**Materials and Methods**

*Study Design*

13 healthy subjects participated in this experiment (age M = 25.7, SD = 7.1 years; 7 male). All subjects were right-handed and scored at least +75% on the Edinburgh Handedness Inventory [1]. None of them had any history of neurological or psychiatric illnesses or had any contraindications to TMS [2]. Subjects gave written informed consent before participation, and the study was approved by the local ethics committee.

We performed hotspot detection and a cortical mapping for each of four stimulation conditions: biphasic latero-medial (BI-90), biphasic posterolateral-anteromedial (BI-45), monophasic latero-medial (MO-90), and monophasic posterolateral-anteromedial (MO-45). Due to the duration of each mapping, recordings were performed in two sessions. Per session, two conditions were assessed. The order of conditions was randomized within each subject.

*Transcranial Magnetic Stimulation*

In each session, TMS was applied with a MagPro X100 with MagOption using Power Mode and a MCF-B70 figure-of-eight coil of 97mm diameter (Magventure, Denmark). Consider that Power Mode increases the maximum stimulator output by 40%. Stimulation was performed based on a template headmodel using the (TMS Navigator, Localite, Germany). We recorded MEPs at the extensor digitorum of the right hand with surface electromyography. The muscle was located by palpation during extension of the wrist and anatomical landmarks. The skin was cleaned using 95% ethanol and abrasive gel. We used self-adhesive electrodes (Neuroline, Ambu, Germany). During the whole experiment, the subject was seated in a comfortable reclining chair and was told to relax his or her muscles.

*Hotspot Detection*

The individual hotspot for EDC representation in the right primary motor cortex was determined for each of the four conditions prior to every mapping. Based on the individual´s head anatomy, we started stimulation at the approximate location of the hotspot. Initial stimulation intensity was set to 40% of maximum stimulator output (MSO) for biphasic stimulation and 65% of MSO for monophasic stimulation [2]. If no MEPs could be elicited, intensity was increased in 5% steps. Coil position was adapted in a random fashion, while coil orientation was fixed based on the respective condition. Finally, the hotspot of each condition was defined as the spot eliciting the highest MEP with shortest latency for a given stimulation intensity.

*Resting Motor Threshold*

At this spot the resting motor threshold (RMT) was determined with the relative frequency method [3], i.e. RMT was defined as the stimulator intensity at which 5 out of 10 stimuli would elicit an MEP with an amplitude larger than 50 µVpp.

*Mapping Grid*

For mapping, a grid was created via Localite with its center 1cm anterior of the previously detected hotspot. In steps of 0.5 cm, we set 7 x 15 grid points. This resulted in a 3 cm wide grid spanning 4.5 cm anterior and 2.5 cm posterior to the hotspot. At each of the 105 grid points, 3 stimuli were applied. This resulted in an average of 15 Stimuli/cm2. The mapping was carried out for each stimulation condition with 110% of the RMT of the primary target. We had to remove 6 measurements for technical reasons, resulting in 46 total and 11 to 13 maps for each of the four conditions.

*Input-Output-Curves*

After mapping, we selected the point in the grid with the highest MEP averaged over 3 stimuli (primary target) and identified the most anterior point eliciting any MEP (anterior target). To assess differences in the respective neuronal structures, we measured the stimulus-response-curve at each target. We stimulated with 7 intensities ranging from 90% - 150% of the respective RMT in steps of 10%, delivering 10 stimuli per intensity. The order of stimulation intensities was randomized within each subject. Consider that 150% of 67% MSO is 100%, suggesting that at RMT higher than 67%, we would not be able to sample for high intensities. To be able to adequately sample the complete input-output curve, we therefore used the following procedure. If the RMT at the primary target was above 67% MSO, we calculated the stimulation intensities for primary and anterior target relative to 67% MSO. If RMT at the primary target was below 67%, we determined the RMT for the anterior target with the relative frequency method [3], and used distinct stimulation intensities for primary and anterior target. We had to remove 15 measurements for technical reasons, resulting in 89 total and 10 to 13 input-output curve measurements for each of the eight conditions.

*Signal Processing*

For all MEPs, latency and amplitude were estimated automatically offline, using costum-written MatLab functions. Additionally, all trials were visually inspected for correctness of the estimation. Incorrect estimates were corrected, and artifacted trials were rejected. Latency and amplitude were averaged for each grid point and for each intensity level. For input-output-curve assessment, we also processed the time-course of MEPs from 5 to 60ms after the TMS pulse. The raw time-course was detrended and baselined in reference to the period from 5 to 17 ms after the TMS pulse.

*Statistical Analysis*

Regarding the input-output-curve, we calculated the influence of the categorical factors coil Orientation (90° vs. 45°) and stimulus Waveform (biphasic vs. monophasic) as well as Target (M1 vs. NPMA) on latency and amplitude, accounting for interactions between the three factors and Subject as a random factor. This test was performed for each stimulus-intensity. For the time-course, we performed this analysis additionally for every time-point. We also calculated the influence of Target on measured RMT between anterior and primary target using an analysis of variance, accounting for Subject as a random factor.

Regarding the mapping, we calculated the influence of the categorical factors coil Orientation and stimulus Waveform on resting motor threshold, latency and amplitude, accounting for interactions between the two factors and Subject as a random factor. This test was performed for each grid-point. The statistical significance of the influence of the factors on latency and amplitude was additionally estimated by contrasting the model coefficients with a permutation analysis using 1000 repetitions. For assessment of the topology, we additionally performed a cluster-based permutation analysis based on the sum of coefficients of neighboring significant grid points. The significance threshold was set to 5% for all statistical tests.

**Results**

*Hotspot Detection*

The average position of the hotspot across subjects used for the definition of the two-dimensional mapping grid origin was centered on X = -36.9, Y = -18.6. This is not different from the position of M1 as established in literature [4] as evidenced by finding no significant differences (t(12) = [0.2, 0.39], p = [0.85, 0.70]). This suggests that, as designed, the average grid origin was 1 cm anterior to M1.

*Resting Motor Threshold*

Inspection of the average motor threshold in %MSO for biphasic stimulation at 90° (M = 38.3, SD = 7.7) and 45° (M = 36.7, SD = 10.7), as well as for monophasic at 90° (M = 65.9, SD = 12.1) and 45° (M = 61.7, SD = 13.0) exhibits the decreased resting motor threshold for biphasic stimulation. Indeed, resting motor threshold was not significantly different for orientation (F(1, 28) = 1.9, p=0.18) , but only for waveform (F(1, 28) = 116.4, p>0.001). Additionally, we found no significant interactions (F(1, 28) = 0.1, p=0.73). We found no significant difference in RMT for anterior versus primary target (F(1,82) = 3.8, P > 0.05).

*Mapping Grid*

We found grid points exhibiting a significant influence of waveform and orientation on MEP parameters (figure 1). Cluster-based permutation test revealed a significant difference for biphasic instead of monophasic stimulation increases amplitude (p = 0.047, figure 1A), mainly pronounced over primary motor areas (centered on X = -33.8, Y = -23.4), while stimulation with 90° decreases amplitude (p = 0.024, figure 1D) over anterior areas (centered on X = -36.6, Y = 3.2). The cluster-based permutation tests also revealed that latency was decreased during stimulation at 90° in contrast to 45° (p = 0.036, figure 1B), mainly over anterior areas (centered on X = -41.1, Y = 7.7). Last, we also found evidence for an interaction between waveform and orientation suggesting that biphasic at 45° and monophasic at 90° reduce latency in contrast to biphasic at 90° and monophasic at 45° (p = 0.001, figure 1C) mainly over primary motor areas (centered on X = -28.8, Y = -22.0).

*Anterior Stimulation Target*

The distribution of the anterior hotspot across subjects is clustered around antero-medial grid points (see figure 2A). We projected the positions also unto a template cortical surface for visual representation (see figure 2B). Anterior stimulation was delivered in average 21.1 mm anterior (CI95% = 18.8 - 23.5 mm) and 4.3 mm medial (CI95% = 1.4 - 7.2 mm) to the M1 [4], with a center of gravity at X = -32.4 and Y = 3.5. The anterior stimulation targets closest classically motor-related area is the dorsal premotor area [4] (see figure 2C), yet it is still 4.7 mm anterior (CI95% = 2.3 - 7.0 mm) to this region.

*Input-Output-Curves*

Based on the permutation test, we found no evidence for an interaction between Orientation, waveform and target at any stimulation intensity (all p > 0.062), suggesting similarity of the response curves for anterior and primary targets. We did find significant modulation for the factors Orientation, waveform and Target at a local 5% alpha error level, but after applying Bonferonni-correction, effects were limited to waveform. Biphasic stimulation exhibited increased amplitude (see figure 3B) and decreased latency (see figure 3E) at moderate stimulation intensities (around 120 - 130 % RMT).

figure1.tif**Figure 1:** *It shows the topography of significance for the two factors Orientation and Waveform on latency and amplitude at the 105 grid points. The maps were interpolated, and colors indicate significance level (red increased, blue decreased). Grey contour lines indicate the threshold for significance at the 5% level. Additionally, we added to each significant cluster a textbox with the estimation of its p-value based on the results of the cluster permutation analysis.* ***A*** *shows the influence of Waveform amplitude, suggesting increased amplitude for biphasic in contrast to monophasic stimulation.* ***B*** *shows the influence of Orientation on latency, suggesting decreased latency for stimulation with 90° in contrast to 45°.* ***C*** *shows the influence of Waveform x Orientation on latency, suggesting decreased latency for stimulation with biphasic 90° and monophasic 45° in contrast to biphasic 45° and monophasic 90°.* ***D*** *shows the influence of Orientation on amplitude, suggesting decreased amplitude for stimulation with 90° in contrast to 45°.*

figure2.tif

**Figure 2:** *It shows the spatial distribution of the anterior stimulation point on the grid (****A****) as well as projected unto a template headmodel (****B****). Colors indicate the probability density estimate.* ***C*** *shows the distance of each subject's anterior stimulation position to several motor-related brain regions with a scatter-cloud and the mean presented as a bar.*

image3.tif

**Figure 3:** *It shows the influence of the factors Orientation, Waveform and Target on latency and amplitude measures for different stimulation intensities. The left column* ***(A-C)*** *shows amplitude with µVpp on the y-axis, and the right column* ***(D-F)*** *shows latency with ms on the y-axis. Data is shown with stimulation intensity on the x-axis in percent of resting motor threshold. Enlarged color-markers indicate significant differences between the respective factor levels as returned by permutation analysis (Bonferonni-corrected for 7 x 6 multiple comparison). Colored patches indicate the bootstrapped 95% confidence intervals.*