Primate Area MST-I is involved in the generation of goal-directed eye and hand movements

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running title: The role of area MST-I for eye and hand movements
Abstract

The contributions of the middle superior temporal area (MST) in the posterior parietal cortex of rhesus monkeys to the generation of smooth pursuit eye movements as well as the contributions to motion perception are well-established. Here, we present first experimental evidence that this area also contributes to the generation of goal-directed hand movements towards a moving target. This evidence is based on the outcome of intracortical microstimulation experiments and transient lesions by small injections of muscimol at identified sites within the lateral part of area MST (MST-I). When microstimulation was applied during the execution of smooth pursuit eye movements, post-saccadic eye velocity in the direction of the preferred direction of the stimulated site increased significantly (93 out of 136 sites tested). When microstimulation was applied during a hand movement trial, the hand movement was displaced significantly in the same direction (28 of 39 sites tested). When we lesioned area MST-I transiently by injections of muscimol, steady state eye velocity was exclusively reduced for ipsiversive smooth pursuit eye movements. In contrast, hand movements were displaced towards the contralateral side, irrespective of the direction of the moving target. Our results provide evidence that area MST-I is involved in the processing of moving targets and plays a role in the execution of smooth pursuit eye movements as well as visually guided hand movements.
Introduction

The correct processing of visual motion is an essential cognitive function. It enables us to identify moving objects, to estimate 3D object configurations, to move our eyes appropriately, and to recover ego-motion in space (for review see Nakayama 1985). In primates, the important role played by area MST in the execution of smooth pursuit eye movements (Ilg et al. 2004; Ilg and Thier 2003; Kawano et al. 1994; Newsome et al. 1988; Thier and Erickson 1992) and in motion perception (Britten and van Wezel 1998; Britten and van Wezel 2002; Celebrini and Newsome 1995, 1994; Heuer and Britten 2004; Ilg and Churan 2004) are well documented.

Area MST receives input from the middle temporal area MT (Maunsell and van Essen 1983; Ungerleider and Desimone 1986), which is believed to carry retinal image motion signals (Lisberger and Movshon 1999). Area MST consists of two sub-areas: a dorsal sub-area and a lateral sub-area (Komatsu and Wurtz 1988). The dorsal part (MST-d) is essential for decoding optic flow (Duffy and Wurtz 1991). The decoding of optic flow is of special relevance for differentiating self-induced and external induced retinal image motion (Zemel and Sejnowski 1998) as well as for estimating heading direction (Hamed et al. 2003; Page and Duffy 2003). On the other hand, the lateral part (MST-l) is important for decoding object motion in space. To recover object motion in space, extra-retinal information related to ongoing eye and head movements is added to retinal image motion signals of the tracked target (Ilg et al. 2004; Ilg and Thier 2003; Kawano et al. 1994; Newsome et al. 1988; Thier and Erickson 1992). As a result, the neuronal activity of a group of neurons in area MST-l represents target movement within an external frame of reference (Ilg et al. 2004).

Based on the results of oculomotor and manual tracking of human subjects in two dimensions, it was earlier hypothesised that manual tracking and smooth pursuit eye
movements use similar control signals and depend on a common neural resource (Engel et al. 1999; Engel and Soechting 2000). In addition, the control strategies for directing the hand and the eyes towards moving targets shared similar dependencies on target predictability (von Donkelaar et al. 1992). The common neural resource might include area MST since it was previously shown that the population vector recorded from area MST represents the target trajectory during a manual tracking task (Kruse et al. 2002). Taken together, an obvious question is whether area MST is a specific pre-processor for visual motion used exclusively for the execution of smooth pursuit eye movements or, alternatively, whether MST processes visual motion guiding eye and hand movements.

The approach taken here is to initially replicate earlier findings on the role of MST-I for smooth pursuit eye movements. Based on these results, we then investigate whether MST-I is also important for generating goal-directed hand movements.
Methods

Two adult male rhesus monkeys (BH and GH) were used in the present study. Using surgical procedures described in detail earlier (Ilg and Thier 1996), they were implanted with a head post, recording chambers, as well as a search coil underneath the conjunctiva. All animal procedures were carried out in accordance with the guidelines laid down by the NIH and German law and were approved by the local ethics committee. The center of each recording chamber was aimed at the lateral parts of area MST (stereotactic coordinates: lateral 19, posterior 3.5, and dorsal 16 mm). The axis of the chamber was tilted 30° upwards with respect to the horizontal in a parasagittal plane. The first penetrations in each hemisphere were performed in the center of the chamber. Well-established single-unit response properties were used to further refine the location of area MST and its two major subdivisions, the dorsal one (=MSTd) and the lateral one (=MSTl) in the individual hemisphere as well as the boundary with neighboring area MT (Komatsu and Wurtz 1988). Details of the single-unit responses recorded from MST of these monkeys have been published in earlier work (Ilg et al. 2004).

Experimental Set Up

Visual Stimulation

The visual stimulation was generated by a PC (1280*1024 pixel, 60 Hz refresh rate) and back-projected (NEC XG-1101G) onto the touch-screen (width 49°, height 30°) which was mounted at a reachable distance (0.33 m) for the monkeys. Targets for eye movements were red dots (luminance 0.5 cd/m², diameter 0.5°) and targets for hand movements were white dots (same luminance and diameter). The stimulation
computer was synchronised with a second personal computer for data acquisition. All experiments were performed in a completely dark chamber. The monkeys could see neither the borders of the screen nor the screen itself. To prevent dark adaptation of the monkeys, bright LEDs were switched on when the liquid reward was provided at the end of each correct trial.

**Eye Movement Recordings**

Horizontal and vertical eye position was measured using implanted search coils (Judge et al. 1980; Robinson 1963). These analogue signals were low-pass filtered at 500 Hz and sampled at a rate of 1 kHz.

**Hand Movement Recordings**

The accuracy of the goal directed hand movements was determined by means of a touch screen (AccuTouch, Elo Touch Systems) connected to the serial port of a PC. The temporal resolution of the touch screen was 60 Hz, identical to the refresh rate of the visual stimulation. We determined the horizontal and vertical finger position on the touch screen in addition to the onset time and offset time of the reaching movement. Since the experiments were performed in total darkness, the monkeys could not see their hands during the pointing movement.

To monitor the monkeys, their actions, and some possible consequences of the microstimulation, we employed IR illumination to enable video control of our monkeys in total darkness.

**Experimental Paradigms**

**Eye Movement Study**
Various saccade and pursuit paradigms were used in this study. All eye movement paradigms began with a fixation period of 1 s (see Fig. 1A). In order to minimise the effect of anticipation (despite this fixed period), we randomised either target position or direction of target movement from trial to trial.

In the pursuit paradigm, the moving target was displayed for 2 s. In the microstimulation experiments, target velocity was fixed to 10°/s and the target moved in one of four possible directions (i.e. 90° circular distance) aligned to the preferred direction of the stimulation site. In the lesion study, we used four different velocities (5, 10, 20, and 30°/s, respectively) to the left or right, respectively. The target always started to move from the position of the stationary fixation target.

In the saccade paradigm, a stationary target was displayed at one out of 8 possible locations (eccentricity 10°) after the fixation target disappeared. The size of the gaze control window was adjusted to the difficulty of the actual task (4° to 8°). If the monkey’s gaze remained within the control window, the animal received a liquid reward (approximately 0.1 ml/trial) at the offset of each correct trial. If the gaze left the control window, the actual trial was instantly aborted and the data were discarded.

**Hand Movement Study**

In the hand movement task, the monkey had to touch a sensor in its chair to start the next trial. In other words, each hand movement trial started with the monkey’s hand located in a constant, pre-defined position in space. After a random fixation period of 500 to 1000 ms, the moving target appeared on the screen (see Fig. 1B). The target appeared at a random position (between center of screen and its border opposite to its direction of movement) and moved at 10°/s along one of the cardinal axes. When
the fixation target’s colour changed from red to blue (randomised 200 to 500 ms after the onset of the moving target), the monkey had to perform a reaching hand movement towards the moving target. Once the monkey’s hand left the sensor in its chair, the moving target was switched off. The analysis of our video material revealed that the monkeys usually touched the surface of the screen with the knuckle of their right index finger. The monkey had to maintain fixation throughout the entire trial; violation of fixation yielded instantaneous trial abortion. In order to instruct the monkeys to perform hand movements as precisely as possible, the amount of reward depended on the size of the pointing error (approximately between 0.1 and 0.6 ml/trial), which was computed as the difference between current target and finger position. The monkeys never received feedback information related to target and finger position.

Microstimulation Protocol

Microstimulation was applied with the same electrode used for the single-unit recordings. We never observed a change of the electrode impedance as a consequence of the stimulation. In the eye movement study, we applied microstimulation (WPI Stimulus Isolator A365) for 200 ms (200 Hz, bipolar pulse length 200 µs, strength from 50 up to 80 µA), 200 ms after target movement onset. During microstimulation, the target for eye movements was either switched off or remained visible. For the hand movement study, microstimulation (with the same parameters as in the eye movement study) began in synchrony with the GO signal and ended when the monkey touched the screen (or after a maximum period of
500 ms). Note that there was a temporal overlap between microstimulation and target presentation during the manual reaction time of every individual trial.

In pilot hand movement experiments (see Fig. 2), we observed no effects of microstimulation on the hand movements if the target was visible during the reaching period. Therefore, we switched off the target as soon as the hand left its initial pre-defined position. As Fig. 2 shows, the mean of the 2D Gaussian function was not affected by this manipulation. Only the width of this function increased during open-loop pointing.

Muscimol Injection Protocol

In order to inject small amounts of muscimol into area MST, we replaced the electrode by a stainless steel canula through which a glass pipette (tip diameter of approx. 50 µm) approached area MST exactly at sites previously explored by single-unit recordings. The pipette was glued onto a Hamilton syringe and was filled with muscimol solution (5 mg/ml in H₂O). The Hamilton syringe itself was filled with paraffin. In one experiment per hemisphere, fluorescent latex microspheres (Lumafluor) were added at the tip of the pipette to reconstruct the center of the injection. Up to 4 µl were slowly injected over approximately 30 min. After a delay of 30 min, the pipette was removed. The behavioural testing was only performed after the micropipette had been removed and the chamber had been closed again. To ensure that we did not measure long lasting effects of the muscimol, we only performed these injections every other day.
Data Processing

All data processing was performed using self-written scripts together with the available toolboxes in Matlab.

Eye Movements

We obtained single trial eye velocity by differentiating and low-pass filtering the eye position profiles. Saccades were automatically detected according to an acceleration threshold algorithm. Saccade parameters such as latency, duration, amplitude, and peak velocity were determined. Eye velocity profiles of single trials were kept empty over the duration of the saccade (i.e. the eye velocity values were replaced by the arithmetic representation of not-a-number) to guarantee that saccades did not influence any other parameter.

In addition to saccade detection, pre-saccadic pursuit initiation was determined based on the eye velocity profiles of single trials. At the time of the initial saccade, the initiation of pursuit is complete and eye velocity is very precisely matched to target velocity (Carl and Gellman 1987; Lisberger 1998). Therefore, we decided to use post-saccadic eye velocity to quantify the effect of microstimulation.

We aligned single trials (10 per condition) to the offset of the initial saccade. Subsequently, we averaged eye velocity over a period of 50 ms immediately after the offset of the initial saccade. The vector difference between post-saccadic eye velocity in stimulated and control trials was used to determine the stimulation effect for a given pursuit direction. The stimulation vector was computed by adding the vectors obtained from pursuit along cardinal axes.

Steady state pursuit velocity was computed by averaging de-saccaded eye velocity from the onset of pursuit until the end of trial. Subsequently, averages were computed for all trials with a given target velocity (from 5 to 30°/s).
Single trial pursuit onset latencies were determined by a threshold algorithm applied to the eye acceleration profiles.

**Hand Movements**

Hand movements were quantified by the following parameters: the time the monkey’s hand left the initial sensor (hand movement latency), the time the finger hit the touch-screen (movement duration, taken as the difference between hit and latency), as well as the position of the finger on the touch-screen. From this position, we calculated the pointing error as the difference between the actual target position and the landing position. The histogram of this pointing error, based on 160 single trials in which the target moved in one of the four cardinal directions, was fitted using the Matlab function lsqnonlin to a 2D Gaussian function:

\[
\text{eqn 1: } f(x,y) = a \exp \left( k \left( (x-x_0)^2 + (y-y_0)^2 \right) \right)
\]

where \( x_0 \) and \( y_0 \) represent the mean horizontal and vertical errors, respectively. The standard deviation (STD) gives the radius of the circle at which the function has fallen to 68.3% of its maximum. The stimulation vector for hand movements was determined by the difference in mean reaching error during stimulated and control trials.

**Comparison of Directions**

In order to compare the preferred direction of the pursuit-related activity at a given stimulation site and the direction of the stimulation vector on eye and hand movements, we performed two analyses. First, we computed the angle between the
preferred direction and the stimulation vector and built a histogram of the angles. Second, we calculated the circular correlation (Fisher 1995) between preferred direction and stimulation vector using the Oriana2 software package (Kovach Computing Services, Anglesey, Wales) and determined whether this correlation was significant.

**Anatomy**

Following the execution of these experiments, the monkeys were sacrificed and their brains were cut frontally into 50 µm sections. Amongst others (in total 8 series), the sections were stained for cell bodies (cresyl violet) or myelin (Gallyas), the latter in order to delineate the borders of area MT in the posterior wall of the superior temporal sulcus (STS), resulting in a spacing between two adjacent stained sections of 400 µm. The reconstruction of stimulation as well as muscimol injection sites was based on relating micro-drive readings to the locations of fluorescent beads and visible traces of the electrode tracks. This confirmed that the manipulations in all three hemispheres were directed towards the lateral part of area MST, located in the fundus of the STS anterior to area MT.
Results

We only started our microstimulation experiments or injected muscimol, respectively, if the electrode picked up pursuit-related activity as shown in Fig. 3. This typical single-unit recorded from the lateral part of MST (MST-l) preferred pursuit to the right and down. The neuronal activity during pursuit in this direction was clearly greater compared to spontaneous activity during stationary fixation. In contrast, during pursuit in the opposite direction, the activity of this neuron fell below spontaneous rate. In every case, the neuronal activity during pursuit in preferred direction was significant larger (p<0.05, t-test) than the activity during pursuit in the opposite direction.

Specific Effects of MST Microstimulation

A typical effect of microstimulation in MST-l on pursuit eye movements is shown in Fig. 4 at the site where the pursuit response presented in Fig. 3 was recorded. If we stimulated during the execution of smooth pursuit eye movements, we observed an increase of eye speed whenever the monkey performed pursuit in the preferred direction (rightward and downward, -25°) of the stimulated site (see red arrow in Fig. 4B). Latencies of the eye movement were not affected by the microstimulation. In the example shown, the pursuit onset latency without stimulation was 145 ms (STD 11 ms) and 153 ms (STD 19 ms) in stimulated trials (n.s., t-test, p=0.2967). The latency of the initial saccades was 372 ms (STD 36 ms) without stimulation and 377 ms (STD 25 ms) in stimulated trials (n.s., t-test, p=0.77). After the cessation of
stimulation, the monkey corrected for the stimulation-induced offset in eye position by backward-directed saccades (see black arrow in Fig. 4B).

In order to quantify this effect of microstimulation, we determined the post-saccadic eye velocity (see Fig 4D) and calculated the stimulation vector as the vector difference in post-saccadic velocity between stimulated and control trials. When pursuit was in the non-preferred direction, there was no significant change in eye speed (see left box in Fig. 5). During pursuit in the direction orthogonal to the preferred direction, the stimulation vector was still directed towards the preferred direction of the stimulation site.

We only observed an effect of microstimulation on eye velocity if the monkeys performed smooth pursuit. If we stimulated during fixation of a stationary target, no effects on eye velocity were observed (see Fig. 6, same stimulation site). Statistical testing of eye velocity with and without stimulation did not result in any significant differences (p>0.05, t-test ).
Overall, we performed microstimulation at 136 sites in area MST-I of our two monkeys and found significant effects on post-saccadic eye velocity at 93 sites (t-test, p<0.05).

On average, the absolute value of the stimulation vectors for all 93 sites with a significant effect was 0.85°/s (STD 0.39°/s) if the target was switched off during stimulation and 0.67°/s (STD 0.45°/s) if the target was continuously visible (see Fig. 7). In other words, in the transient absence of the pursuit target, microstimulation elicited a 27% larger effect. This difference was highly significant (p=0.0048, t-test). Finally, the direction of the stimulation vector computed by the post-saccadic velocity correlated significantly with the preferred direction of the pursuit-related activity recorded at the stimulation site (r=0.037, p=0.0009, circular correlation). The histogram of the deviation between both directions is shown in Fig 7B. For most of the 93 stimulation sites, the deviation between stimulation vector and preferred direction was smaller than 45°.

In contrast to the effect on post-saccadic eye velocity, microstimulation did not affect the latency of the initial saccade (n=93; mean control latency 392 ms (STD 28 ms), stimulated latency 412 ms (STD 52 ms), t-test: p=0.1571). Pursuit onset latency was also not affected by microstimulation (see left panel in Fig. 11).

Hand Movements
The errors in hand movements were fit by a 2D Gaussian function as shown earlier (see Fig. 2). When we stimulated during a hand movement trial, the landing position
of the finger deviated towards the preferred direction of the stimulated site (see Fig. 8A and B). Out of 39 tested stimulation sites in area MST-I of the two monkeys, we found 28 sites with significant effects on the pointing error (t-test, p<0.05). The mean of the absolute values of the stimulation-induced shift in pointing was 1.6° (STD 1.7°, n=28). At these sites, we found significant effects of the microstimulation on the post-saccadic eye velocity. Note that the fixation of the eye was not affected by microstimulation in this condition (t-test of eye position n.s., p>0.05 after Bonferroni adjustment). We did not observe a significant effect of microstimulation on hand movement onset latency (stimulation: 245 ms STD 84 ms; control: 250 ms STD 87 ms) or on hand movement duration (stimulation: 267 ms STD 17 ms; control: 267 ms STD 18 ms; n.s., p>0.05 after Bonferroni adjustment for multiple comparisons).

Similarly to the analysis carried out using stimulation vectors for eye movement data, we applied circular correlation to analyse the relationship between preferred direction and the direction of the stimulation vector for hand movements. We found a significant correlation between both directions (r=0.04, p=0.0116). The histogram of the deviation between both directions is shown in Fig. 8C.

Finally, we asked whether stimulation in MST triggered any other reaction in our monkeys. Based on the inspection of our video material, we never observed any other motor response to the microstimulation than the effects on eye and hand movements described above.

**Specific Effects of Transient MST Inactivation**
Having documented the effects of an artificial increase in neuronal activity on goal-directed behaviour, we next addressed the effects of an artificial decrease in neuronal activity in area MST-I. We performed transient lesions by injections of small amounts of muscimol (<4 µl) at previously explored sites in area MST-I. Typical examples of pre-lesion and post-lesion pursuit eye movements are shown in Fig. 9. Steady-state pursuit velocity to the left (contraversive) was not affected by the injection of muscimol, while steady-state velocity to the right (ipsiversive) was clearly reduced.

When we injected muscimol into area MST of the right hemisphere, the monkeys showed an impairment of steady-state pursuit velocity only if the target moved rightward (ipsiversive, see Fig. 10A and B). If the target moved to the left, no change in eye velocity was observed. In contrast, if muscimol was injected into area MST-I of the left hemisphere, leftward steady-state pursuit was impaired (see Fig. 10C). Overall, we performed these transient lesions at 14 sites in three hemispheres of two monkeys as shown in Fig. 10. After every injection, we observed a reduction in steady-state pursuit velocity whenever the target moved ipsiversively with respect to the injected hemisphere (t-test, p<0.05).
Occasionally, we observed spontaneous nystagmus following muscimol injections in darkness. If this nystagmus occurred, the slow phases were always directed towards the contralateral side (with respect to the lesion).

The deficit in steady-state pursuit eye velocity was the only clear and robust effect of the muscimol injections on the monkeys’ eye movements. For the other parameters (pursuit onset and initial saccade latency), we observed a rather idiosyncratic pattern of effects after each injection. There was a slight individual difference in pursuit onset latency for our monkeys: the latency of monkey BH was 117 ms (STD 15 ms, average across all control conditions shown in Fig. 11), whereas monkey GH started pursuit after 96 ms (STD 6 ms) following the onset of target movement. When we analysed the effect of transient lesions (see Fig. 11), we found in 2 out of 3 injected hemispheres (left hemisphere in BH and right hemisphere in GH) that the pursuit latency increased if the target moved to the contralateral side. Obviously, this increase can be explained by a position effect or scotoma contralateral to the injection, however, it can not be explained by a direction effect. Lesion of the left hemisphere in GH resulted in a significant increase of pursuit latency for both directions of target movement, suggesting the co-existence a position and a direction effect.

We asked whether the changes in pursuit onset latency were also present in the latency of the initial saccades during pursuit initiation. Figure 12 gives the latencies of leftward and rightward saccades following inactivation of the right or left MST, respectively, together with the pre-lesion control values. Again, there was a slight
difference in latency between our monkeys. In contrast to pursuit onset latencies, saccade latency in monkey BH (355 ms STD 24 ms, average across all control conditions) was slightly shorter than for monkey GH (452 ms STD 26 ms).

As Figure 12 shows, the data coarsely follow the pattern of the pursuit onset latencies. For lesions in the right hemisphere in monkey BH and GH, latencies increased for leftward target movement, suggesting a position effect or scotoma. For the left lesion in GH, in which pursuit onset latencies increased for leftward and rightward target movements, only saccade latency to the left increased, indicating an ipsiversive direction effect.

Finally, in order to see whether these changes in saccadic latencies were linked to target motion or to target position, we also analysed saccades towards stationary targets. However, the latency as well as the gain of these saccades did not show a clear lesion-dependent behaviour, as shown in Fig. 13.

The individual difference between both monkeys was also present for saccades directed towards stationary targets. The mean value for all directions in the control condition was 201 ms (STD 16 ms) for monkey BH and 242 ms (STD 11 ms) for monkey GH.

In contrast to the unspecific effects on saccadic latency and gain, we observed consistent effects of microstimulation on goal-directed hand movements. There was
a shift of the landing position towards the left visual field for injections into area MST-I of the right hemisphere (see Fig. 14). The shift in landing position was present and significant (p<0.0001) for all 14 injection sites. In contrast to the directionally selective deficit in pursuit eye movements, this shift in pointing error towards the contralateral visual field did not depend on the direction of target movement. Fig. 14 D and E gives the reaching error for leftward as well as for rightward moving targets. It is important to note that the pre-lesion pointing error depends on target movement direction: leftward target motion yielded an error to the left and vice versa. However, the difference in the horizontal component of the pointing error when the target moved ipsiversively compared to when it moved contraversively was not significant (t-test, p=0.14 for all 14 injection sites).

The transient lesions in area MST did not affect the duration of hand movements in either monkey. Average pre-lesion hand movement duration was 270 ms (STD 11 ms) for monkey BH and 234 ms (STD 9 ms) for monkey GH, while post-lesion duration was 261 ms (STD 16 ms) and 235 ms (STD 4 ms), respectively (p>0.1, t-test).
Discussion

The increase of eye velocity we obtained by microstimulation during the execution of smooth pursuit eye movements is in strong agreement with results reported in earlier studies of areas MT and MST (Born et al. 2000; Groh et al. 1997; Komatsu and Wurtz 1989). The deficit in steady-state eye velocity exclusively for ipsiversive target movement also agrees with earlier findings in monkeys (Dursteler and Wurtz 1988; Yamasaki and Wurtz 1991) and with the description of patients suffering from unilateral lesions of the posterior parietal cortex (Leigh and Tusa 1985; Morrow and Sharpe 1990; Thurston et al. 1988). The similarity between the results from the literature and ours is far from trivial since in the older studies, chronic lesions were made. In the case of chronic lesions, there is a possibility of reorganisation and recovery of function (Yamasaki and Wurtz 1991). In case of transient lesions, the recovery is due to the disappearance of the lesion itself.

Further support of the extra-retinal signals in MST

We obtained larger effects of microstimulation on pursuit eye movements in the temporary absence of the target. As previously shown, MST-I neurons respond during the execution of pursuit eye movements to retinal image motion and eye movement (Ilg et al. 2004). Therefore, in the transient absence of the target, the artificial, stimulation-induced activity solely competes with the eye movement signal. In contrast, during the presence of the pursuit target, the artificial activity has to compete against retinal image motion and eye movement signals. The fact that the neuronal activity of MST is determined by retinal image motion and by extra-retinal signals related to the executed eye movements was recently also documented in humans by means of fMRI (Goossens et al. 2006). With respect to goal-directed hand movements, we failed to observe an effect of stimulation when the target was
continuously present. In this condition, the monkeys most likely did not produce a ballistic hand movement based on a feed-forward mechanism (Ariff et al. 2002); instead they likely used on-line visual feedback to direct their hand (Saunders and Knill 2005).

**Phosphenes - attention - general disturbance**

One might question whether our stimulation effects can be explained by phosphenes elicited by the microstimulation, i.e. that the monkeys simply directed their gaze and hand towards a phosphene. For stimulation in area V1, there is evidence that phosphenes are generated by microstimulation with parameters similar to those we used in our study (for review see (Bradley et al. 2005; Tehovnik et al. 2005)). However, there are two strong arguments against the idea that phosphenes played a role in our experiments. Firstly, we never observed an effect during stationary fixation. During execution of pursuit, the sensitivity of the eye movement control system is greater than during stationary fixation (Schwartz and Lisberger 1994). Secondly, the latency of the stimulation effects would be much longer if the monkeys reacted to phosphenes.

Microstimulation could have either disturbed mechanisms engaged in the focal direction of attention or generally disturbed the monkey by eliciting some level of discomfort. Neither explanation accounts for our findings since microstimulation did not simply reduce the accuracy of goal-directed behaviour, but modified this behaviour in a highly directional-selective manner. In addition, we did not observe any changes in latency due to microstimulation, which would be a clear indication of change in attentional state.

Recently, it was reported that microstimulation in MT resembles the learning of consistent changes in target trajectory (Carey et al. 2005). However, there is a clear
argument that our results cannot be explained by mechanisms of motor learning: since stimulated and control trials were randomised, there was no consistent drive for learning mechanisms.

Finally, one might also ask whether our effects are due to a specific impairment of the motion processing, or, alternatively, due to a more general impairment. The answer to this question is limited by the extent of our experimental data. If the effects were due to an impairment of motion processing, saccades or hand movements elicited by stationary targets should not have been affected by the microstimulation in MST. On the other hand, speed judgments addressed in psychophysical experiments should also have been affected. However, with respect to MST deactivation, gain and latency of saccades directed towards stationary target did not show a clear effect (see Fig. 13). This indicates that we indeed observed a specific impairment of motion processing. Further support of this notion is provided by the similarity of the effects of artificial activation and deactivation of MST on eye and hand movements, together with the clear correlation between the preferred pursuit direction and the stimulation vector.

Differentiation between effective and ineffective sites of microstimulation

The difference between effective and ineffective stimulation sites might be explained by the presence of cortical columns containing neurons with similar response properties (Mountcastle 1957). Effective sites, i.e., sites at which microstimulation resulted in significant changes in post-saccadic eye velocity, were found when the position of the microelectrode was centred within a single cortical column or cluster in area MST (see (Britten 1998)). In this case, only neurons with similar properties were activated and therefore an effect on the ongoing eye or hand movement was detectable. Ineffective sites, i.e., sites at which microstimulation did not produce
significant changes in eye velocity, resulted from an electrode position close the border between two columns, thereby stimulating neurons with different response properties. However, since we do not have experimental data supporting the hypothesis of a columnar or clustered structure in MST-I, this explanation remains speculative.

A second and alternative explanation for the existence of ineffective stimulation sites relates to the fact that extra-retinal responses of MST neurons are not only selective for direction, but also for velocity (Churchland and Lisberger 2005b). If the preferred velocity of a given stimulation site would have been close to target velocity (10°/s), stimulation would not have been effective. Otherwise, we can assume that effective sites are characterised by representing higher preferred velocities as 10°/s. Since we did not determine the preferred velocity of each stimulation site, this explanation is similar speculative as the first explanation.

Size of affected tissue

Microstimulation and injections of muscimol most likely affected different amounts of tissue in area MST-I. Microstimulation at effective sites most likely affected neuronal activity within a single cortical column. Assuming passive spread of current, it can be approximated that stimulation at 80 µA (used at most sites) affected cortical tissue within a radius of 0.1 mm (Stoney et al. 1968). However, a recent study using fMRI to reveal the size of the affected tissue showed that tissue within a radius of up to ten times greater may have been affected (Tolias et al. 2005). The size of a column in area MST was estimated to be approximately 0.5 mm (Britten 1998). The amount of tissue affected by the muscimol injection was much larger, autoradiographic estimation of the extent of affected tissue (Martin 1991) suggest a radius of up to approximately 3 mm. Therefore, the effect of the transient lesions on eye movements
was due to the reduction of activity within a large portion of MST-I in the targeted hemisphere, definitively not only of a single cortical column or cluster.

**Common substrate of eye and hand movement control**

This processing of visual motion is not only related to the generation of smooth pursuit eye movements, but also to the generation of hand movements directed towards a moving target. As explained in the introduction, there are arguments for a common neuronal substrate for eye and hand movements (Engel et al. 1999; Engel and Soechting 2000; von Donkelaar et al. 1992). In the same vein, it was previously shown that the population vector of area MT and MST represents the target trajectory during a manual tracking task (Kruse et al. 2002). Similar results were obtained in a recent fMRI study addressing brain activity when subjects performed either eye or hand movements (Oreja-Guevara et al. 2004): hMT+ was similarly activated during both tasks. In addition, it has recently been shown that disturbing processing in the posterior parietal cortex by transcranial magnetic stimulation (TMS) prevents subjects from adapting to a velocity-dependent force field in a manual tracking task (Della-Maggiore et al. 2004). These results, together with our own results, indicate that MST plays a role in the generation of goal-directed eye and hand movements. The question that arises here is how does the information from MST in the extra-striate cortex reach the motor cortex? There are two possibilities which need to be considered: first, cortico-cortical projections may enable the information transfer. Projections from MST to the frontal eye field are well-documented (Boussaoud et al. 1990; Churchland and Lisberger 2005a). Alternatively, and more plausibly, the cortico-ponto-cerebello-thalamo-cortical pathway might be responsible for the information transfer (Ramnani 2006). There are projections from MST to the pontine nuclei (Hoffmann et al. 2002; Ilg and Hoffmann 1993). Single-unit recordings from
pontine nuclei have demonstrated responses to eye and hand movements (Matsunami 1987; Tziridis et al. 2004). Furthermore, Purkinje cells in the cerebellum are driven during manual tracking (Roitman et al. 2005) and during pursuit (Lisberger et al. 1987). Finally, the information could be sent from the cerebellum via thalamic relay to the motor cortex (Kelly and Strick 2003). It remains up to future poly-synaptic connectivity studies to prove or disprove this possible scheme of cortico-cortical information transfer.

Acknowledgement

We thank Ute Grosshennig for technical support throughout the entire study, Theresa Cooke for language assistance, Peter Thier for many discussions, and Gabriella Ugolini and Werner Graf for the anatomy of both monkeys. This work was financially supported by DFG SFB 550 A3, a DFG Heisenberg fellowship to U.I., and the Hermann and Lilly Schilling Foundation.
Figure legends

Fig. 1: Experimental paradigms used in this study. Event sequences during the microstimulation paradigm are shown. To examine the effects of transient inactivation of area MST-I, identical paradigms were applied without microstimulation. A shows the eye movement task; B shows the hand movement task. [color figure].

Fig. 2: Precision of pointing. The errors of hand movements of individual trials (1280 in each condition) are shown in the presence of the target (A) and in the absence of the target (B). The horizontal error (i.e., difference between target and finger position) is shown on the x-axis; the vertical error is shown on the y-axis. C and D give the resulting 2D Gaussian functions. The function shown in C had $x_0=0.3^\circ$, $y_0=0^\circ$, and STD = 2°. The function shown in D had $x_0=-0.8^\circ$, $y_0=-0.7^\circ$, STD = 2.7°. Note that the pointing was more precise if the target was present.

Fig. 3: Pursuit-related single-unit activity recorded from MST-I. Target and eye movement (green indicates horizontal target position; red indicates vertical target position) are shown for 8 different pursuit directions (velocity 10°/s). Neuronal activity is shown as a raster display and as spike density functions (standard deviation 40 ms) for each direction. In the central polar diagram, the steady-state activities are plotted together with the resulting von Mise distribution,

\[
f(x) = \frac{a}{(2\pi)^{1/2}\Gamma(0,k)} \exp\left(k^\ast\cos(x-T)\right)
\]

The arrow gives the preferred direction. The grey circle shows spontaneous activity measured during initial fixation of the stationary target. [color figure].
Fig 4: Effect of microstimulation on smooth pursuit eye movements. A shows target and eye position in the control condition directed in the preferred direction of this site (rightward and downward, -25°). B shows superimposed eye position during stimulation (in red) and control trials (in black). The red horizontal bar shows the stimulation period. Note that microstimulation did not produce a change in the latency of the eye movement. The red arrow marks the stimulation-induced overshoot in eye position; the black arrow marks the backward compensation. C shows eye velocity of control and stimulated trials. The red horizontal bar gives the stimulation period. D gives mean eye velocity. Trials are aligned on the offset of initial saccade. The black lines indicate the 200 ms stimulation interval of every individual stimulation trial. The two grey rectangles mark the time (50 ms) and mean values of the post-saccadic eye velocity in stimulated and control trials, respectively. These values are used to determine the stimulation vector.

Fig. 5: Post-saccadic velocities. The effect of stimulation on horizontal and vertical post-saccadic eye velocity (mean and standard deviation based on 10 trials in each condition) during pursuit in four different directions (approximately as arranged in the figure) is shown. The preferred direction of the stimulation site was to the right and down (-25°). Significant differences between stimulation (black bars) and control trials (grey bars) are marked (*: p<0.05, t-test). Target velocity was 10°/s. The resulting stimulation vector on post-saccadic eye velocity is shown in the central polar plot as a black arrow. In addition, the preferred direction of the pursuit-related activity at this stimulation site is shown as a grey arrow.
**Fig. 6 Effects of stimulation during fixation.** Mean horizontal eye velocity during microstimulation is shown by the grey trace. The stimulation interval is marked by the grey horizontal bar. The black trace represents the control condition without stimulation. The mean profiles were computed on 10 single trials each. The target was switched off during microstimulation. If the monkey was not engaged in pursuit, no effect of microstimulation on eye velocity was observed.

**Fig. 7: Stimulation effect in the presence and absence of the moving target.** A shows the absolute values of the stimulation vectors ($n=93$) if the target was present or absent during microstimulation. The mean value in the absence of target is given by the horizontal dashed line. The mean value in the presence of the target is given by the vertical dashed line. B shows the histogram of the deviation of the stimulation vector and the preferred direction for all 93 sites with significant effects.

**Fig. 8: Effect of microstimulation on hand movements.** Microstimulation resulted in a shift of hand movement towards the preferred direction of the stimulation site as shown. The pointing errors of single trials are shown in A together with the mean values of the Gaussian fits (control trials are in grey; stimulated trials are in black). B shows the fitted Gaussian functions (control: $x_0= -0.4^\circ$, $y_0= 0^\circ$, STD= 2.6°; microstimulation: $x_0= 0.8^\circ$, $y_0= 0.6^\circ$, STD= 2.8°). The black surface represents the Gaussian function fit to data from stimulated trials; the grey surface corresponds to control trials without stimulation. The direction of the stimulation-induced shift was 25°; the preferred direction at this site was 37°. The two distributions are significantly different ($p=0.00049$, t-test). The solid black line marks the zero-point. C shows the histogram of the deviations between the preferred direction of the pursuit-related
activity and the stimulation vector for the 28 sites with significant effects on hand movements.

**Fig. 9: Pre-lesion and post-lesion pursuit eye movements.** A and B show target (thick grey lines) and single-trial eye position traces for leftward (A) and rightward (B) pursuit. Black traces show pre-lesion pursuit traces for monkey BH (injection into right hemisphere) and red traces show post-lesion data. C and D provide target velocity (grey lines), mean smooth eye velocity and its standard deviation, computed upon de-saccaded velocity profiles. Note that target motion onset was at 500 ms.

[color figure].

**Fig. 10: Effects of transient deactivation of area MST on smooth pursuit eye movements.** The steady-state pursuit velocity is plotted against the stimulus velocity (5, 10, 20, and 30°/s, respectively) for monkey BH (A) and GH (B and C). Each grey band shows the monkey’s pre-lesion data based on 5 experiments, each consisting of at least 10 trial repetitions. The width of the grey band gives the standard deviation of pre-lesion steady-state eye velocity, centred at its mean value. Note that the steady-state pursuit velocity was only reduced compared to pre-lesion data if the target moved towards the lesioned side.

**Fig. 11: Pursuit onset latencies.** The left panel gives pursuit latencies during control and microstimulation (e-stim) averaged for all directions (mean and standard deviation). The middle panel gives pursuit latencies for leftward target movement.
The right panel shows latencies for rightward target movement during control and muscimol injections into either the right or left hemispheres.

**Fig. 12:** Pre-lesion and post-lesion latencies of the initial saccades during pursuit initiation. A, C and E show leftward target movement. B, D and F show rightward target movement. Black bars represent pre-lesion latencies, and grey bars represent post-lesion data. In addition to the histograms, mean and standard deviation are given by the horizontal bars. P values from t-tests are given.

**Fig 13:** Saccades towards stationary targets. A, B and C correspond to saccade latencies for 8 different target locations. D, E and F show saccade gain for identical target locations. Each data point represents the mean for 10 saccades. A and D are taken from monkey BH, right hemisphere. B and E are taken from monkey GH, right hemisphere. C and F are taken from monkey GH, left hemisphere. The grey band shows the mean and its standard deviation of pre-lesion data based on 100 saccades in each condition (*: p<0.05, t-test).

**Fig. 14:** Errors in hand movements towards a moving target. A shows pointing errors in the visually-guided hand movements of monkey GH while reaching for a moving target before (black symbols) and after muscimol injection (grey symbols). The crosses show the mean of the Gaussian fit. The data are based on upward, downward, leftward, and rightward target trajectories. Each condition was repeated 40 times. The raw data were fitted by a 2D Gaussian function as shown in B (black prelesion, $x_0$= -0.2°, $y_0$= 1.2°, STD= 1.9°; grey post-lesion, $x_0$= -4.4°, $y_0$= -0.6°, STD= 2.1°). The lesion-induced difference in the pointing error was significant (p<0.0001, t-
test). **C** shows the mean and standard deviation of pointing error pre-lesion (dark grey) and in six transient lesions in area MST (light grey) in the right hemisphere of monkey BH for all directions of target movements. The differences for all six injections were significant (p<0.0001, t-test). In **D**, the pointing error resulting from exclusive leftward (contraversive) target movements is shown. **E** shows the pointing error for rightward (ipsiversive) target movements.
References


Churchland AK and Lisberger SG. Discharge properties of MST neurons that project to the frontal pursuit area in macaque monkeys. *J Neurophysiol* 94: 1084-1090, 2005a.


Groh JM, Born RT, and Newsome WT. How is a sensory map read out? Effects of microstimulation in visual area MT on saccades and smooth pursuit eye movements. *J Neurosci* 17 (11) 4312-4330, 1997.


Maunsell JH and van Essen DC. The connections of the middle temporal visual area (MT) and their relationship to a cortical hierarchy in the macaque monkey. *J Neurosci* 3: 2563-2586, 1983.


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