Anisotropy in the visual cortex investigated by neuronavigated transcranial magnetic stimulation

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Responses to transcranial magnetic stimulation (TMS) of the motor cortex depend on the direction of the induced current with an optimum perpendicular to the orientation of the precentral gyrus. Little is known about anisotropy in other cortical regions. We measured phosphene thresholds in the visual cortex using a frameless neuronavigation system. Comparing horizontal and vertical current orientation as well as monophasic and biphasic pulses in 7 subjects, we found lower thresholds with biphasic pulses and a tendency for lower thresholds with horizontal currents. When varying current directions in steps of 45° centered on a hot spot over the occipital cortex, in 10 out of 12 measurements optimal current orientation ran perpendicular to the underlying gyrus (mean deviation 14.6°). Optimal current orientation was determined as the orientation of the second eigenvector from the covariance matrix of the stimulation sites that had been shifted along the respective current direction by the amount of the measured threshold. Individual cortical architecture was obtained by segmentation of a 3d anatomical MR scan, with large interindividual differences among the orientations of the stimulated gyrus. As with the motor system, the optimum threshold with biphasic pulses was flipped about 180° compared to the optimum with monophasic pulses (p < .02) throughout subjects, suggesting both similar anisotropic properties of networks in the visual and motor cortices and the existence of anisotropic behaviour in any cortical region. As a consequence, optimal TMS application should always take into account the individual orientation of the gyrus to be stimulated.

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Introduction

Transcranial magnetic stimulation is a well established method in basic and clinical neuroscience. However, despite 20 years of research most of the physiological details of the stimulation process are still unclear. Within a roughly estimated cortical and probably subcortical area of 1–4 cm² the induced gradient of the electric field interacts with a neuronal network consisting of about 1 to 5 billion (10^9) of cells (Rockel et al., 1980). The network response depends on many different factors, such as the physical properties of the stimulator (Barker et al., 1991; Di Lazzaro et al., 2001a; Kammer et al., 2001b), or the dynamic state of the network, as indicated by spontaneous EEG fluctuations (Hess et al., 1987; Rossini et al., 1991; Fadiga et al., 1995). One particular piece in the complex puzzle is the electric anisotropy of the cortex which is well documented in the motor system. An induced current oriented perpendicular to the course of the precentral gyrus evokes a motor response more efficient compared to an orientation in parallel to the course of the gyrus (Brasil-Neto et al., 1992; Mills et al., 1992).

Furthermore, the different current directions evoke qualitatively different stimulus patterns in the pyramidal tract (Werhahn et al., 1994; Di Lazzaro et al., 2004). Since axons oriented in parallel to the electric field gradient have the lowest threshold (Rushton, 1927) a common explanation for this phenomenon is based on the histological observation that the orientation of certain interneuronal axons in the motor cortex is not equally distributed but tend to run in a direction perpendicular to the precentral gyrus (Landgren et al., 1962; Marin-Padilla, 1970; Jones, 1975; Gatter and Powell, 1978).

Cortical anisotropy in the context of TMS has also been reported from two other regions, the prefrontal cortex and the occipital cortex. In a memory-guided saccade task Hill et al. (2000) demonstrated cortical anisotropy for the prefrontal cortex. TMS disturbed the saccade task most effectively with an antero-lateral current direction over the right prefrontal cortex. In the visual system, phosphene thresholds depend on the orientation of the current direction. Lower phosphene thresholds were observed with currents oriented from lateral to medial, as compared to currents from medial to lateral (Meyer et al., 1991; Kammer et al., 2001a). This finding was recently confirmed with a short-term double pulse paradigm (Sparing et al., 2005), but not with single pulses. Furthermore, it remains unclear whether vertical currents are less or more effective than horizontal currents (Kammer et al., 2001a; Sparing et al., 2005).

In the present study we set out to investigate cortical anisotropy in the occipital cortex in more detail, using frameless stereotactic
navigation. We first systematically compared phosphenes for the two vertical and two horizontal current directions. Besides current direction we included monophasic and biphasic pulse forms as a second independent parameter. In a second experiment, we mapped phosphenes at one stimulation site over the full range of 360° in steps of 45°. Results were related to the individual geometry of cortical gyri using anatomical MR data.

**Methods**

**Subjects and setup**

Fifteen healthy subjects (age 21 to 40 years, 4 male, 11 female) participated in the studies after giving written informed consent. The study was approved by the local internal review board of the Medical Faculty, University of Ulm.

Subjects were stimulated with a Medtronic Magpro stimulator (Skovlunde, Denmark, year of manufacture 1995, green box), using a figure-of-eight coil, MC-B70, fixed on a tripod. Maximal stimulation rate was restricted to 0.25 Hz. Coil position in relation to the head was monitored and registered continuously in all six degrees of freedom (three translational and three rotational) with the frameless stereotactic positioning system BrainView 2 (see Appendix A). Brain anatomy of the individual subjects (second experiment) was measured on a Siemens Sonata 1.5 T MR-scanner (TR = 1.3 s, TE = 3.22 ms, TI = 660 ms, flip angle = 15°, 192 sagittal slices, matrix 256 × 256, voxel size = 1×1×1 mm). A 3d model of the cortical surface was calculated by segmentation of the border between the grey and white matter using the automatic segmentation tools from BrainVoyager 2000 (V 4.9, Brain Innovation, Maastricht, The Netherlands).

In a first session subjects were familiarized with the occipital stimulation and the observation of phosphenes. Subjects were sitting in a comfortable chair and fixated a spot on a white screen in a slightly illuminated room. Phosphenes perception had to fulfill the following three criteria (Kammer et al., 2005): a) dependence on the stimulated hemisphere, i.e. perception in the left visual field while the subject was stimulated with a suprathreshold intensity of the interhemispheric cleft as a vertical plane. All current directions reported here indicate the initial fast rising phase of the current pulse induced in the brain (Kammer et al., 2001b).

Orientations of the coil were visually referred to the orientation of the interhemispheric cleft as a vertical plane. All current directions reported here indicate the initial fast rising phase of the current pulse induced in the brain (Kammer et al., 2001b).

**Experimental procedure**

Phosphenes perception thresholds from the left occipital pole were measured following a previously established protocol (Kammer et al., 2001a). First, the stimulation site was determined by moving the coil in steps of about 5 mm over the left occipital pole while the subject was stimulated with a suprathreshold intensity known from the familiarization procedure (biphasic pulse form, induced current direction latero-medial) until he or she observed a sharply delineated phosphenes clearly restricted to the right visual field (“hot spot”). This coil position was stored in the navigation system. Then, for each stimulation condition 100 magnetic stimuli were delivered at 10 different stimulator output intensities in steps of 3%. All stimuli were randomly intermixed (method of constant stimuli). A computer program presented the actual stimulation intensity on a screen and the experimenter transferred it to the stimulator manually. Then the subject released the magnetic stimulation with a keypress, observed the white screen and reported the presence or the absence of a phosphen after each stimulus (‘yes–no’) by pressing one out of two response keys. After the response, the delay time of 4000 ms started and the experimenter transferred the next stimulation intensity to the stimulator. An acoustical signal indicated the end of the delay time allowing the subject to release the next magnetic stimulus. Thus, a trained subject reached an average stimulation frequency of less than 0.2 Hz (4000 ms + decision time + reaction time to the acoustical signal).

In experiment 1 thresholds were measured for the two horizontal and the two vertical current directions left–right (latero-medial, LM), right–left (medio-lateral, ML), down-up (caudo-rostral, CR), and up-down (rostro-caudal, RC), and for the two pulse forms monophasic and biphasic, giving eight measurements in total per subject. Different current directions were obtained by rotating the coil around the axis perpendicular to the coil plane. For each measurement the focus of the actual coil position was carefully readjusted to the position stored after completing the search procedure described above. During the threshold measurement coil position was monitored online with the navigation system and readjusted in case of deviation. No anatomical data were used in experiment 1.

In experiment 2 the initial coil position was selected as in experiment 1 by determining a “hot spot” for the induction of a phosphen. In addition, the individual cortical anatomy of the subject was considered. The focus of the coil was navigated over an occipital gyrus with a straight, non-bent course. In addition to the eight current directions of experiment 1 eight intermediate current directions were adjusted by coil rotation, resulting in eight current directions in steps of 45°. Thresholds were determined for all eight current directions with monophasic and biphasic pulse forms (16 measurements).

Orientations of the coil were visually referred to the orientation of the interhemispheric cleft as a vertical plane. All current directions reported here indicate the initial fast rising phase of the current pulse induced in the brain (Kammer et al., 2001b).

**Data analysis**

A sigmoidal function (Boltzmann, see Kammer et al., 2001a) was fitted to the reported responses over stimulation intensity. The stimulation intensity at 50% ‘yes’-responses was taken as the phosphen threshold.

In experiment 2 the effect of current direction on thresholds was calculated in Matlab (R14, The MathWorks, Natick, MA, USA) as follows. First, the mean stimulation site was determined as the mean of the eight individually stored coil positions in all three dimensions (Fig. 1a). Then eight new sites were calculated by shifting the mean stimulation site into the direction of the induced current by an amount equal to the threshold measured for the particular current direction. The three mutually orthogonal eigenvectors of the (3 × 3) covariance matrix from the N = 8 new sites were calculated. First and second eigenvector represent current orientations associated with lowest and highest threshold, respectively. Estimated lowest and
highest thresholds were a function of the eigenvalues corresponding to the respective eigenvectors: If $L$ is an eigenvalue and $N$ the number of samples, than with an equal spacing of orientations, threshold is $(2LN/(N−1))^{1/2}$. The third and smallest eigenvalue represents the variance among coil orientations, i.e. tiltings of the coil plane. The first two eigenvectors, together with their eigenvalues, determine an ellipse in 3d.

Original and new coil positions were generated as 3d surface files with arrows indicating the induced current direction and visualized together with the reconstructed cortical surfaces in BrainVoyager (Figs. 1 and 3). From the mean stimulation site, four straight lines were plotted into the direction of the first and second eigenvectors and the opposite directions, ending at the circumference of the ellipse. A gradient was determined as the ratio of the first and second eigenvalue, representing highest and lowest thresholds. The orientation of the underlying gyrus was determined visually (white bars in Fig. 3) in order to measure the angle between the first eigenvector and the orientation of the gyrus (Table 1).

The inference statistics was done using Statistica (V 7.1, StatSoft, Tulsa, OK, USA).

Results

Experiment 1

Nine subjects participated in experiment 1. Overall stimulation conditions phosphene thresholds varied between subjects in a large range from 14.1% to 89.7%. Thresholds were subjected to a repeated measure ANOVA with the factors PULSE FORM (monophasic, biphasic) and CURRENT DIRECTION (LM, ML, CR, RC). A significant main effect was obtained for PULSE FORM ($F(1,8)=13.4, p<.01$). Monophasic pulses reached a higher threshold (49.2%) than biphasic pulses (36.7%). While no main effect was observed for CURRENT DIRECTION ($F(1,8)=1.1, p=.37$), the interaction of PULSE FORM×CURRENT DIRECTION was significant ($F(3,24)=7.0, p<.005$, Fig. 2).

For the monophasic pulses post-hoc tests (LSD) showed that the horizontal current LM was significantly lower than ML (45.3% vs. 49.9%, $p<.001$). LM was also significantly lower than the vertical currents CR (50.2%, $p<.001$) and RC (51.2%, $p<.0001$). For the biphasic pulses ML was lower than LM (35.2% vs. 37.5%) but did not formally reach significance ($p=.07$). The highest threshold was obtained with the vertical current direction RC

![Fig. 1. Method to calculate the main current axes in experiment 2. View on the occipital pole from left lateral (left column) and from above (right column). (a) Light blue arrows: superposition of the 8 coil positions over the stimulation focus from varying current directions in steps of about 45°. The arrows indicate induced current directions. Yellow line: orientation of mean coil position. Dark blue arrows: mean stimulation site shifted into the direction of the induced current. The amount of shifting reflects the measured phosphene threshold. (b) Red line parallel to the yellow line: center of the covariance matrix from the 8 shifted coil positions. Blue and red lines: orientation of first and second eigenvector, respectively. The longer segment is drawn as thick line, the shorter segment is drawn as thin line. The asymmetry of the axes results from the shift of the center (red line) to the mean stimulation site (yellow line). Data from JM biphasic.](image-url)

![Table 1 Phosphene thresholds, orientation of gyrus and eigenvector transformation (experiment 2)](table-url)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pulse form</th>
<th>Mean threshold (%)</th>
<th>Gyrus orientation (°)</th>
<th>First eigenvector orientation (°)</th>
<th>Delta angle (°)</th>
<th>Gradient</th>
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<td>98</td>
<td>1</td>
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<tr>
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<td>6</td>
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<td>62</td>
<td>82</td>
<td>1.08</td>
</tr>
</tbody>
</table>

mono: monophasic, bi: biphasic. Gyrus orientation as determined visually on the reconstructed brain surfaces in the mean coil plane. Zero degree is a horizontal orientation of the gyrus, positive angle is oriented counterclockwise. Delta angle is the deviation of the first eigenvector from gyrus orientation, gradient is the ratio of first and second eigenvalue, see text.

![Fig. 2. Influence of pulse form and current direction on phosphene thresholds, given as percent of maximum stimulator output. Data show mean values±S.E.M. from 9 subjects. The abscissa indicates different current directions. LM latero-medial, ML medio-lateral, CR caudo-rostral, RC rostro-caudal. For statistics, see text.](image-url)
(38.4%) that significantly differed from CR (35.8%, \( p < .05 \)) and from ML (\( p < .05 \)). Post-hoc differences between monophasic and biphasic pulses were all significant.

**Experiment 2**

Nine subjects including four subjects from experiment 1 participated in experiment 2. Due to technical difficulties, complete datasets with thresholds for all 8 current directions in steps of 45° were only obtained from 5 subjects in the monophasic mode, and from 7 subjects in the biphasic mode. The orientation of the first and second eigenvector in relation to the orientation of the underlying gyrus is shown in Fig. 3. The orientation of the gyrus under the focus of the magnetic stimulation site (Fig. 3, white bars) varied considerably among subjects (Table 1). In 10 out of 12 datasets (83%) the first eigenvector representing highest phosphene thresholds is oriented close to the course of the gyrus. The absolute values of deviation range from 1° to 26°, mean deviation was 14.6°. In the remaining 2 datasets (EG bi, and JG bi) deviations range from 63° to 82°, indicating that not the first but the second eigenvector is orientated close to the course of the gyrus. To estimate the amount of asymmetry in the eigenvector transformation a gradient was calculated as the ratio between the estimated highest and lowest thresholds. The mean gradient was 1.08, range 1.04 to 1.13.

The relation of monophasic and biphasic pulses was determined from the measured phosphene thresholds in the pairs of opposite current directions next to the second eigenvector. Data from all 7 subjects were included since the corresponding monophasic values in the incomplete datasets (JG mono and EG mono) did exist. In Fig. 4, mean values for these optimal and non-optimal current directions were shown for both pulse forms. The optimal current direction was defined as the direction with the lower threshold in the threshold pair from monophasic pulse form. Optimal current direction was flipped with biphasic pulses compared to the monophasic ones (interaction PULSE × CURRENT DIRECTION, \( F(1,6) = 11.1, p < .05 \)).

No significant relation between threshold level and correspondence of current directions to gyrus orientation was found (\( p = .55 \)).

**Discussion**

Our results show that biphasic pulses have lower thresholds for phosphenes than monophasic pulses. Concerning optimal current directions, in experiment 1 an inconsistent pattern with respect to the pulse forms was obtained. With monophasic pulses a latero-medial current direction revealed lower thresholds compared to medio-lateral direction and the two vertical current directions. With biphasic pulses thresholds tended to be lower with latero-medial currents compared to medio-lateral currents (almost significant difference). Vertical current directions were significantly different for biphasic pulses, but showed no difference with monophasic pulses. Experiment 2 gave an explanation for the inconsistent pattern observed in experiment 1. The optimal current direction for both biphasic and monophasic pulses strongly depends on the individual orientation of the occipital gyrus underlying the stimulation site. In 10 out of 12 recordings the lowest thresholds were obtained with current directions perpendicular to the gyrus.

![Fig. 3. Optimal current directions projected onto individual visual cortices in 12 cases (7 subjects). The main direction of the stimulated gyrus is depicted as a white bar. Blue arrows indicate the mean stimulation sites shifted by the amount of measured phosphene threshold into the direction of the induced current. Orientations of the first and second eigenvector are shown as blue and red lines. The second eigenvector indicates reconstructed current direction with lowest phosphene thresholds. Symbols and colors are similar to Fig. 1. Each view is oriented perpendicular to the mean stimulation plane. Angles measured are given in Table 1.](image-url)
This orientation varies considerably among individuals, with no preference to any range of orientations. A systematic but different relation between optimal current direction and gyrus orientation for monophasic and biphasic pulses was found: The optimal current direction for monophasic pulses was non-optimal for biphasic pulses and vice versa.

**Neuronavigated TMS and current directions**

Frameless stereotactic systems that control coil position relative to a subject's head and brain have become the advanced standard in TMS research (cf. Herwig et al., 2001; Thielscher and Kammer, 2002; Sack and Linden, 2003). In most neuronavigated experiments the position of the coil's focus has been controlled with the target region defined either in terms of neuroanatomy or by means of functional imaging. The present study applies neuronavigation in a more advanced way. The online monitoring facility allowed us to change coil orientation carefully in steps of 45° while keeping the focus of the coil constant. Data analysis was based on the stored coil positions using all six degrees of freedom. Individual neuroanatomy was considered with respect to the site and orientation of the stimulated gyrus in the occipital cortex. Visual determination of the gyrus’ orientation in a 2d view might be afflicted by an error. The main uncertainty here is due to curved gyri, as with subjects JG and EG. It might explain the non-correspondence between orientations of gyrus and major axis in those subjects.

**Optimal current orientation for phosphenes—relation of experiment 1 and experiment 2**

The dependence of optimal coil position to evoke phosphenes from the orientation of the underlying gyrus may explain the inconsistencies among results obtained previously. Some studies used a vertical position with currents oriented cranio-caudally or caudo-cranially (e.g. Boroojerdi et al., 2000; Stewart et al., 2001; Fumal et al., 2002) while others preferred a horizontal position (Kammer et al., 2001a; Kammer and Beck, 2002; Gerwig et al., 2003). As a preliminary result from 3 subjects we reported higher thresholds in vertical than in latero-medial current directions, but lower thresholds in the opposite medio-lateral current directions (Kammer et al., 2001a). In a recent study comparison of phosphene perception with horizontal and vertical current directions independently of gyrus orientation showed no difference for single pulses but an advantage for latero-medial currents with double pulses at an inter-stimulus-interval of 12 ms (Sparing et al., 2005). In the visual system, response differences due to different current orientations were smaller than in the motor system (Kammer et al., 2001b). Polar plots were less elongated and less asymmetric than those calculated from gradients in the motor system (Brasil-Neto et al., 1992; Mills et al., 1992). Nevertheless the gradients obtained with phosphene thresholds were sufficient to determine optimal and non-optimal current directions perpendicular and parallel to the orientation of the underlying gyrus in the majority of our cases. This may explain the weak result of experiment 1 as well as the inconsistent findings in the literature. The orientation of the gyrus stimulated at the occipital pole highly differs interindividually, in contrast to the orientation of the precentral gyrus. Therefore, a rule of thumb for the optimal coil orientation to evoke phosphenes does not exist without knowledge about the individual orientation of the stimulated gyrus.

**Influence of pulse form on phosphene threshold**

Two observations in the present study parallel the findings in the motor cortex. (i) A biphasic pulse is more efficient compared to a monophasic pulse (Claus et al., 1990; Niehaus et al., 2000; Kammer et al., 2001b; Sommer et al., 2006). The polarity change in the field gradient seems to recruit more neurons, thus lowering the threshold. This could either be caused by a sequence of hyperpolarization and depolarization recruiting more sodium channels compared to a pure depolarization (Maccabee et al., 1998). An alternative explanation would be that optimal neuronal responses depend on the duration of the induced current in its optimal orientation (Maccabee et al., 1998; Davey and Epstein, 2000). (ii) The optimal current direction with monophasic pulses differs from the optimal current direction with biphasic pulses by 180° (Kammer et al., 2001b; Sommer et al., 2006). This flip could be explained within the same framework. The second phase of the biphasic pulse seems to need the same orientation as the monophasic pulse in order to evoke neuronal responses at lowest thresholds. Using visual masking a similar flip between monophasic and biphasic pulse forms has been observed in the visual cortex (Corthout et al., 2001). In general, the finding that neuronal responses depend on current directions points to the fact that cortical networks have anisotropic properties.

**Anisotropy of the cortex**

Our finding that phosphene thresholds are lowest with currents oriented perpendicular to course of the stimulated gyrus in the visual cortex exactly parallels the constellation in the motor cortex (Brasil-Neto et al., 1992; Mills et al., 1992). In the motor system anisotropy for TMS manifests with two different observations: (i) motor thresholds and muscle action potentials depend on current direction and pulse form (Niehaus et al., 2000; Kammer et al., 2001b; Sommer et al., 2006), (ii) the pattern of cortico-spinal response varies with current direction (Werhahn et al., 1994) and pulse form (Di Lazzaro et al., 2001a). The second
finding suggests that with different current orientations different neuronal populations were excited. Direct recording of the cortico-spinal tract (pyramidal cells from motor cortex) demonstrates a response pattern characteristic for the motor system that contains a burst of waves at a frequency of about 600 Hz. The waves are generated by an excitation of the pyramidal axon close to the cell body (direct or D-wave with the shortest latency, Patton and Ammassian, 1954) as well as by transsynaptic excitation of the pyramidal neurons (indirect or I-waves with longer latencies, for review see Ziemann and Rothwell, 2000; Di Lazzaro et al., 2004). An important observation in this context is that TMS at threshold does not evoke a D-wave but rather, dependent on current orientation, an I1- or I3-wave (Day et al., 1962; Marin-Padilla, 1970; Jones, 1975; Gatter and Powell, 1978) as well as by transsynaptic depolarization horizontally oriented axons from grey matter inter-neurons projecting to pyramidal cells. Fibers of intrinsic neurons, e.g. large multipolar basket cells, have longer horizontal axons perpendicular to the orientation of the gyrus (Landgren et al., 1962; Marin-Padilla, 1970; Jones, 1975; Gatter and Powell, 1978) thus resulting in the observed electrical anisotropy.

The visual cortex does not generate D- and I-waves as an output signal. Nevertheless, the observation of a preferred direction perpendicular to the course of the gyrus as well as the asymmetry of phosphene thresholds along the optimal direction suggest a common underlying principle of anisotropy. In some anatomical studies on the visual cortex anisotropic projections have been observed in the grey matter (Kritzer et al., 1992; Budde and Kisvarday, 2001). However, a homogeneous distribution of these cells with respect to macroscopic cortex orientation has not been described yet. It should be noticed that the gradients forming anisotropy in the visual cortex are much lower than the gradients known from the motor system (Brasil-Neto et al., 1992; Mills et al., 1992) where the polar plots determined by responses to different current directions were much more elongated. Taken together, we might expect certain common aspects in the architecture of motor and visual cortices causing electrical anisotropy as well as certain specific differences in architecture, that account for the quantitative differences and for differences in the output patterns.

Two modeling approaches to cortical effects induced by TMS consider cortical anisotropy. With a cortico-thalamic loop including a 3-layered model of the motor cortex Esser et al. (2005) were able to generate I-wave patterns as well as other TMS-related phenomena in the motor system. They modeled differences in I-wave patterns due to different current orientations on assumption that currents with postero-anterior orientation depolarize cortical interneurons whereas currents with antero-posterior orientation mainly depolarize somatosensory afferents to the precentral gyrus. Miranda et al. (2003) put forward a different model. Using finite elements to calculate induced field distributions, they demonstrate that the boundary effects between cerebrospinal fluid, grey matter, and white matter remarkably distort the electric field. Considering the homogeneous orientation of axons in the white matter close to the grey matter border they argue that this electrical anisotropy might enhance or attenuate electric field gradients acting in the grey matter. Here the course of pyramidal axons from the cortical banks within a sulcus might play a crucial role. Starting from the cell body axons are initially oriented perpendicular to the course of the gyrus until they reach white matter. The majority then bends about 90° to leave the cortex forming the large bundles in the deep white matter. These initial parts of pyramidal axons are distributed in a highly anisotropic fashion in accordance with the observed preferred current direction. However, this hypothesis does only account for the orientation preference, but not for the observed asymmetry along the optimal current orientation. Furthermore, the pattern differences in I-waves observed in the motor system cannot be explained by the approach of Miranda et al. (2003).

**Conclusion**

Cortical networks seem to behave anisotropically when depolarized by TMS. In both the motor and the visual system optimal current direction is perpendicular to the course of the underlying gyrus. Since anisotropic effects have been observed also in prefrontal regions (Hill et al., 2000) it is very likely that they occur all over the cortex as a general feature. Therefore, coil orientation should not only be considered in motor experiments but in any cortical region stimulated. This holds true in particular if interindividual variability of gyrus orientation is as high as in the occipital cortex. Further investigations considering different current orientations are required to estimate the amount of anisotropy in different cortical regions.

**Appendix A. BrainView: a frameless stereotactic system for TMS navigation**

The TMS navigation system BrainView (Version 2) has been developed by the Fraunhofer Institute for Manufacturing Engineering and Automation (IPA) in Stuttgart, Germany, in cooperation with the Max Planck Institute for Biological Cybernetics, Tuebingen, Germany, and the University of Ulm, Germany. The software is running on a Windows-PC with an Open-GL graphics board. Any 3D tracker providing relative coordinates between two freely moving tools (six degrees of freedom) can be integrated into the system, in the Ulm installation the Image Guided tracker (Image Guided Technologies Inc., Boulder, CO, USA) is used. The following features are implemented in the system:

1. Display of the brain anatomy on the basis of an T1 data set (isotropic voxels of 1 mm3) with three orthogonal slices and a composed 3D view on the three slices. The file format is compatible to BrainVoyager vmr.
2. Display of a 3D cortical surface model from BrainVoyager (srf file).
3. Display of statistical maps from functional MRI studies,
4. Online tracking and display of a coil position relative to the head,
5. Storage of any coil position and display of stored positions together with the online coil position to reorient the coil to a known position,
6. Subject registration by two reference procedures.

Prior to any navigation session a 3D reference tool is attached to the handle of the TMS coil. Origin and orientation of the coil with respect to the reference tool are defined with a 3D pointer. The 3D tool is attached to the subject’s head, here by means of an elastic strap.
Coil positions are referred to a head coordinate system defined by the three anatomical landmarks as fiducials: left ear (−X) right ear (+X) and nasion (+Y). As ear points we chose the incision between crus helicis and tragus. The Y axis is perpendicular to the X axis, the Z axis perpendicular to X and Y pointing upwards (right hand coordinate system). In a first step the three landmarks are defined in a subject with a 3D pointer and the distances are stored. Any additional anatomical landmark (e.g. inner canthi, bases of alae nasi) can be defined in relation to the head coordinate system. The exact positions of the landmarks should be photographed with the initial registration while pointing on them. Furthermore, to prepare a proper reference to an MR-picture (step (ii) see below), contours of the surface can be stored with the initial registration of a subject. Re-referencing (e.g. a new session with the same subject) requires at least the redefinition of the three basic anatomical landmarks with the pointer. The actual transformation matrix is determined with a least square fit on the stored distances. Deviations to the originally stored positions are shown in millimeters. This reference procedure is sufficient to store any coil position with respect to the head coordinate system in order to reposition the coil in a subsequent session. No MR picture of the subject is required.

The head coordinate system is referred to the individual anatomical MR picture with a second transformation matrix. In the actual version of the software the determination of this second matrix is not implemented yet but has to be done with BrainVoyager. On the surface model of the skull (*.srf) the position of the anatomical landmarks are marked and with a least square fit the transformation matrix is determined. Though not formally tested our impression is that a surface matching using the stored contours of the face and skull (see above) results in a transformation matrix much more precisely compared to the sole fiducial fit. We did not use the raw T1 data set but reoriented it according to the AC–PC axis and the interhemispheric axis using BrainVoyager tools. We did not normalize the individual anatomical data sets.

The quality of the reference procedures are controlled as follows:

(i) The precision of re-referencing is given by the deviations of the anatomical landmarks to the originally stored constellation (Euclidian distance). It is quite easy to reach deviations below 3 mm. With a little practice deviations below 2 mm are possible. In the present study a criterion to start an experimental session was to keep the maximum deviation below 2 mm.

(ii) The quality of the reference to the MR picture can only be controlled on-line using a pointer device. Any position at the skull should be at the skull level in the MR picture, too. We are not aware of a more formal procedure to test the quality of this reference procedure.

Fig. A1. Screenshot of a navigation situation in BrainView. The three orthogonal slices of an anatomical MR scan are displayed in the upper two quadrants and in the lower right quadrant. In the lower left quadrant the 3d view composed by the three orthogonal slices is shown. It can be rotated with the mouse. The relative size of the quadrants can be changed. Two superimposed coil positions over the occipital pole are depicted by colored coordinate systems. One position (marked by white dots) has been stored, the other depicts the actual coil position. In the marked coil position the induced current is oriented latero-medially, in the other position the coil is rotated by 45°. Long green axis — handle position, red axis — orientation of the two wings, blue axis — axis perpendicular to the coil surface: focus of the induced electric field. The two blue axes are parallel to each other but not match each other. In the present experiments the actual coil position carefully would have been corrected in order to match the blue axis of the stored coil position.
The on-line navigation of a coil position allows to adjust the center of the coil with the maximum field strength over a target region of the brain. This could be a cortical region determined by functional MRI. Alternatively, any anatomical landmark defined in the individual anatomical scan may serve as a target region. In the composed 3d view the coil positions were visualized by means of a self-defined geometrical model consisting of lines and circles (Fig. A1). The most descriptive model of a focal coil contains the handle of the coil as well as the two wings. However, for the purpose of navigation we prefer a simple 3d coordinate system with separate colors for each of the axes. The geometrical models are stored and can be changed during navigation. In order to place the coil over a target region, the axis perpendicular to the coil plane should reach the region. Furthermore, the orientation of the coil plane has to be tangentially to the surface of the skull. Therefore, all three axes of the coil should be visualized.

Repositioning of a coil position stored previously just requires to align the online coil position with the display of the stored position. Here, a simple coordinate system with separate colors for each of the axes is much more informative compared to a naturalistic coil model. In the present study we used this kind of coordinate system in order to rotate the coil around the axis perpendicular to the coil’s plane centered over the occipital gyrus chosen for stimulation.

References


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