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The temporal characteristics of motion processing in hMT/V5+: Combining fMRI and neuronavigated TMS

Alexander T. Sack,[a,*,1] Axel Kohler,[b,c,1] David E.J. Linden,[b,d,e] Rainer Goebel,[a] and Lars Muckli [b,c]

[a]*Department of Neurocognition, Faculty of Psychology, Maastricht University, PO Box 616, Postbus 616, 6200 MD Maastricht, The Netherlands*
[b]*Department of Neurophysiology, Max Planck Institute for Brain Research, Deutschordenstr. 46, 60528 Frankfurt am Main, Germany*
[c]*Brain Imaging Center Frankfurt, Schleusenweg 2–6, 60590 Frankfurt am Main, Germany*
[d]*School of Psychology, University of Wales, Bangor, UK*
[e]*Department of Psychiatry, Johann Wolfgang Goethe University, Heinrich-Hoffmann-Str. 10, 60528 Frankfurt am Main, Germany*

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Functional imaging has demonstrated the specific involvement of the human middle–temporal complex (hMT/V5+) during processing of moving stimuli. Some studies applied transcranial magnetic stimulation (TMS) to investigate the causal relevance of hMT/V5+ for motion perception. Although the studies used similar visual stimuli and TMS parameters, the critical time point of functionally relevant hMT/V5+ activity differed by 100 ms and more.

The present study aimed to elucidate further the temporal characteristics of motion processing in hMT/V5+ by investigating all critical time windows currently debated in the literature. In contrast to previous studies, we used TMS neuronavigation based on individual fMRI results of five participants to target hMT/V5+, applying single-pulse TMS at 24 different time windows (−50 till +200 ms relative to stimulus onset).

We revealed that TMS significantly impaired motion perception when applied over hMT/V5+ at 40 to 30 ms before as well as 130 to 150 ms after onset of the moving stimuli. While the late effective time window conforms to results from previous experiments, we did not find evidence for an early time window around 0 ms that has been reported in other studies.

Our neuronavigation approach enabled us to quantify the inter-individual variance in the exact location of hMT/V5+ and the respective TMS target position on the skull of the participants. Considering that shifting the TMS coil position only by a few millimeters can already lead to a complete loss of TMS effects, our study clearly demonstrates the utility of neuronavigated TMS when investigating specific neuronal effects as in the case of motion processing.

Introduction

The perception of motion represents one of the most crucial abilities of our visual system, enabling us to survive in a dynamically changing environment. A large number of electrophysiological (Albright et al., 1984; Cook and Maunsell, 2002; Diogo et al., 2003; Snowden et al., 1991; Van Essen et al., 1981; Zeki, 1974) and functional imaging studies (Gulyas et al., 1994; Heeger et al., 1999; Huk et al., 2002; Rees et al., 2000; Seiffert et al., 2003; Tootell et al., 1995b; Watson et al., 1993; Zeki et al., 1991; see Orban et al., 2004 for a review) have shown that within the network of specialized regions in the visual system, the extrastriate visual area hMT/V5+, located in the occipitotemporal cortex, is specifically activated during the processing of moving stimuli.

Although likely, this evidence alone does not allow inferences on the causal role of neuronal activity in hMT/V5+ for motion perception. A causal relationship can only be inferred if a documented change of activity in hMT/V5+ causes a related change in motion perception. Neuronal activity in hMT/V5+ can be disrupted due to lesions or it can be manipulated experimentally by transcranial magnetic stimulation (TMS) in humans (Sack and Linden, 2003) or cooling in animals (Hupé et al., 2001). Several lesion (Baker et al., 1991; Newsome and Paré, 1988; Vaina and Cowey, 1996; Zihl et al., 1983) and TMS studies (Anand et al., 1998; Beckers and Hömberg, 1992; Beckers and Zeki, 1995; d'Alfonso et al., 2002; Hotson et al., 1994; Hotson and Anand, 1999; Walsh et al., 1998) have confirmed a causal relation between hMT/V5+ activity and performance on visual motion tasks. The demonstration that TMS over hMT/V5+ can also disrupt the storage and perception of the so-called motion after-effect (Théoret et al., 2002; see also Antal et al., 2004b) and even produce moving phosphenes (Antal et al., 2003, 2004a; Campana et al., 2002; Stewart et al., 1999) complements the evidence that this area is functionally relevant for the processing of moving stimuli.

* Corresponding author. Fax: +31 43 3884125.
E-mail address: a.sack@psychology.unimaas.nl (A.T. Sack)
[1] These authors contributed equally to this work.
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The systematic use of single-pulse TMS (spTMS) with its high temporal resolution in the millisecond range allows not only the identification of the functional relevance of a particular brain region, but also the assessment of the exact time point at which this neuronal activity is critical. Accordingly, spTMS has been used to induce transient deficits in visual motion detection (Beckers and Hömberg, 1992). The authors displayed a pattern of randomly moving dots and observed a modulation of motion detection when stimulating hMT/V5+ within a time window of 10 ms before and 10 ms after stimulus onset. The magnetic stimulation of area hMT/V5+ selectively impaired motion perception without interfering with basic recognition of the stimuli or color perception in a control task. A similar study replicated the general result but identified as the critical time window for the impairment of motion detection the interval from 100 to 150 ms after stimulus onset (Hotson et al., 1994). On the basis of further studies on the temporal aspects of effective TMS in visual processing, Beckers and Zeki (1995) speculated that there might be two different visual pathways to area hMT/V5+. Signals would be relayed to hMT/V5+ both directly from the thalamus and indirectly via V1. This might account for the different time windows identified by Beckers and colleagues (Beckers and Hömberg, 1992; Beckers and Zeki, 1995) and Hotson et al. (1994).

A complementary or alternative interpretation of the differing results on critical time windows in motion processing is that TMS affected different brain regions in different studies. All of the aforementioned spTMS studies were limited in their spatial accuracy in that they localized the target site for TMS merely on the basis of an anatomical-landmark criterion. The authors of these studies argued that there are several lines of evidence suggesting that a site 5 cm lateral and 3 cm dorsal to the inion would overlie hMT/V5+. The main argument for this assumption was that imaging studies had demonstrated the correspondence of those coordinates with hMT/V5+ (Watson et al., 1993; Zeki et al., 1991). Hence, these spTMS studies simply used the scalp position based on the anatomical landmarks in every participant, assuming that the cranial coordinates would generally identify the appropriate stimulation site for hMT/V5+ and that the effects of interindividual variance in anatomical and functional organization are negligible. Yet, Watson et al. (1993) also demonstrated that the position of area hMT/V5+ itself is not constant from one individual to the next and can vary by as much as 27 mm in the left, and 18 mm in the right hemisphere.

An earlier attempt to account for intersubject variability in determining the stimulation site for MT (d'Alfonso et al., 2002) was based on the individual interference maps obtained by TMS. In their procedure, the center of a two-dimensional matrix of 3×3 cm (nine points) was placed over the T5 electrode position, according to the international 10–20 EEG system. Each of the nine points was stimulated for 10 different delays, while participants performed a motion-direction discrimination task. Their method of localizing hMT/V5+ used the center-of-gravity position and thus provided an individual map of functional activity in the hMT/V5+ area. Yet, as the authors themselves acknowledged, the most accurate method of localizing the stimulation site for TMS would rather be based on the individual activation map of every participant revealed by functional imaging.

In this study, we used functional magnetic resonance imaging (fMRI) to precisely map the area hMT/V5+ in every single participant. Based on this individual functional activation map, we neuronavigated the TMS coil for magnetic stimulation to the appropriate cranial coordinates, using a frameless stereotaxic co-registration system developed by our group. We applied spTMS at 24 different time intervals, covering all critical time windows that are debated in the literature. In particular, our aim was to determine whether the motion perception is disrupted by hMT/V5+ stimulation during the very early (around stimulus onset) or during the later period (around 150 ms) and whether both time windows might be critical in the same individuals.

Materials and methods

Participants

Five volunteers (mean age = 29.2 years; range = 25 to 37 years) participated in the study. All participants were right-handed and had normal or corrected-to-normal ($N = 1$) vision, and had no history of neurological or psychiatric disorder. The participants were informed about MRI and TMS and received a questionnaire to check for potential health risks and contraindications. Volunteers gave their informed consent after being introduced to the procedure. The experiments were conducted in accordance with the Declaration of Helsinki.

Overall study design

In our study, we experimentally combined the methods of functional and anatomical MRI with TMS. This approach enabled us to define the site for TMS stimulation based on the individual fMRI measurements and to use a neuronavigation device to guide the TMS coil to the respective target locations. Participants were tested in six separate sessions: In the first session, functional and anatomical MRI measurements were obtained from all participants. The data were used to localize hMT/V5+ and to co-register an anatomical reconstruction of the participant's head with stereotaxic data recorded with an ultrasound digitizer (see below for details). In the second session, psychophysical thresholds were determined for the random-dot paradigm in individual participants and surface points from the participant's head were recorded with a digitizer. In the four TMS sessions, participants were stimulated with single-pulse TMS while they performed the random-dot task.

MRI measurements

Stimuli

Stimuli were generated with a custom-made program based on the Microsoft DirectX library and back-projected onto a frosted screen with a liquid-crystal-display projector. Participants viewed the screen through a mirror fixed on the head coil. Two stimulus conditions were used to identify hMT/V5+: (1) 400 white dots moving radially outward on a dark screen (visual field, $30 \times 23°$; dot size, $0.06 \times 0.06°$; dot velocity, 3.6–14.4°/s), and (2) 400 stationary white dots with the same stimulus parameters as in condition (1). The moving-dot stimulus is known to produce strong activation in hMT/V5+, in contrast to stationary dots (Goebel et al., 1998; Rees et al., 2000; Tootell et al., 1995b).

Design

The two experimental conditions (moving and stationary dots) were presented alternately in blocks of 16.6 s. The

conditions were always separated by a fixation period of the same length. Three blocks of moving dots and two blocks of stationary dots were used. Participants were asked to fixate and be attentive during the whole measurement. Eye movements were not monitored during MR imaging.

MR imaging

Functional MRI was done on the 1.5 T Siemens Magnetom Vision MR tomograph (Siemens, Erlangen, Germany) at the University Hospital, Frankfurt am Main, Germany, or the 3 T Siemens scanner at the Brain Imaging Center Frankfurt, Frankfurt am Main. For the scans at 1.5 T, a gradient-recalled echo-planar-imaging (EPI) sequence was used with the following parameters: 16 slices, oriented approximately parallel to the calcarine sulcus; TR, 2081 ms; TE, 60 ms; FA, 90°; FOV, 210 mm; in-plane resolution, 3.44×3.44 mm; slice thickness, 4 mm; gap thickness, 0.4 mm. At 3 T, parameters were: 34 slices; TR, 2080 ms; TE, 30 ms; FA, 85°; FOV, 210 mm; in-plane resolution, 3.3×3.3 mm; slice thickness, 3 mm; gap thickness, 0.3 mm. In addition, a high-resolution T1-weighted anatomical scan was acquired using a Siemens fast low-angle-shot (FLASH; Siemens Vision scanner) or magnetization-prepared rapid-acquisition gradient-echo (MPRAGE; Siemens Trio scanner) sequence.

MRI data analysis

Data were analyzed using the BrainVoyager 4.9 and BrainVoyager QX 1.4 software package (BrainInnovation, Maastricht, The Netherlands). The first four volumes of each scan were discarded to allow for T1 saturation. Preprocessing of the functional data included the following steps: (1) three-dimensional motion correction using the Levenberg–Marquardt algorithm, (2) linear-trend removal and temporal high-pass filtering at 0.01 Hz, (3) slice-scan-time correction with sinc interpolation. The statistical analysis was performed with multiple linear regression. For every voxel, the time course was regressed on a set of dummy-coded predictors representing the experimental conditions. The predictor time courses (box-car functions) were convolved with a gamma distribution to account for the shape and delay of the hemodynamic response (Boynton et al., 1996).

Co-registration of stereotaxic and MRI data

Stereotaxic data for the localization of the TMS stimulation site were recorded with the electrode positioning system CMS30P (zebris, Tübingen, Germany). This system consists of several miniature ultrasound transmitters, which are attached to the participant's head as well as the TMS coil. These ultrasound markers continuously transmit ultrasonic pulses to a receiving sensor device. The measurement of the relative spatial position of these transmitters in 3-D space is based on the travel time of the transmitted ultrasonic pulses to three microphones built into the receiving sensor. In a next step, local spatial coordinate systems are created by linking the relative raw spatial position of the ultrasound senders to a set of fixed additional landmarks on the participant's head. The specification of these fixed landmarks is achieved via a digitizing pen that also hosts two transmitting ultrasound markers in order to measure its relative position in 3-D space. The three anatomical landmarks we used to define the local coordinate system were the nasion and the two incisurae intertragicae. The system now provides topographic information of the head ultrasound transmitters relative to a participant-based coordinate frame. Similarly, the TMS coil also hosts a set of ultrasound transmitters whose relative spatial positions are linked to fixed landmarks specified on the coil in order to calculate another local coordinate system. After having defined the local spatial coordinate system for the participant's head and the TMS coil in real 3-D space, these coordinate systems have to be co-registered with the coordinate system of the MR space. For TMS-fMRI co-registration, the same landmarks digitized on the participant's head are specified on the head representation (mesh) of the participant in the fMRI software. Hence, using the BrainVoyager software, the anatomical landmarks were identified in the MRI scan of the participant's head and co-registered with the coordinates from the digitizer. As an additional constraint for the co-registration, a set of points covering the whole head were recorded from each participant. To correct for measurement errors (MRI and ultra-sound distortions), an algorithm fitted the additionally recorded surface points to the outer boundary of the MRI head reconstruction. After the landmarks specified on the real head are co-registered with those on the mesh head, events occurring around the head of the participant in real space are registered online and visualized in real-time at correct positions relative to the participant's anatomical reconstruction of the brain. By superimposing the functional data on the anatomical reconstruction of the brain, the TMS coil can be neuronavigated to a specific anatomical and/or functional activation area of every participant. TMS neuronavigation was based on data in AC-PC space (rotating the cerebrum into the anterior commissure-posterior commissure plane) in order to avoid any additional transformations that could distort the correspondence between MRI and stereotaxic points.

Although the described fMRI-based TMS neuronavigation represents the optimal methodological approach for positioning the TMS coil relative to an individual fMRI activation cluster, it should not be neglected that the spatial resolution of localized TMS is still hampered by the spatial distribution of the applied magnetic field itself. Hence, while the full digitization of the received sonic signals during the fMRI-TMS co-registration procedure guarantees a high measuring accuracy in the millimeter range in terms of exact TMS coil positioning, the spatial distribution of the applied TMS pulse still limits the accuracy of magnetic brain stimulation. This latter limitation in spatial accuracy is solely defined by the specific geometry of the used TMS coil and can only partly be addressed by TMS neuronavigation. Nonetheless, it has been shown that despite the limited spatial resolution of the applied magnetic field, a shift of the TMS coil position by only few millimeters can already result in a complete loss of the TMS-induced behavioral effect (Beckers and Hömberg, 1992; d'Alfonso et al., 2002).

Functional MRI data of individual participants were used to localize hMT/V5+ according to standard criteria (Muckli et al., 2002). A contrast between the motion and the static condition was computed (in participant P3, the contrast motion vs. static was too weak; we therefore used motion vs. baseline). The known anatomical landmarks and Talairach coordinates were used as additional constraints for the identification of hMT/V5+ (Dumoulin et al., 2000; Watson et al., 1993). The TMS stimulation site for hMT/V5+ was defined as the surface point whose normal vector intersected the hMT/V5+ cluster (see Figs. 1 and 2). The normal vector was approximated locally using

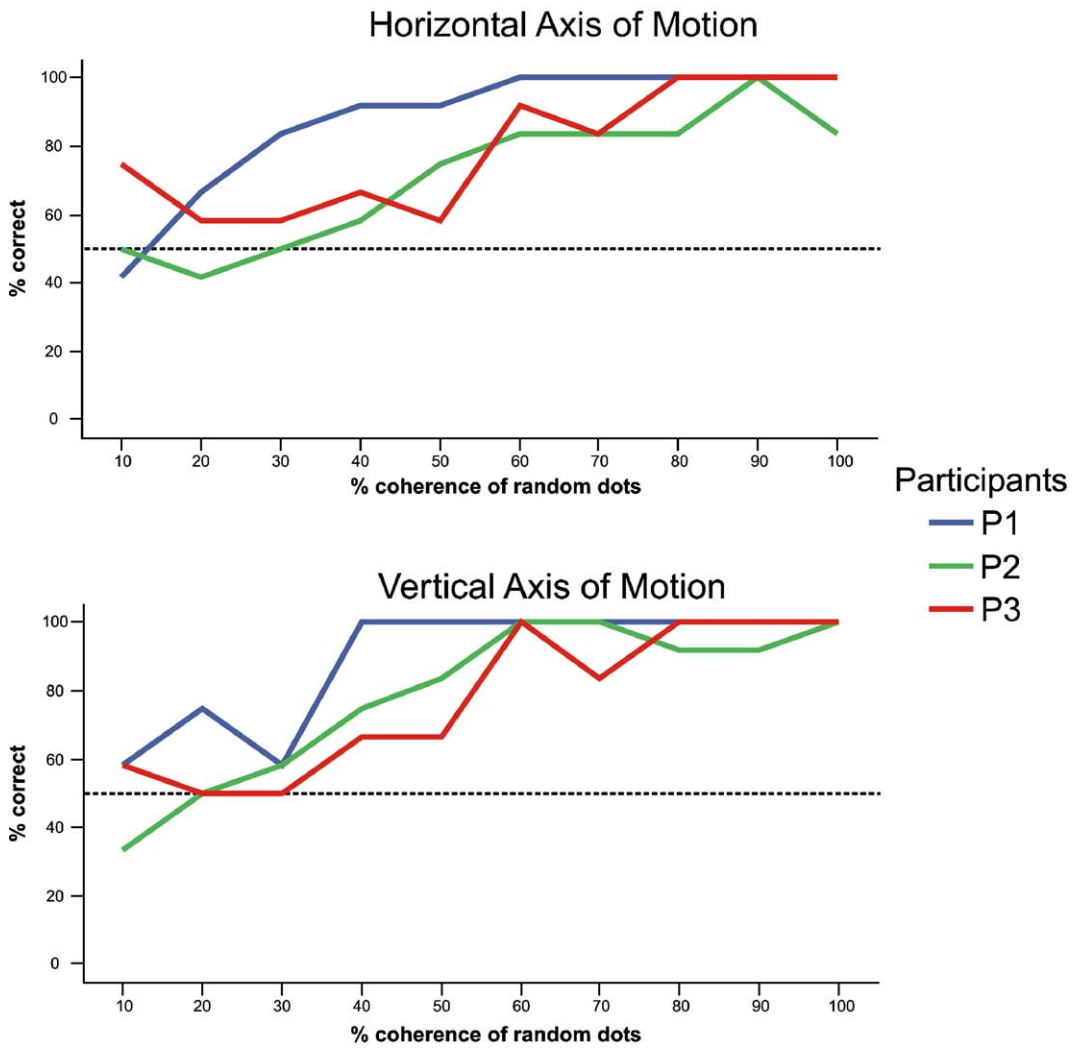

Fig. 1. Performance for three out of five participants in the random-dot task. In separate trials, vertical (up and down) and horizontal motion (left and right) were tested using the method of constant stimuli. At low coherence, performance dropped to chance levels (indicated with the dashed horizontal lines). At coherences of 40% to 60%, participants' performance reached almost perfect levels.

neighboring surface points. In addition, a control location was defined at a distance of 4 cm in the parasagittal plane superior to the individual hMT/V5+ surface point (Fig. 2). This site was chosen as a control site because it was sufficiently close to the hMT/V5+ site to mimic the non-neuronal effects of TMS (coil click and skin stimulation) while the stimulated brain area did not show any motion-specific activation.

TMS measurements

TMS apparatus and stimulation parameters

Biphasic TMS pulses were applied with a figure-of-eight coil (MC-B70, the inner and outer radii of the two coil loops are 1.2 and 5.4 cm, respectively) and a Medtronic-Dantec MagPro stimulator (Medtronic Functional Diagnostics A/S, Skovlunde, Denmark; maximum stimulator output, 2 T). The coil was fixed on a tripod and placed tangentially on the skull with a custom-made coil holder. The handle was oriented parallel to the horizontal plane pointing towards the occiput. The coil position was monitored on-line with the ElGuide (zebris, Tübingen, Germany) or BrainVoyager software and adjusted to the respective target location. Single-pulse TMS was applied at 70% of maximum stimulator output. To avoid computer monitor artifacts and to provide accurate timing, the TMS pulses were applied in the vertical refresh period of the cathode-ray-tube (CRT) monitor.

Stimuli

Stimuli were generated with a custom-made program based on the Microsoft DirectX library and presented on a CRT monitor. The distance between the participants' eyes and the monitor was 47 cm. The stimulus consisted of a frameless square window of moving random dots located to the right of the fixation cross at an eccentricity of 11.0° visual angle (distance from the fixation cross to the center of the square); the side length of the square was 7.4°. Comparable stimuli have been used in previous TMS, neurophysiological, and neuropsychological studies of motion perception (Baker et al., 1991; Beckers and Hömberg, 1992; Beckers and Zeki, 1995; Hotson et al., 1994; Newsome and Paré, 1988). The random-dot moving pattern was presented for five consecutive frames, corresponding to an approximate presentation duration of 50 ms

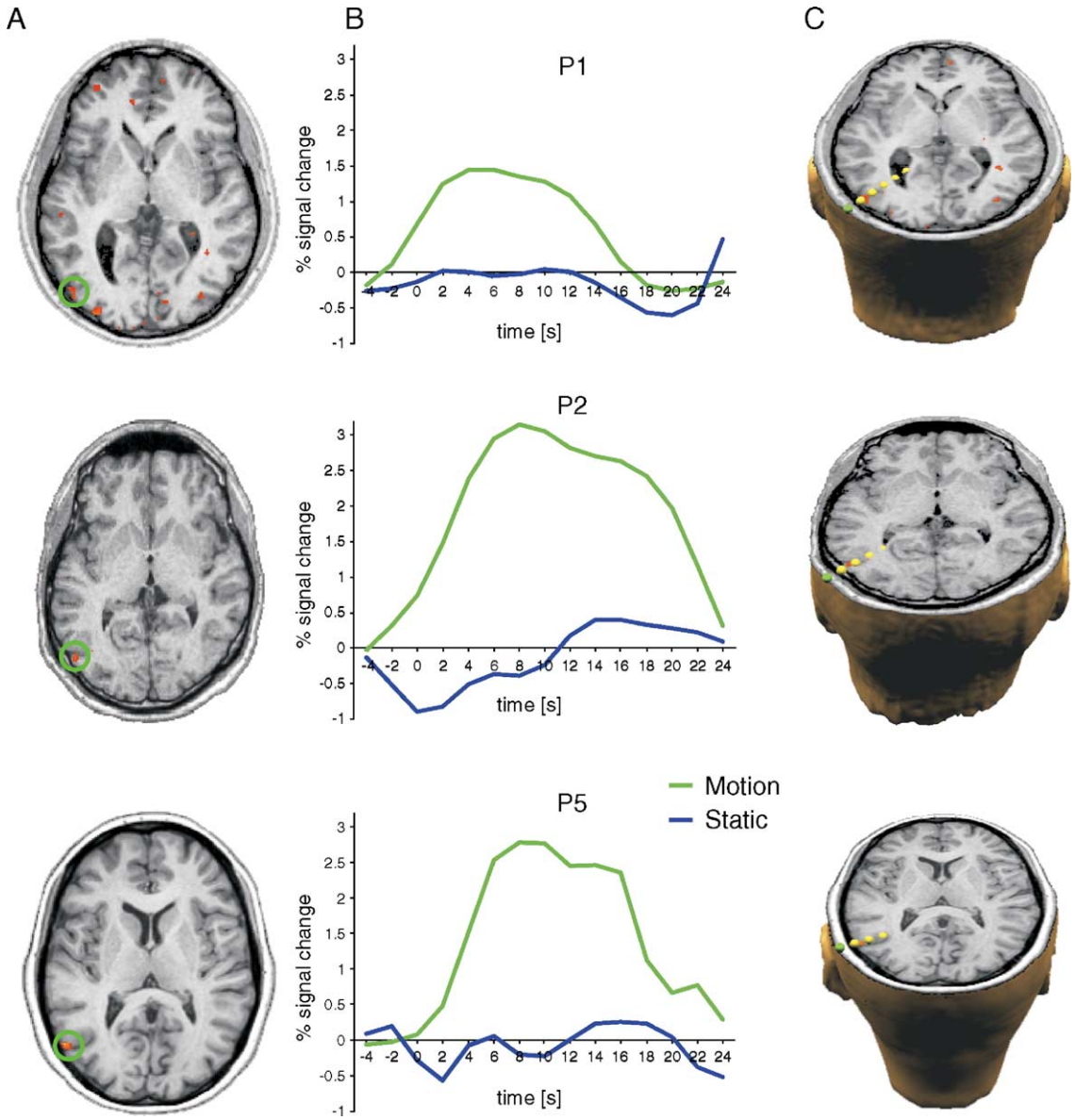

Fig. 2. Location of left hMT/V5+ and the corresponding TMS site in three out of five participants. (A) Axial slices at the level of hMT/V5+ (marked with a green circle). Voxels with significant activation (P (uncorrected) < 0.005) in response to random-dot motion vs. static dots (see Materials and methods) are marked in false colors. (B) BOLD time courses from hMT/V5+. The response to coherently moving dots (green line) is markedly larger than the response to static dots (blue line). (C) Axial slices through head reconstructions showing the hMT/V5+ stimulation site (green sphere) relative to motion-specific activation. The yellow spheres visualize the orientation of the TMS coil. The imagined line through the spheres corresponds to the normal vector originating from the TMS focus (distance between spheres is 1 cm).

(refresh rate, 100 Hz). During the first stimulus frame, 100 white dots (0.15° visual angle) were presented at random positions in the presentation window. For the following frames, a subset of points was displaced coherently either in the horizontal direction (right and left) or, in separate runs, in the vertical direction (up and down). The displacement corresponded to a speed of 9.0°/s. To avoid strong local motion cues, the subset of coherently moving dots was randomly selected for each frame separately, i.e., previous movement of a dot had no predictive value for subsequent movement. Points that were not selected for coherent displacement were redistributed randomly inside the window. In case a coherently moving dot was projected to a position outside the window, the dot was inserted at the opposite side. The percentage of coherent dots was adjusted individually in a preceding psychophysical session.

Psychophysics session

The goal of the preliminary psychophysics was to determine an adequate coherence level in the random-dot paradigm for individual participants. In three participants, we used the method of constant stimuli with coherence levels ranging from 10% to 100%. For each coherence level and each of the four motion directions (up, down, left, and right), participants performed six trials, resulting in 240 trials overall. The trials were divided into two blocks with vertical motion and two blocks with horizontal motion. The stimulus-onset asynchrony between trials was 4 s. Participants had to indicate the movement direction accurately and as quickly as possible pressing one of two keys (either up and down, or right and left). Reaction times and button presses were recorded by the stimulation program. For the TMS session, a

Table 1
Talairach coordinates of hMT/V5+ in the left hemisphere of individual participants

Participant	x	y	z
P1	−45	−76	6
P2	−50	−70	2
P3	−46	−72	3
P4	−42	−75	7
P5	−52	−65	10

Notes. Talairach conventions: *x*—left to right, *y*—back to front, *z*—bottom to top.

coherence level was selected at which participants obtained at least 90% correct responses. For two participants, we used an abbreviated procedure to find an appropriate coherence level. We started at a coherence of 80% and adjusted the level in steps of 5% until criterion was reached (90% correct responses).

TMS sessions

Participants were tested with single-pulse TMS on four different days (split evenly between vertical and horizontal random-dot patterns). On each day, they performed a total of 480 trials in eight blocks of 60 trials. In four of the eight blocks, participants were stimulated at hMT/V5+, in the other four blocks at the control site. The sequence of stimulation sites was counterbalanced across participants. For each motion axis, 24 different stimulus pulse asynchronies (SPA = TMS-pulse onset minus stimulus onset in milliseconds) were tested. The SPAs ranged from −50 ms to 200 ms in steps of 10 ms. The SPAs of 50 ms and 70 ms were omitted to reduce the overall number of TMS pulses, since no effects had been reported in previous studies at or near these intervals (Beckers and Hömberg, 1992; Hotson et al., 1994).

Before the first TMS session started, coherence levels of the random-dot stimuli were again adjusted to assure high performance levels for the task. Participants had to complete blocks of 24 trials without TMS until they reached an accuracy of 90% or higher, starting with the coherence level that had been determined in the preliminary psychophysics. When participants failed to reach the desired performance level in two subsequent blocks, the coherence level was raised in 5% steps. The resulting coherence level was used for all following measurements.

Data analysis

The logic of our study was to investigate a small number of participants with maximum precision and a high number of trials. We analyzed our single-trial count data with a hierarchical log-linear analysis (Howell, 2004). A log-linear analysis allows to handle multi-way contingency tables and to test for main and interaction effects. It uses an iterative procedure to generate maximum-likelihood estimates. The variables of interest in our analysis were axis of motion (horizontal vs. vertical), TMS stimulation site (hMT/V5+ vs. control), SPA (24 intervals), and correctness of responses (correct vs. incorrect). The data were analyzed with the HILOGLINEAR algorithm of the SPSS 12.0 software package (SPSS Inc., Chicago, IL, United States of America). The algorithm was applied with backward elimination of variables and an elimination criterion of $P = 0.05$. The maximum number of iterations was set to 20. To identify SPAs at which the number of correct responses was reduced at hMT/V5+ relative to the control site, we performed post hoc chi-square tests for the 24 SPAs with Bonferroni correction for multiple comparisons.

Results

Co-registration of fMRI and stereotaxic data

We used a co-registration procedure to target hMT/V5+ based on previously measured fMRI data from individual participants. This neuronavigation approach allowed us to accommodate interindividual differences in hMT/V5+ localization and to validate independently the appropriateness of the TMS stimulation site. The average Talairach coordinates of left hMT/V5+ for our five participants were −47, −72, 6; the centers of mass are provided in Table 1. The maximal Euclidean distance between Talairach coordinates of our participants was 14 mm. The coordinates were in agreement with previous reports (Goebel et al., 1998; Muckli et al., 2002; Tootell et al., 1995a; Watson et al., 1993).

In other studies investigating motion processing in hMT/V5+ (Beckers and Hömberg, 1992; Beckers and Zeki, 1995; Hotson et al., 1994), the location of the TMS stimulation site was defined relative to the inion, an anatomical landmark at the back of the head. To compare our stimulation sites to these studies, we computed the distance of the hMT/V5+ surface point to the inion (Table 2). On average, the TMS stimulation site for hMT/V5+ was located 59 mm left to the midsagittal plane, 27 mm anterior to the inion, and 35 mm above the AC-PC plane, resulting in an Euclidean distance of 74 mm. This corresponds to the guidelines described in the literature, according to which hMT/V5+ is located 5–6 cm lateral to and 3–4 cm above the inion (Beckers and Hömberg, 1992; Beckers and Zeki, 1995; Hotson et al., 1994; Stewart et al., 1999). Still, the location of the optimal TMS stimulation site for hMT/V5+ can vary considerably between participants. In our group, the maximal difference for the position relative to the inion was 15 mm. Although the spatial resolution of TMS is limited due to the non-focal nature of the magnetic field, a shift of 15 mm can lead to the complete loss of TMS effects (Beckers and Hömberg, 1992; d'Alfonso et al., 2002).

Psychophysics

Participants were tested with the random-dot paradigm in a preliminary session to find a coherence level at which they showed almost perfect performance. In a range from 10% to 60% coherence, performance increased almost linearly in all participants, leveling off at higher coherences with perfect performance (Fig. 3). For our purpose, threshold was defined as the coherence level with a performance at or above 90%. In the first TMS session, coherence levels were again adjusted to ensure high performance. In all participants, coherence had to be increased by another 10% to 20%, except for horizontal motion in participant P2 (reduction of

Table 2
Distance from site of TMS stimulation (hMT/V5+) to the inion of each individual participant in mm

Participant	x	y	z	Euclidian distance (mm)
P1	54	20	34	67
P2	63	26	37	78
P3	61	35	21	73
P4	55	20	41	71
P5	63	33	40	82

Notes. Coordinates: *x*—left to right in the ACPC plane, *y*—back to front in the ACPC plane, *z*—bottom to top orthogonal to the ACPC plane.

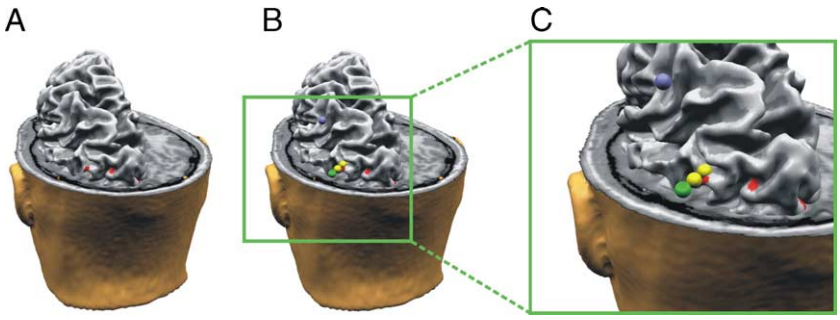

Fig. 3. Stimulation sites relative to left hMT/V5+ and the sulcal anatomy in participant P1. (A–C) Reconstruction of the head as well as the white-/gray-matter boundary of participant P1. Areas with motion-specific responses are marked in red. (B) TMS site targeting hMT/V5+ is indicated with a green sphere. The yellow spheres visualize the orientation of the TMS coil (normal vector originating from the TMS focus; distance between spheres is 1 cm). The light blue sphere is located at the stimulation site for the control condition. (C) Close-up view of the hMT/V5+ section marked in panel B.

5%). The coherence ranged from 50% to 85% for horizontal motion and from 50% to 90% for vertical motion in our participants. This coherence level was used for all following measurements.

TMS sessions

The effect of TMS over hMT/V5+ on motion processing was tested at 24 different time intervals between stimulus onset and the TMS pulse, ranging from 50 ms before to 200 ms after stimulus onset. The same set of intervals was also tested with TMS at the control site in parietal cortex. All participants showed a decrease of correct responses for TMS at hMT/V5+ relative to the control condition (Fig. 4A). The reductions were not evenly distributed among the time intervals. There were distinct time periods with reductions of up to 20%. For other time periods, there was no difference between the hMT/V5+ and the control condition. In two time windows, −40 to −20 ms and 110 to 160 ms, there was a consistent effect in all five participants. The reaction-time data did not show a corresponding effect across participants (Fig. 4B).

For statistical analysis of the correct responses, we performed a hierarchical log-linear analysis with the following factors: direction axis (horizontal vs. vertical), stimulation site (hMT/V5+ vs. control), stimulus-TMS asynchrony (from −50 to 200 ms), and correctness of response (correct vs. incorrect). Although the number of incorrect responses was higher for horizontal vs. vertical motion (interaction 'direction axis' × 'correctness of response'; $\chi^2(1) = 11.601$, $P < 0.001$), the interactions of the factor 'direction axis' with other factors were insignificant ($P > 0.25$), meaning that the pattern of results was similar for the vertical and horizontal motion directions. The final model of the log-linear analysis included two additional interaction terms: 'condition' × 'correctness of response' ($\chi^2(1) = 113.165$, $P < 0.001$) as well as 'stimulus-TMS asynchrony' × 'correctness of response' ($\chi^2(23) = 55.759$, $P < 0.001$). As expected, this implies that the percentage of correct responses was significantly different for the two TMS conditions, as well as for different stimulus-TMS asynchronies. The three-way interaction 'stimulus-TMS asynchrony' × 'condition' × 'correctness of response' showed a trend towards significance ($\chi^2(1) = 32.549$, $P = 0.09$).

To identify the time intervals in which the number of correct responses was reduced in the hMT/V5+ condition relative to the control site, we performed post hoc chi square tests with Bonferroni correction for the different stimulus-TMS asynchronies. With a conservative threshold of P (Bonferroni) < 0.05, we found five time intervals with a significant reduction of correct responses: In the early time window, the effect was significant at −40 and −30 ms; for the late time window, the differences at 130, 140, and 150 ms reached significance.

Discussion

We show that targets for TMS application can be reliably selected on the basis of individual activation patterns from an fMRI experiment. Area hMT/V5+ was identified in individual participants using a motion-mapping paradigm. Anatomical and functional MRI data were co-registered with stereotaxic data from the participants' heads, and TMS was applied to the individually defined stimulation sites. TMS at hMT/V5+ but not at a parietal control site led to a significant reduction of correct motion discriminations in an early (−40 to −30 ms) and a late (130 to 150 ms) time window. Motion axis (vertical vs. horizontal) did not have a significant influence on the TMS effects.

Our data confirm that hMT/V5+ is of special relevance for the visual processing of moving stimuli. Earlier studies in macaques and humans have shown that lesions in hMT/V5+ produce severe deficits in motion perception (Baker et al., 1991; Newsome and Paré, 1988; Zeki et al., 1991; Zihl et al., 1983). We produced 'virtual lesions' (Cowey and Walsh, 2001) with spTMS in a time-dependent manner. In contrast to earlier TMS studies (Anand et al., 1998; Beckers and Hömberg, 1992; Beckers and Zeki, 1995; d'Alfonso et al., 2002; Hotson et al., 1994), we were able to explicitly target hMT/V5+ with a neuronavigation device based on fMRI data from individual participants. This is an important methodological improvement because, first, the exact location of hMT/V5+ can vary considerably between participants (Watson et al., 1993) and, second, moving phosphenes, which are used to identify hMT/V5+ functionally, can be produced only in a small percentage of participants (Pascual-Leone and Walsh, 2001). Moreover, moving phosphenes as a location criterion are not specific for hMT/V5+ since they also result from stimulation at other cortical sites (Fernandez et al., 2002). In addition, there is another motion-processing region (kinetic occipital, KO; Orban et al., 1995) in the vicinity of hMT/V5+, which might be relevant for direction discrimination in random-dot patterns and could accidentally be targeted in TMS studies investigating hMT/V5+.

In addition to the effective time window at 130–150 ms, we found an early decrement of performance at −40 to −30 ms SPA for stimulation over hMT/V5+. If one assumes that the impact of

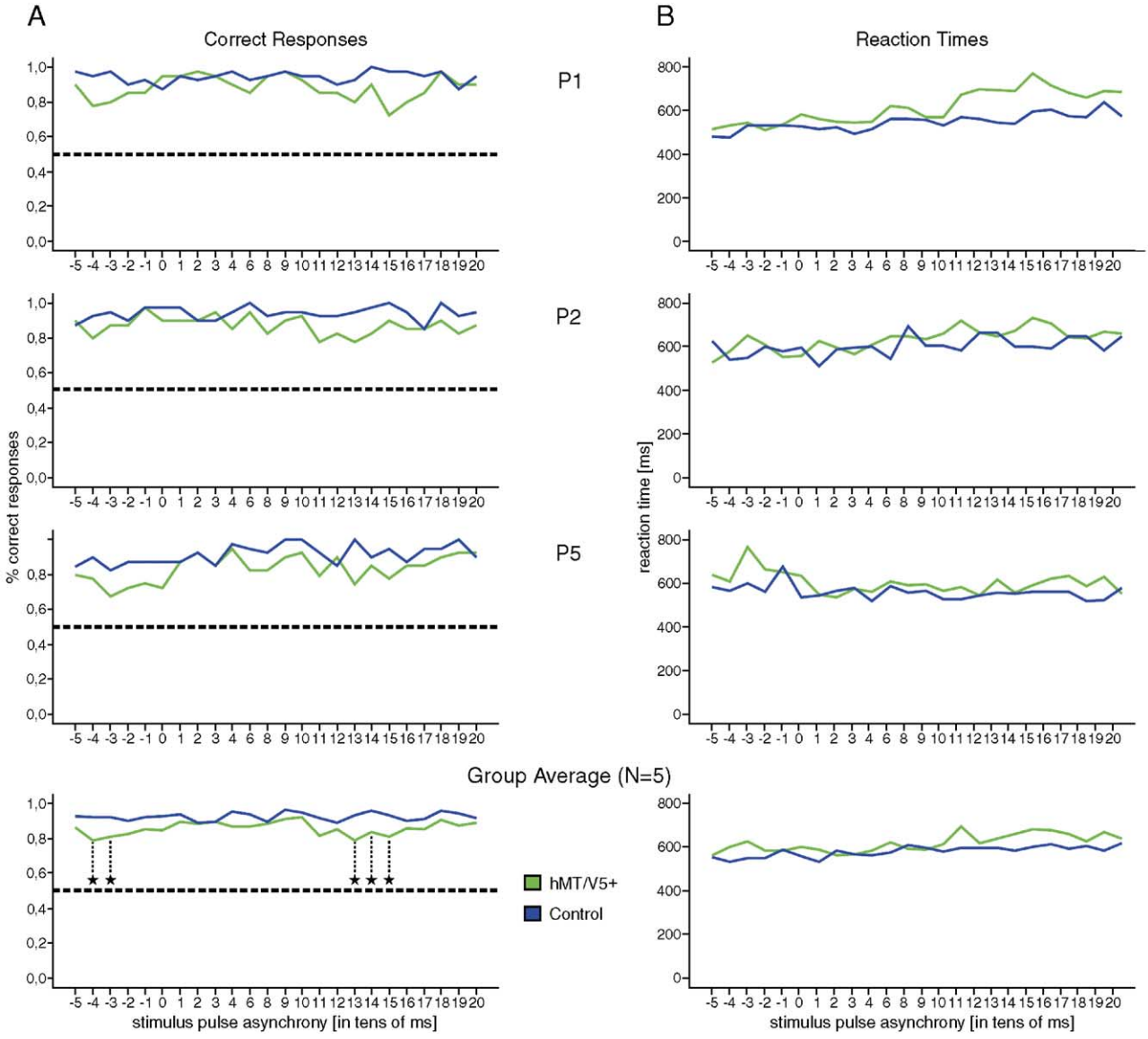

Fig. 4. Percent correct responses and reaction times for three out of five participants and average values. (A) Percent correct responses of participants P1, P2, and P5, and the group average value ($N = 5$) for the 24 different stimulus-pulse asynchronies (SPA). The dashed lines indicate performance at chance level. The performance of participants is impaired for stimulation over hMT/V5+ (green line) compared to stimulation over the control site (blue line). There is a selective reduction of correct responses in two major time windows in all participants: an early interval at −40 to −20 ms SPA and a late window around 110–160 ms SPA. Although the exact time point of maximal effectiveness differs between participants, we found significant average differences between conditions in both time intervals (−40 and −30 ms as well as 130–150 ms; marked with an asterisk). There were no significant effects at any other time window. (B) Reaction times do not show a consistent trend across participants.

TMS is limited to milliseconds after TMS onset (Walsh and Cowey, 2000) and takes into account the response latencies of cells in the visual cortex (Nowak et al., 1995), this is most likely a non-neuronal effect that is nevertheless specific to stimulation at hMT/V5+. The effect occurs in a time window in which previous studies have described decreased performance due to reflex blinking of the eyelids (Amassian et al., 1998; Beckers and Hömberg, 1991; Corthout et al., 2003). We did observe in our experiments that stimulation at hMT/V5+ led to muscle twitches with a higher probability than at the parietal control site. This, in turn, could have increased the probability of blinks, which interfered with the initial processing of the visual stimulus.

Previous experiments that have studied the relevance of hMT/V5+ for processing of random-dot patterns with TMS showed mixed and partly contradictory results (Anand et al., 1998; Beckers and Hömberg, 1992; Beckers and Zeki, 1995; d'Alfonso et al., 2002; Hotson et al., 1994). Two studies found a disruptive effect on motion perception with an SPA around 0 ms (Beckers and Hömberg, 1992; Beckers and Zeki, 1995), while others reported an effective SPA of 100 to 150 ms (Anand et al., 1998; Hotson et al., 1994; see also Walsh et al., 1999). In one study, the TMS effects were topographically specific to one or a few stimulation sites but showed mixed results in terms of the temporal distribution (d'Alfonso et al., 2002). Beckers and Hömberg (1992) tested both the early (0 ms) and late (100–150 ms) time windows in the same experiment but only found a significant impairment around 0 ms and no effect for 100–150 ms. It is unclear how these diverging results can be explained. The parameters used by Hotson et al.

(1994) were different from those used in the experiments of Beckers and colleagues (Beckers and Hömberg, 1992; Beckers and Zeki, 1995). In the latter studies, the size of the random dots as well as the size of the presentation window were markedly smaller, and the window was presented at less eccentric locations in the visual field. But it is unlikely that these factors would shift the relevant time window by 100 to 150 ms, thereby fundamentally changing the temporal dynamics of stimulus processing.

In contrast, our own data indicate that the functionally critical time point of specific neuronal activity in hMT/V5+ during motion perception lies between 130 and 150 ms following stimulus onset. Thus, our results confirm the relevance of the late time window but not of the early window around 0 ms (the first time window at −40 to −30 ms revealed in our study is clearly separate from the debated 0-ms delay). Our data are in good accordance with MEG results measuring the temporal pattern of neuronal activity during motion processing (Ahlfors et al., 1999; Scherg et al., 1999). By using an fMRI-guided MEG source analysis, Ahlfors et al. (1999) revealed an onset latency of hMT/V5+ activation at around 130 ms with a first peak at around 150–180 ms after stimulus onset. However, other combined EEG/MEG results (ffytche et al., 1995) suggest two activation peaks depending on the speed of the motion stimulus. In this study, the neuronal response to faster moving stimuli (22°/s) occurred at around 50 ms, while the response to slower moving stimuli (< 6°/s) occurred later in time. The authors speculated that the faster moving stimuli might be processed via a non-sequential parallel input to hMT/V5+, which by-passes V1 (dynamic parallelism). However, although this study showed that stimulus speed can have a strong impact on neuronal response latencies, we could not find any evidence for a functionally critical time point representing this parallel visual motion input to hMT/V5+. Although one might argue that our stimuli were not moving fast enough (9°/s) to evoke the earlier neuronal response, the speed of our motion stimuli did not differ from those of Beckers and colleagues and thus cannot account for the contradictory finding between the different TMS studies.

Area hMT/V5+ is not the only cortical region implicated in the processing of moving patterns. Other prominent motion-selective regions are V3A and KO (Orban et al., 1995; Rees et al., 2000; Tootell et al., 1997; for review see Culham et al., 2001). Their motion selectivity is not as pronounced as that seen in hMT/V5+ and there are also qualitative differences in the response properties of these areas. KO has been first described as being especially sensitive to kinetic boundaries (Orban et al., 1995). Furthermore, when testing hMT/V5+, KO, and V3A with different coherences of random-dot motion, Rees et al. (2000) could show that hMT/V5+ shows a linear relationship between motion coherence and activation, whereas the relationship for KO and V3A was U-shaped. Despite those differences, it is still possible that all those areas are necessary for the detection of motion directions in a random-dot pattern. Specific targeting of KO and V3A with neuronavigated TMS in future studies might reveal the functional contribution and relevant time windows for random-dot motion processing in those regions.

Our methodological approach enabled us to reveal and quantify the interindividual variance in the exact location of hMT/V5+ and the respective TMS target position on the skull of the participants. Considering that shifting the TMS coil position only by a few millimeters can already lead to a complete loss of TMS effects (Beckers and Hömberg, 1992; d'Alfonso et al., 2002), our study clearly shows the benefits of a neuronavigated TMS procedure that is based on individual functional imaging data.

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