one experimental session consisted of several stages or protocols. A summary in the form of a checklist is given in section A.2.1. After a setup check before participant arrived, the participant was introduced to the experiment and setup. Possible questions were answered until participant was comfortable with the experiment. Next step was signing of informed consent and filling in of the TMS safety questionnaire (section A.2.2).
**Preparation of participant**

First, the participant had to be seated comfortably with his arm in the PoPe clamps and head resting in right position. Then, the FCR muscle location was checked and electrode surface placed in line with the muscle. Electrode contacts were checked on the Porti PC with Portilab (TMSi, Enschede, NL). If no or bad signal was recorded during contraction and/or bending of the wrist, electrode surface was replaced carefully. Next step was neuronavigation initialization, participant got a head band with markers placed on his head and three points (nose bridge, middle of both ears) were registered in ANT Visor (ANT, Amsterdam, NL). As a last step, the arm of participant was fixed in the PoPe clamps using moldable foam pads. Maximum voluntary torque (MVT) was measured with pins attached in the PoPe, so the handle was mechanically blocked from moving.

**TMS initialization**

Different parameters for the use of TMS had to be defined. First, the hotspot had to be determined by applying stimuli over the head and checking the resulting motor evoked potential (MEP) size at the Porti PC. Stimulation was started at 50% stimulator output and increased in steps of 5% if necessary to evoke a good response. Hotspot search was done with a resting muscle, eventually with slight contraction to check location. Each TMS pulse was delivered with at least 5 seconds in between. Once the hotspot was found, this was registered in ANT Visor.

With coil positioned over the hotspot in 45 degree angle from midline, using the coil stand, the protocol to find active motor threshold (AMT) was started. Participant had to go to a 10% MVT contraction, with help of the force feedback screen. After approximately 1 second of sustained contraction, the TMS device was automatically triggered. The researcher increased and decreased the stimulation intensity manually in accordance with the recorded EMG. If 5 out of 10 stimuli did not present a visually detectable MEP in the EMG (Rossini et al., 1994), this intensity was taken as AMT.

Last TMS parameter was MEP onset time, for which a stimulation intensity of 130% AMT was used in all participants. Ten stimuli were given subsequently, and the mean of their EMG response was calculated on the Porti PC. Onset was taken as first time instance higher than three time standard deviation of the mean background EMG, rounded to nearest integer in milliseconds.

**Stretch reflex testing**

For the stretch perturbations, the pins of the PoPe had to be detached so the handle could move freely. Participants first were allowed to experience perturbations with a short test protocol consisting of 10 stretches of different durations. When comfortable, the reflexes were tested at durations of 40 and 80 ms (velocity of 1.5 rad/s), each duration applied 10 times in random order. Again, a pre-contraction of 10% MVT was used. Response was calculated at the Porti PC, and used to check if a sufficient stretch reflex, and especially long latency (M2) response, could be induced. By looking at filtered and normalized EMG responses, and comparing size of M2 response for both stretch durations, it was decided if the reflexes could be affected by duration and therefore TMS.
TMS and stretch reflexes combined

The main part of the experiment was the combination of TMS and stretch reflexes. In total 120 trials were applied, 10 non-reflex trials, 10 non-TMS trials and 10 trials for each of 10 intervals between stretch onset and TMS pulse. These trials were applied in a random order, with a pre-contraction of 10% MVT. Participants were allowed to take a short break in between every 20 trials, with between trials a mandatory rest of 3 seconds. Instructions were to let the muscle rest in these breaks, and contract again for each new trial.

After 120 trials, another short protocol was run to study the effect of TMS at stimulation intensities of 90% and 110% AMT. Each intensity was repeated 10 times in a row, first 90% and then 110% AMT. Interval between stretch onset and TMS pulse was taken as interval of largest inhibition for 90% AMT and interval of largest excitation for 110% AMT, as derived from results of the 120 trials.
A.2.1 Protocol Checklist

For consistency and smoothness of the experiments, a checklist was used as reminder of the most important steps.

**Before experiment**

1. Set-up ready: chair, neuronavigation, TMS, EMG (+ computer) positioned
2. All cables connected to TMS/PoPe/EMG
3. Turn on neuronavigation, check visibility of coil/pen at head’s position, load Visor/ASA
4. PoPe controller initialized
5. PoPe pins attached
6. EMG prepared: electrodes with sticker, gel ready, kit close by.

**Preparations with participant**

1. Explain protocol, TMS, EMG
2. Answer questions
3. TMS questionnaire, sign informed consent
4. Adjust chair, screen for participant. Make sure seating is comfortable!
5. Scrub and clean skin
6. Place electrode surface (look at muscle line), check connectivity in Portilab
7. Place head markers (reference)
8. Initialize neuronavigation, check visibility of coil and reference together
9. Fix lower arm

**During measurements**

1. MVC measurement (three times), check EMG (and force) again
2. Hand triggered TMS, find hotspot with ‘Stimulate’ in Visor, check recordings, write down time of hotspot stimulus.
3. Set up ‘Reproduce stimuli’ with hotspot recording, coil in positioning handle
4. Automatic triggered AMT finding
5. MEP characteristics, get latency from EMG computer
6. CHANGE TRIGGER FROM TMS TO POPE
7. Deattach PoPe pins
8. Stretch test session
9. Stretch duration testing at Ts = 40 and 80 ms, check results at EMG computer
10. Check TMS positioning, eventually short break for participant
11. Run TMS/stretch protocol
   (a) Check TMS positioning while running!
   (b) Allow short breaks with head movement after 20 trials, then reposition coil very carefully
12. Get ISI-M2 curve, define max. inhibition and excitation
13. Run TMS/stretch IE protocol
14. Reattach PoPe pins
15. MVC end check
A.2.2 TMS safety questionnaire

Contra-indications were screened using a safety questionnaire based on (Keel et al., 2001). Fifteen YES/NO questions were presented, if the answer was YES specifications had to be written down to check relevancy to the experiment. No participants were excluded from the experiment, also because exclusion criteria were checked during recruitment. Medication used had no cortical or neuronal effects (blood pressure or gastric juice pills were most used). The questions asked are presented here, the official questionnaire (in Dutch) is available on the data DVD.

Safety questionnaire

1. Have you ever had any side effects of TMS?
2. Have you ever had an epileptic seizure?
3. Has anyone in your direct family a history of epilepsy? If YES, what is your relation to this person?
4. Have you ever had an electroencephalogram (EEG) as part of a medical procedure?
5. Have you ever had any serious damage to your head?
6. Do you have any metal in your head (outside the mouth), like surgery clips or particles due to metal processing or welding?
7. Do you have a medical aid implanted, like pace maker, cochlear implant, medicine pump?
8. Have you had neurosurgery (including eye surgery)?
9. Do you often (at least once a month) suffer from severe head aches?
10. Have you ever had severe heart problems?
11. Do you use any prescribed medication?
12. Did you drink more than three glasses of alcohol in the past 24 hours?
13. Did you use (recreational) drugs in the past 24 hours?
14. Have you had very little sleep last night?
15. Is there a chance that you are pregnant?
References


Appendix B

Pilot Studies on Stretch Duration

Long latency stretch reflex (M2) response depends on stretch duration, opposed to the velocity dependent short latency (M1) component. To shorten duration of the experiment, it was decided not to test the stretch duration dependency of M2 for each subject. Rather, one fixed stretch duration was chosen, based on literature. An overview of previous research is given in table B.1. Based on this data and five pilot tests, a fixed stretch duration of 40 ms was determined for this study. Also the Master’s thesis work of Mark van de Ruit indicated that 40 ms was a good choice. During the measurements, one participant did not respond to TMS but agreed to test stretch duration dependency. This dataset is also presented along the pilot tests in figure B.2.

The chosen stretch duration is halfway the slope present in stretch duration dependency, independent of stretch velocity (Schuurmans et al., 2009). This is an indication that the response size is not maximum or minimum and thus can decrease and increase depending on the cortical stimulation (see figure B.1).

Table B.1 – Stretch duration dependency in literature. Muscles: ECR = extensor carpi radialis, FCR = flexor carpi radialis, BB = biceps brachii.

<table>
<thead>
<tr>
<th>Study</th>
<th>Muscle</th>
<th>Velocity [rad/s]</th>
<th>Critical duration [ms]</th>
<th>Slope [ms]</th>
<th>Joint torque</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee and Tatton (1982)</td>
<td>ECR</td>
<td>7-9</td>
<td>43.8</td>
<td>n.a.</td>
<td>0.5 Nm</td>
</tr>
<tr>
<td>Lewis et al. (2005)</td>
<td>BB</td>
<td>4</td>
<td>36 ± 5</td>
<td>n.a.</td>
<td>5% MVC</td>
</tr>
<tr>
<td>Schuurmans et al. (2009)</td>
<td>FCR</td>
<td>1.5, 2, 3, 5</td>
<td>n.a.</td>
<td>20-60</td>
<td>1.0 Nm</td>
</tr>
<tr>
<td>Meskers et al. (2010)</td>
<td>FCR</td>
<td>2</td>
<td>n.a.</td>
<td>30-70</td>
<td>1.0 Nm</td>
</tr>
</tbody>
</table>
Figure B.1 – Stretch duration dependency of M2 response for different velocities. Error bars indicate standard error of the mean. Highlighted is dependency curve with slope between 30-60 ms, and the chosen duration of 40 ms (Adapted from Schuurmans et al., 2009).
Figure B.2 – Stretch duration effect on M1 and M2 for six datasets (Mean ± standard error).
References


Appendix C

EMG overview per participant
**Figure C.1** – Normalized rectified EMG recordings averaged for participant TMS01. Combined trials (thick line) for 10 intervals ($T_{\text{MEP}}$, indicated by red dot) are plotted with averaged stretch-only perturbation (thin line in each plot). Dark grey shaded area between lines indicates increase in response after subTMS pulse. Light grey shaded areas indicate M1 (20-50 ms) and M2 (55-100 ms) time windows.
Figure C.2 – Normalized rectified EMG recordings averaged for participant TMS02. Combined trials (thick line) for 10 intervals (T\textsubscript{MEP}, indicated by red dot) are plotted with averaged stretch-only perturbation (thin line in each plot). Dark grey shaded area between lines indicates increase in response after subTMS pulse. Light grey shaded areas indicate M1 (20-50 ms) and M2 (55-100 ms) time windows.
Figure C.3 – Normalized rectified EMG recordings averaged for participant TMS03. Combined trials (thick line) for 10 intervals ($T_{\text{MEP}}$, indicated by red dot) are plotted with averaged stretch-only perturbation (thin line in each plot). Dark grey shaded area between lines indicates increase in response after subTMS pulse. Light grey shaded areas indicate M1 (20-50 ms) and M2 (55-100 ms) time windows.
Figure C.4 – Normalized rectified EMG recordings averaged for participant TMS04. Combined trials (thick line) for 10 intervals ($T_{MEP}$, indicated by red dot) are plotted with averaged stretch-only perturbation (thin line in each plot). Dark grey shaded area between lines indicates increase in response after subTMS pulse. Light grey shaded areas indicate M1 (20-50 ms) and M2 (55-100 ms) time windows.
**Figure C.5** – Normalized rectified EMG recordings averaged for participant TMS05. Combined trials (thick line) for 10 intervals ($T_{MEP}$, indicated by red dot) are plotted with averaged stretch-only perturbation (thin line in each plot). Dark grey shaded area between lines indicates increase in response after subTMS pulse. Light grey shaded areas indicate M1 (20-50 ms) and M2 (55-100 ms) time windows.
Figure C.6 – Normalized rectified EMG recordings averaged for participant TMS06. Combined trials (thick line) for 10 intervals ($T_{\text{MEP}}$, indicated by red dot) are plotted with averaged stretch-only perturbation (thin line in each plot). Dark grey shaded area between lines indicates increase in response after subTMS pulse. Light grey shaded areas indicate M1 (20-50 ms) and M2 (55-100 ms) time windows.
Figure C.7 – Normalized rectified EMG recordings averaged for participant TMS08. Combined trials (thick line) for 10 intervals ($T_{MEP}$, indicated by red dot) are plotted with averaged stretch-only perturbation (thin line in each plot). Dark grey shaded area between lines indicates increase in response after subTMS pulse. Light grey shaded areas indicate M1 (20-50 ms) and M2 (55-100 ms) time windows.
Figure C.8 – Normalized rectified EMG recordings averaged for participant TMS09. Combined trials (thick line) for 10 intervals ($T_{MEP}$, indicated by red dot) are plotted with averaged stretch-only perturbation (thin line in each plot). Dark grey shaded area between lines indicates increase in response after subTMS pulse. Light grey shaded areas indicate M1 (20-50 ms) and M2 (55-100 ms) time windows.
Figure C.9 – Normalized rectified EMG recordings averaged for participant TMS10. Combined trials (thick line) for 10 intervals ($T_{MEP}$, indicated by red dot) are plotted with averaged stretch-only perturbation (thin line in each plot). Dark grey shaded area between lines indicates increase in response after subTMS pulse. Light grey shaded areas indicate M1 (20-50 ms) and M2 (55-100 ms) time windows.
Figure C.10 – Normalized rectified EMG recordings averaged for participant TMS11. Combined trials (thick line) for 10 intervals ($T_{\text{MEP}}$, indicated by red dot) are plotted with averaged stretch-only perturbation (thin line in each plot). Dark grey shaded area between lines indicates increase in response after subTMS pulse. Light grey shaded areas indicate M1 (20-50 ms) and M2 (55-100 ms) time windows.
Figure C.11 – Normalized rectified EMG recordings averaged for participant TMS12. Combined trials (thick line) for 10 intervals ($T_{MEP}$, indicated by red dot) are plotted with averaged stretch-only perturbation (thin line in each plot). Dark grey shaded area between lines indicates increase in response after subTMS pulse. Light grey shaded areas indicate M1 (20-50 ms) and M2 (55-100 ms) time windows.