Responses of MSTd and MT Neurons during Smooth Pursuit Exhibit Similar Temporal Frequency Dependence on Retinal Image Motion

When our eyes are in constant motion, the world around us remains perceptually stable; although eye movements produce slips of the visual scene on our retinal images. In our previous study, we suggested that visual motion in space is served by neurons, which compensate retinal-image motion due to pursuit eye movements, in the dorsal part of the medial superior temporal (MSTd) area. Additionally, neurons in the middle temporal (MT) area respond to retinal-image motion. In the present study, to further elucidate the visual properties of MSTd/MT neurons, we investigated the neuronal response to the motion of checkerboard patterns (CBPs) in addition to the random-dot pattern used in the previous study. We found that neuronal responses in both areas decreased regardless of fixation or pursuit when the temporal frequency of the CBPs exceeded 20 Hz on the retina. Our results support the idea that pursuit-speed compensation observed in area MSTd might be formed by the reception of retina-based visual information from MT neurons because both areas MT and MSTd were dependent on retina-based information during pursuit eye movements.

Keywords: area MSTd, area MT, monkey, smooth pursuit, visual motion

Introduction

Our eyes are in constant motion, but the world around us appears to remain stationary. To achieve this, the visual system must reconstruct a stable world in spite of retinal-image motion resulting from eye movements. We recently reported evidence for visuospatial stability during pursuit eye movements using a neurophysiological study in nonhuman primates (Inaba et al. 2007). We investigated correlates of visual motion in cortical regions of the dorsal part of the superior temporal sulcus (STS), that is, the middle temporal (MT) and dorsal part of medial superior temporal (MSTd) areas of macaque monkeys. Both of these regions have been known to be rich in motion-sensitive neurons and closely linked to the perception of visual motion (Maunsell and Van Essen 1983a, 1983b; Sakata et al. 1983, 1985; Saito et al. 1986; Newsome et al. 1988; Duffy and Wurtz 1991; Britten et al. 1992; Celebrini and Newsome 1994; Graziano et al. 1994; Williams et al. 2003).

In our previous study, we measured the speed tuning of MT and MSTd neurons to a large-field textured background that moved briefly at various speeds during smooth pursuit or stationary fixation. As a result, we found that most MSTd neurons were more sensitive to the actual motion of the background in space than to its motion on the retina, whereas most MT neurons were sensitive to retinal-image motion (Inaba et al. 2007). This result suggested that pursuit speed compensation observed in area MSTd may be formed by utilizing the information encoding eye movements and the retina-based visual information. In fact, it has been suggested that area medial superior temporal (MST) encodes extraretinal information related to the eye movements (Sakata et al. 1983; Newsome et al. 1988). Further, area MST receives strong fiber projections from area MT (Maunsell and Van Essen 1983a, 1983b; Ungerleider and Desimone 1986); thus, it is likely that area MT is the source of visual information for MSTd neurons. However, it is unclear whether the visual properties of MSTd-MT neurons due to their retinal origin would be similar or not.

MSTd neurons have been reported to prefer high stimulus speeds and exhibit saturation in firing rates in response to a random-dot pattern (RDP) as a large-field visual stimuli (Kawano et al. 1994). Furthermore, it was also reported that the responses of the MST neurons decreased when the monkey was exposed to a moving checkerboard pattern (CBP), and its temporal frequency exceeded 20 Hz (Kawano et al. 1994). It is currently unknown, however, whether such temporal frequency dependence of MST neurons is evident even during pursuit eye movements. Thus, in the present study, we investigated the neuronal responses in areas MSTd and MT to moving CBPs, instead of the RDP that was used in our preceding study (Inaba et al. 2007), during both pursuit and fixation. To examine whether their response characteristics are dependent on the temporal frequency of the image motion on the retina or on the screen, the animal was required to either fixate a stationary target or pursue a moving target.

Similar to our previous study, we found that when the temporal frequency of the moving CBPs was lower than 20 Hz on the retina, MSTd neuronal responses were correlated with their motion in space in most cases. However, when the background stimulus was moved at higher speeds on the retina and its temporal frequency exceeded 20 Hz on the retina, their neuronal responses decreased regardless of fixation or pursuit. The MT neurons showed a similar characteristic dependence on the temporal frequency of the visual stimulus on the retina. Therefore, the similar response properties of areas MSTd and MT to visual motion indicate that the characteristic dependence of MSTd neurons during pursuit eye movements might be formed by utilizing the retina-based information mediated by MT neurons.

Materials and Methods

The procedures used in the present study are similar to those previously described (Kawano et al. 1992; Inaba et al. 2007). All experimental protocols were approved by the Animal Care and Use Committee of Kyoto University.

Animal Preparation

Two male rhesus monkeys (monkeys S and K; Macaca mulatta) weighing 7 and 9 kg were used in this study; however, monkey S was

Naoko Inaba and Kenji Kawano

Department of Integrative Brain Science, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan
also used in our previous study (Inaba et al. 2007). Prior to surgery, all animals were trained to track a small target spot, and anatomical pictures were obtained using magnetic resonance imaging (MRI) scans (Signa Horizon). Surgeries were performed under general anesthesia using pentobarbital sodium. Under sterile conditions, scleral search coils were chronically implanted for measuring eye position (Judge et al. 1980), and a head holder and recording chambers were embedded in a dental acrylic cap that covered the top of the skull. The recording chambers were stereotaxically placed to allow for a dorsal approach to the posterior parietal cortex in the vertical orientation (stereotaxic coordinates: anteroposterior -2 to -4 mm, mediolateral ± 16-18 mm).

**Recording Technique and Histology**

Initial mapping penetrations were made with handmade glass-coated tungsten electrodes and located at the STS; MRI scans were used to confirm the location of the STS. Within the STS, neurons were identified as either MSTd or MT neurons according to previously published reports of their location relative to the STS and their receptive-field characteristics (Gattass and Gross 1981; Komatsu and Wurtz 1988). Single-unit recordings were obtained with tungsten microelectrodes (Microprobe, FHC or Nano Biosensors). Vertical microelectrode penetrations were made via transdural guide tubes inserted in the grid hole using a guide-tube grid system (Crist Instruments, Hagerstown, MD).

Histological sections through the STS of monkey S were obtained at the end of the experiments as previously described (Kawano et al. 1994). Histological verification of the recording sites indicated that these neurons were located in either area MSTd or MT (Maunsell and Van Essen 1983a, 1983b; Komatsu and Wurtz 1988; Kawano et al. 1994). In the other animal (monkey K), histological verification of the recording sites has not been obtained, as recordings are still under way.

**Behavioral Paradigms and Visual Stimuli**

In a dark room, the animal was seated in a primate chair with its head fixed by a head holder. The animal faced a translucent, tangent screen. In a dark room, the animal was seated in a primate chair with its head holder. The animal faced a translucent, tangent screen. In a dark room, the animal was seated in a primate chair with its head holder. The animal faced a translucent, tangent screen. In a dark room, the animal was seated in a primate chair with its head holder. The animal faced a translucent, tangent screen. In a dark room, the animal was seated in a primate chair with its head holder. The animal faced a translucent, tangent screen.
160 deg/s), was turned on for 200 ms. The positive-negative value of the speed indicates motion in the preferred-antiprefed direction. An electromechanical shutter in the light path was used to turn the textured background stimulus on and off. After 1000 ms (the total duration of each target ramp motion), the target spot was turned off, which signaled the end of the trial. The animal then received a fluid drop as a reward for maintaining its eyes within 2.5 deg of the target spot throughout the trial.

For measuring and mapping the visual receptive field, we displayed a target (0.3 deg) and random dots (1 deg x 1 deg to 10 deg x 10 deg), created by MATLAB PsychToolbox (Pelli 1997), on the screen using a mirrored reflection digital light-processing projector (WT600, NEC). Visual receptive-field mapping was conducted while the animal looked at the central fixation target as random dots moved at a constant velocity (20-80 deg/s) in the preferred direction.

Data Collection and Analysis
The stimulus presentation and data collection were controlled by a personal computer (PC) using the real-time experimental system (Hays et al. 1982). Eye movements were measured with the electromagnetic search-coil technique (Fuchs and Robinson 1966). Voltage signals encoding the horizontal and vertical components of the eye position were low pass filtered with R-C circuitry (170 Hz, -3 dB) and digitized to a resolution of 12 bits with a 1-kHz sampling rate. All eye position were low pass filtered with R-C circuitry (170 Hz, -3 dB), and eye velocity traces were derived from the 2-point backward difference of the eye-position data in order to identify saccades using velocity criteria; eye movements were determined to be saccades if the eye velocity was greater than ±25 deg/s from the each target velocity (0 deg/s for fixation and 20 deg/s for pursuit). A time-amplitude window discriminator was used to measure spikes with a time resolution of 1 ms. Spike-density histograms were calculated by convolving the spike trains with a Gaussian function (sigma = 10 ms) (Richmond et al. 1987). Neuronal responses to the motion of the textured background were measured as the average of the spike densities from 50 to 250 ms after the onset of the background-stimulus presentation (i.e., from 550 to 750 ms after the onset of the target ramp motion). For all analyses, at least 3 repetitions of each stimulus condition (8.2 ± 3.6 trials mean ± standard deviation, SD) were required for each neuron. One repetition consisted of 14 trials per block (i.e., 16 speeds of the background stimulus by 3 patterns of the textured background image [RDP and CBPs of 0.5 and 0.25 cycles/deg] by the 3 tracking conditions [fixation and pursuits in preferred and antipreferred directions]). We exposed the animal to these stimulus conditions in a pseudorandomized order. The speed of the background stimulus on the retina was determined to be saccades if the eye velocity was greater than ±25 deg/s from the each target velocity (0 deg/s for fixation and 20 deg/s for pursuit).

Results
We recorded the activity of 117 neurons (75 in area MSTd and 42 in area MT) in the STS in 4 hemispheres of 2 awake, behaving monkeys. All of these neurons responded to brief movement of a large textured background with directional selectivity as evidenced by increased average firing rates in the preferred directions (≥1.5 times higher) compared with firing rates in the antipreferred directions. We identified the neurons in areas MSTd and MT using physiological criteria, such as the receptive-field properties of the recorded neurons, and their relative locations to the STS (Gattass and Gross 1981; Komatsu and Wurtz 1988).

Effects of 2 Different Background Images on the Responses of an MSTd Neuron
Figure 2A shows sample responses of an MSTd neuron to the brief movement of an RDP while the animal was fixating a target that was stationary (gray lines) or moving at 20 deg/s in the same (solid lines) or opposite (dashed lines) direction as the leftward moving (the neuron’s preferred direction) RDP. Data are shown from the time the target started to move (0 ms to focus on both the rising and falling gradients of the speed tuning curves. Therefore, we adopted the Gabor function analysis to calculate their speed-tuning curves within a wider range of speeds (between ~20 and +100 deg/s on the screen or the retina).

Moreover, to ask whether the decrease in neuronal response was dependent on the speed of the image motion on the retina or on the screen, we used CBPs in addition to the RDP and measured the peak of each speed-tuning curve. To calculate the speed-tuning curve of each neuron, we fitted a “Double-sigmoid function” described by the following formula:

\[ R(s) = a_1 + (d_2 - a_1) \times \frac{1}{1 + \exp^{-b_1(s-a_0)}} + \frac{1}{1 + \exp^{-b_2(s-a_0)}} \]

Parameters \(a_1\) and \(a_2\) correlated with the baseline firing rate and the peak firing rate of the neuron in response to the background motion, respectively. The speed of the background-image motion was denoted by \(s\). The slopes of the rising and falling gradients (steepness or gentleness) of the speed-tuning curves corresponded to \(b_1\) and \(b_2\), respectively. Parameters \(a_0\) and \(d_2\) correlated with the most sensitive speed change at the rising and falling gradients of the speed-tuning curve, respectively.

We quantified potential differences between using RDP and CBPs among the tracking conditions. First, we normalized the responses to the CBPs of each neuron with respect to its best response to the RDP for the 3 tracking conditions. Second, the rate of the decrease of the normalized response was calculated for each tracking condition by dividing the response to the CBP by the response to the RDP at each stimulus speed. If the dependence of the neuronal responses to the stimulus speed of the CBP was the same as of the RDP, then the rate of decrease should be 1.0, that is, no decrease. Otherwise, because the temporal-frequency contents of the CBPs are limited in contrast to the RDP, the response would decrease when the temporal frequency of the moving stimulus exceeds the system’s upper limit. We fitted the following formula to the rate of the decrease of the normalized response for each tracking condition:

\[ R'(s) = a_1 + (d_2 - a_1) \times \frac{1}{1 + \exp^{-b_1(s-a_0)}} \]

Interestingly, the function \(R'(s)\) is the same as part of the tuning function \(R(s)\) in the Double-sigmoid function analysis that accounts for the falling gradient of the speed-tuning curve. The parameter \(a_0\) determines the 50% temporal cut-off frequency on the retina and the slope of the rate of decrease for the normalized responses. The fitting was performed using self-written scripts in addition to available package programs in MATLAB (function ‘fmisearch’).
on the time base), and the moving background was visible only for 200 ms (500–700 ms). In Figure 2Aa–e, the RDP was stationary (0 deg/s) and moved at 20, 40, 80, and 160 deg/s in the preferred direction. The neuron increased its firing rate in response to the background motion with latencies of approximately 40–50 ms. As the speed of the RDP on the screen increased, the neuronal responses increased and saturated at high stimulus speeds (80 deg/s; Fig. 2Ad) for the 3 tracking conditions. Interestingly, the speed-tuning curves were similar despite different tracking conditions.

Using various speeds of the background stimulus on the screen, a similar pattern of neuronal behavior was observed in the 3 tracking conditions. We recorded the responses of the MSTd neuron to movement of the RDP (16 speeds between –160 to +160 deg/s) during the 3 tracking conditions: pursuit at 20 deg/s in the preferred and antipreferred directions and fixation. Figure 2C illustrates the neuronal responses of the MSTd neuron (Fig. 2A) with changing stimulus speeds of the RDP as the background image. As the speed of the RDP increased on the screen, the neuronal responses increased up to saturation, which occurred at a stimulus speed of 80 deg/s with the 3 tracking conditions (Fig. 2C). To quantify the relationship between the neuronal responses and the stimulus speeds, we calculated response shifts of the speed-tuning curves using a Double-sigmoid function analysis (see Materials and Methods). The shifts \( \omega_{1}, \omega_{2}, \omega_{3} \) in the rising gradient of each response curve for the 3 tracking conditions (preferred direction [solid line], antipreferred direction [dashed line], and fixation [gray line]) were estimated to be \( \{27.3, 19.8, 20.2\} \) along the x-axis for the background-stimulus speed on the

![Figure 2](https://example.com/figure2.png)

Figure 2. Effects of changing the speed of the background-stimulus presentation on the responses of an MSTd neuron. An RDP was used in (A,C,D) and a CBP of 0.5 cycles/deg was used in (B,E,F). The responses of an MSTd neuron to a moving RDP and CBP of 0.5 cycles/deg during fixation (gray lines), and pursuit in the preferred direction (black lines) and in the antipreferred direction (dashed lines) are presented in (A,B), respectively. The stimulus velocity, from the left to the right, was (a) 0, (b) 20, (c) 40, (d) 80, and (e) 160 deg/s on the screen in (A,B). The speed-tuning curves of the MSTd neuron in relation to the stimulus speed on the screen are presented in (C,E) and on the retina in (D,F). The pursuit target moved at 20 deg/s in the preferred (black circles and solid lines) and antipreferred (open circles and dashed lines) directions or remained stationary in the center of the screen (gray circles and lines).
screen. Considering that the maximum difference in the speed of eye movements was 40 deg/s, the data indicate that the rising gradient of response curves for the 3 tracking conditions exhibited essentially the same stimulus-speed dependence but were shifted horizontally with respect to each other. In addition, the MSTd neuron preferred high speeds and its firing rate tended to saturate when the RDP was used as the background. Thus, the shifts \( a_g \) of the falling gradient in the 3 tracking conditions were insignificant because the values of \( b_2 \) in each curve, which determined the slope of the falling gradient (i.e., steepness or gentleness) of the speed-tuning curves, were less than 0.0005.

This observation that the 3 tracking conditions exhibited stimulus-speed dependence on the screen was further confirmed by the following analysis: replotted the neuronal response data shown in Figure 2C as a function of the background speed on the retina (Fig. 2D). In Figure 2D, the data sets obtained for the 3 tracking conditions are widely separated, commensurate with the differences in the speed of the background image on the retina. To quantify the relationship between the neuronal responses and the stimulus speeds, the response data were fitted by a Double-sigmoid function. The shifts \( \{ a_g, b_2, o_g \} \) in the rising gradient of each response curve were clearly separated with large horizontal shifts which were estimated to be \([9.9, 38.6, 19.6]\) along the \( x \)-axis representing the stimulus speed on the retina (Fig. 2D). The differences among these shifts indicate that the activity of this neuron was "not" correlated with the background motion on the retina. To summarize, the MSTd neuron encoded the image motion on the screen (Fig. 2C) rather than the retinal-image motion (Fig. 2D) when the RDP was used as the background image.

We further examined the responses of the same MSTd neuron by substituting a CBP of 0.5 cycles/deg for the usual RDP. The relationship between the neuronal responses and stimulus speed differed when the stimulus was the CBP of 0.5 cycles/deg (Fig. 2B) as compared with the RDP used in the data represented in Figure 2A. Under the fixation condition (gray lines), the firing rate of the neuron increased with increases in stimulus speed from 0 to 40 deg/s on the screen (Fig. 2Ba-c). However, when the stimulus speed was 80 and 160 deg/s (Fig. 2Bd,e), the neuronal responses decreased. In contrast, during pursuit in the preferred direction (solid line), the neuronal response survived at a stimulus speed of 80 deg/s (Fig. 2Bd). During pursuit in the antipreferrred direction (dashed lines), the neuronal responses at stimulus speeds of 80 and 160 deg/s (Fig. 2Bd,e) were lower than that at 40 deg/s (Fig. 2Bc).

In Figure 2E, the mean firing rates of the same neuron are plotted as a function of the velocity of the CBP on the screen for the 3 tracking conditions. Compared with the neuronal responses under the conditions using the RDP (Fig. 2C), the neuronal responses in the same neuron were clearly different when the background image was the CBP and moved at high speeds (Fig. 2E). Under the fixation condition (gray circles), the responses of the MSTd neuron increased with increases in stimulus speed of the CBP up to \( \sim 40 \) deg/s on the screen. However, when the speed of the CBP was 60 deg/s, the neuronal responses were lower than at 40 deg/s. Moreover, the firing rate of this neuron during fixation decreased to near spontaneous levels when stimulus speeds of the CBP were between 80 and 160 deg/s. On the other hand, under the pursuit condition with the target moving in the preferred direction (black circles), the neuronal response at 60 deg/s was at the peak, and the responses were near baseline when the stimulus speed of the CBP was over 100 deg/s (Fig. 2E). Additionally, under the pursuit condition with the target moving in the antipreferred direction (open circles), the neuronal responses were lower than those during the fixation condition. The neuronal responses increased as the speed of the CBP increased to \( \sim 20 \) deg/s on the screen. When the speed of the CBP exceeded 60 deg/s, the neuronal responses were near baseline. Thus, when the CBP of 0.5 cycles/deg was presented as the background and the stimulus speed was over 20 deg/s (arrow in Fig. 2E), the responses of the MSTd neuron differed considerably depending on the tracking conditions.

The speed-tuning responses were fitted by the Double-sigmoid function analysis to examine whether the neuronal response was dependent on the image motion on the retina or on the screen when the CBP of 0.5 cycles/deg was used as the background image. In this case, we used the same \( a_g \) and \( b_1 \) that were calculated for the responses when the RDP was presented as the background. The remaining parameters \( a_1, a_2, b_2, o_g \) were set as free parameters. The shifts in the response curves \( \{ o_g, b_2, o_g \} \) and \( \{ b_2, b_2, b_2 \} \) were estimated to be \([86.2, -18.9, 62.6]\) and \([0.11, 0.04, 0.09]\) along the \( x \)-axis for the stimulus speed on the screen (Fig. 2E). In contrast to the data using the RDP as the background, these data indicated that the function \( R(s) \) of each tracking condition had a significant peak speed within our examined speed range of the background motion. Further, the falling gradients of the 3 tracking conditions were significantly separated along the \( x \)-axis for the stimulus speed on the screen. We then calculated the peak speed of the tuning curve in each tracking condition: the preferred and antipreferrred directions using both pursuit and fixation. As a result, the peak speeds for the 3 tracking conditions \([58, 27, 42]\) were also separated clearly along the \( x \)-axis representing the stimulus speed on the screen (Fig. 2E). The results suggest that pursuit eye movements in the preferred and antipreferrred directions caused the peak speed to shift along the \( x \)-axis representing the stimulus speed on the screen.

This observation that the peak speeds for the 3 tracking conditions were separated on the screen was further confirmed by the following analysis: re-plotting the neuronal response data as a function of the speed of the CBP on the retina. Figure 2F illustrates the dependence of the neuronal responses on the retinal speed of the CBP of 0.5 cycles/deg in each of the 3 tracking conditions. The data sets during the 3 tracking conditions seemed to decrease in a similar manner when the CBP was moved at speeds over \( \sim 40 \) deg/s on the retina. We also fitted the Double-sigmoid function in each tracking condition. The shifts \( \{ o_g, b_2, o_g \} \) were estimated to be \([67.6, 1.0, 62.1]\) along the \( x \)-axis for the stimulus speed on the retina (Fig. 2F). These data indicated that the speed-tuning curves in the 3 tracking conditions had peak speeds within our examined speed range of the background motion. The peak speeds in the response curves for the 3 tracking conditions in the preferred and antipreferrred directions and fixation were estimated to be \([40, 46, 42]\) along the \( x \)-axis for the stimulus speed on the retina (Fig. 2F). When the CBP was moved at over \( \sim 40 \) deg/s on the retina, the data indicate that the responses decreased with increases in the speed of the CBP on the retina regardless of fixation or pursuits (Fig. 2F). Therefore the peak speed in each tracking condition was dependent on the visual
property of stimulus motion on the retina rather than that on the screen. However, the neuronal responses were dependent on the motion on the screen until the speed of the CBP of 0.5 cycles/deg reached a certain limit of the stimulus speed on the retina (~40 deg/s).

Response Characteristics of MSTd Neurons

We recorded responses of 75 MSTd neurons (53 from monkey S and 22 from monkey K) to the background image moved at speeds of -160 to +160 deg/s during the 3 tracking conditions: pursuit at 20 deg/s in the preferred and antipreferred directions and fixation. Using both the Gabor function analysis and the Double-sigmoid function analysis, we calculated the shifts of the rising gradients of response curves of each neuron for the pursuit conditions with the target moving in the preferred or antipreferred direction relative to that of the response curve obtained for the fixation condition. These results are summarized in Figure 3 as frequency histograms of the means of the relative response shifts derived from the fitting of the Gabor function (Fig. 3A,B) and Double-sigmoid function (Fig. 3C,D) to the sets of the response data during the 3 tracking conditions. Using the Gabor function analysis on data collected with the RDP, the means of the response shifts of 75 MSTd neurons for the pursuit conditions in the preferred $\omega_p$ and antipreferred $\omega_f$ directions relative to the fixation condition $\omega_0$, on the basis of the background speed on the screen, were 9.8 ± 20.2 and 9.1 ± 21.3 deg/s (means ± SD), respectively (Fig. 3A; paired t-test: $P > 0.01$). On the other hand, the means of the relative response shifts {\{\omega_p-\omega_0, \omega_f-\omega_0\}} of MSTd neurons based on the retinal coordinates were -6.9 ± 19.3 and 26.9 ± 19.1 deg/s (means ± SD) (Fig. 3B; paired t-test: $P < 0.001$). These results were consistent with our previous study (Inaba et al. 2007).

Double-sigmoid function fitting was conducted for MSTd neurons, and the distribution of relative response shifts $\omega_0$ was summarized as frequency histograms (Fig. 3C,D). The shifts of the rising gradient of neuronal responses for the pursuits in the preferred $\omega_p$ and antipreferred $\omega_f$ directions relative to the fixation condition $\omega_0$, on the basis of the background speed on the screen, were distributed around 5.2 ± 38.2 and -2.0 ± 43.5 deg/s (means ± SD), respectively (Fig. 3C; paired t-test: $P > 0.01$). On the other hand, the means of the relative response shifts {\{\omega_p-\omega_f, \omega_f-\omega_f\}} of MSTd neurons based on the retinal coordinates were -14.9 ± 46.2 and 20.7 ± 55.8 deg/s (means ± SD), respectively (Fig. 3D; paired t-test: $P < 0.001$). Thus, the results of the Gabor function analysis and Double-sigmoid function analysis corroborated the findings of the MSTd response properties shown in Figure 2: During pursuit eye movements, most neurons in area MSTd were more sensitive to the motion of the RDP in space than to its motion on the retina.

When using the CBP of 0.5 cycles/deg, the decrease in response of the MSTd neurons was dependent on the stimulus speeds on the retina regardless of pursuits or fixation. The mean shifts of peak speeds for the pursuit conditions in the preferred and antipreferred directions relative to the fixation condition on the basis of the background speed on the screen were distributed around 10.4 ± 53.2 and -35.9 ± 105.5 deg/s (means ± SD), respectively (Fig. 3E; paired t-test: $P < 0.001$). On the other hand, the means of the relative shifts of the peak speeds based on the retinal coordinates were -10.7 ± 51.3 and -26.0 ± 105.3 deg/s (means ± SD), respectively, and were not significantly different (Fig. 3F; paired t-test: $P > 0.01$). These data suggest that the peak speed of each MSTd neuron depended on the property of visual motion on the retina rather than on that on the screen.

Response Characteristics of MT Neurons

In our previous study (Inaba et al. 2007), we revealed that the responses of MT neurons were dependent on the retinal speed of the RDP in contrast to the responses of MSTd neurons. In the present study, to investigate whether the neuronal dependence on the motion of the CBP at high speeds of areas MSTd and MT is similar, we also recorded responses of 42 MT neurons (33 from monkey S and 9 from monkey K). The Gabor function analysis of the neuronal responses to the RDP revealed that the means of the relative response shifts {\{\omega_p-\omega_0, \omega_f-\omega_0\}} of the MT neurons, on the basis of the background speed on the screen, were 18.9 ± 4.6 and -15.7 ± 6.0 deg/s (means ± SD), respectively (Fig. 4A; paired t-test: $P < 0.001$). On the other hand, the means of the relative response shifts {\{\omega_p-\omega_0, \omega_f-\omega_0\}} of the MT neurons based on the retinal coordinates were 1.8 ± 4.6 and 2.5 ± 5.7 deg/s (means ± SD), respectively (Fig. 4B; paired t-test: $P > 0.01$). Additionally, the Double-sigmoid function analysis revealed that the means of the relative shifts of the rising gradient of speed-tuning responses {\{\omega_p-\omega_f, \omega_f-\omega_f\}} of the MT neurons on the basis of the background speeds on the screen were 21.2 ± 12.6 and -7.7 ± 43.7 deg/s (means ± SD), respectively (Fig. 4C; paired t-test: $P < 0.001$).
function analysis coincided with those of the Gabor function analysis. Thus, most neurons in area MT were more sensitive to the retinal motion of the RDP than its motion on the screen regardless of the tracking conditions.

When using the CBP of 0.5 cycles/deg, the shifts of the peak speeds for the pursuits in the preferred and antiprefred directions relative to the fixation based on the screen coordinates were distributed around 24.6 ± 38.5 and −10.3 ± 39.2 deg/s (means ± SD), respectively (Fig. 4E; paired t-test: P < 0.001). Again, the results of the Double-sigmoid function analysis coincided with those of the Gabor function analysis. Thus, most neurons in area MT were more sensitive to the retinal motion of the RDP than its motion on the screen regardless of the tracking conditions.

When using the CBP of 0.5 cycles/deg, the shifts of the peak speeds for the pursuits in the preferred and antiprefred directions relative to the fixation based on the retinal coordinates were 5.6 ± 39.5 and 8.6 ± 46.2 deg/s (means ± SD), respectively (Fig. 4F; paired t-test: P > 0.01). The data suggest that the peak speed of each MT neuron depended on the property of visual motion on the retina rather than that on the screen, which is similar to the MSTd neurons as shown in Figure 3F.

Population Responses to the Background Motion in Neurons from Areas MSTd and MT

Figure 5 illustrates the effects of changing the stimulus speed of the background images on the mean responses of the MSTd neurons (N = 75). The mean responses were normalized with respect to the best response of each neuron to the moving RDP. The mean responses of the 75 MSTd neurons were fitted by the Double-sigmoid function. The shifts \( \{ \omega_{f_1} - \omega_{f_1}, \omega_{f_1} - \omega_{f_1} \} \) of the rising gradient of the speed-tuning curves for the 3 tracking conditions with the RDP were estimated to be \{26.8, 18.9, 15.9\} along the \( x \)-axis for the background-stimulus speed on the screen (Fig. 5A). On the other hand, the shifts \( \{ \omega_{f_1} - \omega_{f_1}, \omega_{f_1} - \omega_{f_1} \} \) on the basis of the stimulus speed on the retina were estimated to be \{7.8, 37.3, 15.3\} (Fig. 5B). The results suggest that as a population, the responses of the MSTd neurons were sensitive to the motion of the RDP on the screen rather than that on the retina.

We examined the relationship between the mean responses of MSTd neurons and stimulus speed using the CBP of 0.5 cycles/deg (Fig. 5C,D). When the CBP of 0.5 cycles/deg was presented as the background and the stimulus speed was over

---

**Figure 4.** Frequency histograms of the relative response shifts of 42 MT neurons. (A,B) Distributions of the relative response shifts of the speed-tuning curves fitted by the Gabor function for pursuit in the preferred (solid line) and antiprefred (dashed line) directions on the basis of the stimulus speed on the screen (A) and on the retina (B). (C,D) Distributions of the relative shifts of the rising slope of the speed-tuning curves fitted by the Double-sigmoid function on the basis of the stimulus speed on the retina (C) and on the retina (D). (E,F) Distributions of the relative shifts of peak speeds of neuronal responses to the motion of the CBP of 0.5 cycles/deg on the basis on the stimulus speed on the screen (E) and on the retina (F).

**Figure 5.** Effects of using an RDP and CBP of 0.5 cycles/deg on the responses of 75 MSTd neurons during pursuit. (A,B) The speed-tuning curves of the mean responses of MSTd neurons in relation to the speed of the RDP on the screen (A) and on the retina (B). (C,D) The speed-tuning curves of the mean responses of MSTd neurons in relation to the speed of the CBP on the screen (C) and on the retina (D). The pursuit target moved at 20 deg/s in the preferred (black circles with solid lines) and antiprefred (open circles with dashed line) directions or remained stationary in the center of the screen (gray circles with gray lines).
20 deg/s (arrow in Fig. 5C), the mean responses of the MSTd neurons differed considerably depending on the tracking condition. When the stimulus speed exceeded 40 deg/s on the retina, the neuronal responses began to rapidly decrease regardless of eye movements (arrow in Fig. 5D). The peak speeds of the speed-tuning curves of the MSTd neurons were {49, 22, 31} on the screen (Fig. 5C) and {30, 40, 30} on the retina (Fig. 5D). The results indicate that as a population, the peak speed of the responses of the MSTd neurons depended on the property of visual motion on the retina rather than that on the screen.

When we used the RDP as the background stimulus, the MT neuronal population (N = 42) consistently encoded the background image motion on the retina rather than that on the screen (Fig. 6A,B). The mean shifts {ω₆, ωₓ, ωᵧ} of the rising gradient of neuronal responses were {26.1, −10.3, 6.4} and {6.9, 8.4, 6.1} on the screen and on the retina, respectively. The results suggest that the mean responses of the MT neurons were sensitive to RDP motion on the retina rather than on the screen. Lastly, the peak speeds of the speed-tuning curves of the mean responses of the MT neurons in the 3 tracking conditions were estimated to be {54, 25, 38} and {41, 44, 39} on the screen and on the retina, respectively. The results suggest that the mean responses of the MT neurons were sensitive to RDP motion on the retina rather than on the screen. When we used the CBP of 0.5 cycles/deg as opposed to the RDP as the background stimulus, MT neurons exhibited a sharper decrease in mean responses to the increased stimulus speed (Fig. 6C,D). The neuronal responses in the 3 tracking conditions increased with stimulus speeds of up to ~20 deg/s on the retina. When the stimulus speed exceeded 20 deg/s on the retina, the neuronal responses began to decrease regardless of fixation or pursuits. Moreover, the neuronal responses in the 3 tracking conditions approached baseline levels at stimulus speeds over ~100 deg/s on the retina (Fig. 6D). Thus, the mean responses of the MT neurons decreased when the background-stimulus speed increased from 20 to 100 deg/s on the retina. Additionally, the peak speeds of the speed-tuning curves of the mean responses were {39, 8, 21} on the screen and {26, 27, 22} on the retina (Fig. 6C,D). These results confirm a similar property of MSTd and MT neurons of decreasing responses when the CBP was used as the background stimulus and moved at high speeds on the retina.

Effects of Temporal Frequency of the Background Image

When the CBP was used as the background stimulus, the dependence of both MSTd and MT neurons on the retinal image motion appeared to be similar at high stimulus speeds. To investigate whether the decrease in neuronal responses at higher speeds is due to the upper limit of the temporal frequency of the retinal image motion, the neuronal responses to the motion of a CBP of an additional spatial frequency (0.25 cycles/deg) were studied. We recorded responses of 26 of the 75 MSTd and 16 of the 42 MT neurons to the CBPs of both 0.5 and 0.25 cycles/deg to quantify potential differences between using RDP and CBPs among the tracking conditions. We calculated the rate of the decrease of the normalized responses and fitted the formula $R(s)$ to the rate of the decrease of the normalized response for each tracking condition (see Materials and Methods). Results for the neuronal responses in both areas are shown in Figure 7. The normalized responses in area MSTd decreased rapidly when the temporal frequency of the CBP of 0.5 cycles/deg exceeded ~20 Hz (0.5 cycles/deg × 40 deg/s) on the retina irrespective of the tracking condition (Fig. 7A). Over this frequency, the normalized responses decreased sharply in proportion to the temporal frequency of the visual motion on the retina regardless of pursuits and fixation. As a result, the shift {ω₆, ωₓ, ωᵧ} and {b₂, b₂, bₙ} were estimated to be {30.5, 28.6, 30.8} and {0.13, 0.32, 0.21}, respectively (Fig. 7A). Therefore, these data indicate that the normalized responses in area MSTd decreased to 50% irrespective of the tracking condition when its temporal frequency of the CBP of 0.5 cycles/deg exceeded approximately 30 Hz on the retina.

This observation that the decrease for the normalized responses was dependent on the temporal frequency of the visual motion on the retina was corroborated using a CBP with

![Figure 6](image_url)

**Figure 6.** Effects of using an RDP and CBP of 0.5 cycles/deg on the responses of 42 MT neurons during pursuit. (A,B) The speed-tuning curves of the mean responses of MT neurons in relation to the speed of the RDP on the screen (A) and on the retina (B). (C,D) The speed-tuning curves of the mean responses of MT neurons in relation to the speed of the CBP on the screen (C) and on the retina (D). The pursuit target moved at 20 deg/s in the preferred (black circles with solid lines) and antipreferred (open circles with dashed line) directions or remained stationary in the center of the screen (gray circles with gray lines).
a different spatial frequency (i.e., 0.25 cycles/deg compared with 0.5 cycles/deg). Figure 7B shows the rate of decrease for the normalized responses in area MSTd. The shifts \( \{b_0, b_1, b_2, b_3, b_4\} \) and \( \{b_1, b_2, b_3, b_4\} \) were estimated to be \( \{29.5, 27.3, 32.1\} \) and \( \{0.11, 0.14, 0.10\} \), respectively (Fig. 7B): The normalized responses in area MSTd decreased to 50% when its temporal frequency of the CBP of 0.25 cycles/deg exceeded approximately 30 Hz on the retina. Actually, the properties of the decrease in the normalized responses were similar between the CBPs of 0.5 and 0.25 cycles/deg. Although the CBPs contain a wide range of spatial frequencies in terms of a Fourier decomposition of the background stimulus, the responses decreased to 50% when the fundamental temporal frequency of the stimulus exceed \( \sim 30 \) Hz (0.5 cycles/deg \( \times 60 \) deg/s, 0.25 cycles/deg \( \times 120 \) deg/s) on the retina.

A similar pattern was observed in the neuronal behavior of area MT (Fig. 7C,D). The normalized responses in area MT decreased rapidly when the temporal frequency of the CBP of 0.5 and 0.25 cycles/deg exceeded \( \sim 20 \) Hz on the retina irrespective of the tracking condition. The normalized responses of MT neurons also decreased to 50% cut-off frequency irrespective of the tracking condition when its temporal frequency exceeded 30 Hz on the retina (Fig. 7C,D); this is similar to that exhibited by MSTd neurons. Actually, the shifts \( \{b_0, b_1, b_2, b_3\} \) and \( \{b_1, b_2, b_3, b_4\} \) were estimated to be \( \{31.6, 29.4, 30.5\} \) and \( \{0.18, 0.12, 0.16\} \), respectively, when using the CBP of 0.5 cycles/deg (Fig. 7C), and \( \{26.9, 26.5, 30.0\} \) and \( \{0.12, 0.07, 0.11\} \), respectively, when using the CBP of 0.25 cycles/deg (Fig. 7D). The normalized responses in area MT decreased to 50% when its temporal frequency of the CBPs exceeded approximately 30 Hz on the retina.

The results revealed that the upper limit of the temporal frequency for the visual responses in both areas was \( \sim 20 \) Hz (40 deg/s with a CBP of 0.5 cycles/deg and 80 deg/s with that of 0.25 cycles/deg) on the retina. However, the decline in the neuronal responses with CBPs in area MT seemed to be slightly less severe than that observed in area MSTd, for example, when the CBP of 0.5 cycles/deg was used, the mean of \( b_1 \) for the normalized responses in area MSTd was 0.22, whereas that in area MT was 0.15. To reconcile this observation, we compared the distribution of the best speeds of the RDP within the neurons recorded in areas MSTd and MT (Fig. 7E). Similar to the previous report using a large-field RDP (90 \( \times \) 90 deg) as the visual stimulus (Kawano et al. 1994), most of the MSTd neurons showed their best responses at high stimulus speeds (Fig. 7E; black bars; 93.0 \( \pm \) 61.0 deg/s). On the other hand, most of the MT neurons exhibited their best responses at lower speeds than those exhibited by MSTd neurons (Fig. 7E; white bars, 49.0 \( \pm \) 43.4 deg/s). This suggests that the difference in the means of \( b_1 \) (MSTd 0.22, MT 0.15) might be due to the difference between the distributions of the best response speeds for these neurons.

Hence, we selected the 14 MSTd and 13 MT neurons of which the peak speeds were between +10 and +60 deg/s when the RDP was used and recalculated the rate of the decrease of the normalized responses and fitted the formula \( k'(s) \) to the rate of the decrease of the normalized responses for each tracking condition. The normalized response of the 14 selected MSTd neurons also decreased in proportion to the temporal frequency of the visual motion on the retina regardless of pursuits and fixation when the CBP of 0.5 cycles/deg was used, and the shifts \( \{b_0, b_1, b_2, b_3, b_4\} \) and \( \{b_1, b_2, b_3, b_4\} \) were estimated to be \( \{30.0, 24.9, 28.1\} \) and \( \{0.12, 0.25, 0.27\} \), respectively. The shifts \( \{b_0, b_1, b_2, b_3, b_4\} \) and \( \{b_1, b_2, b_3, b_4\} \) of the 13 MT neurons were estimated to be \( \{30.3, 25.6, 29.6\} \) and \( \{0.33, 0.11, 0.21\} \), respectively. For these selected neurons, the mean of \( b_2 \) for the normalized responses of the MSTd neurons was 0.21 (N = 14), whereas that of the MT neurons was 0.22 (N = 13). The result supports the idea that the \( b_2 \)-differences in the MSTd and MT neurons are due to their different dependence on the stimulus speed.

**Discussion**

In our previous study, we suggested that visual motion in space is served by MSTd neurons that use retina-based information and the information encoding eye movements (Inaba et al. 2007). It has been reported that area MST receives strong fiber projections from area MT (Maunsell and Van Essen 1983a, 1983b; Ungerleider and Desimone 1986). Accordingly, the
most likely source of the retina-based signals on the MSTd neurons is area MT. In the present study, to investigate whether the MSTd and MT neurons show a similar dependence on the temporal frequency of the visual stimuli during pursuit and fixation, an RDP and CBPs (0.5 and 0.25 cycles/deg) were presented as the visual stimuli. As a result, we found that neuronal responses in both areas MSTd and MT exhibited similar temporal-frequency dependence on the retinal image motion during pursuit eye movements. Our study suggests that pursuit-speed compensation observed in area MSTd might be formed by utilizing the retina-based information mediated by MT neurons.

In the present study, we asked whether the decreases in the neuronal responses were dependent on the speed of the image motion on the retina or on the screen by moving the background image at a wider range of speeds (between −160 and 160 deg/s) than previously used (Inaba et al. 2007). We adopted the Gabor function analysis, which we used in the previous study, and calculated the speed-tuning curves for the neuronal responses to visual motion of the RDP on the screen or the retina; this was conducted for the range of stimulus speeds between −20 and +100 deg/s. Although the speed range of the RDP was wider than previously used (between −40 and +60 deg/s), the data in the present study are consistent with our previous study. Additionally, a Double-sigmoid function analysis was also conducted for MSTd and MT neurons to confirm the dependence of the neuronal responses on the visual properties of the moving background image. Despite the differences in the methods, the distributions of response shifts $\omega$, obtained by the Gabor function analysis, and $\omega_0$, obtained by the Double-sigmoid function analysis, corresponded with each other. Both analyses confirmed the findings of our previous study (Inaba et al. 2007): Neuronal responses in area MSTd were correlated with the speed of visual motion in space, whereas the responses of MT neurons were correlated with the speed of retinal image motion.

Because we focused largely on the falling gradients in the speed-tuning curves for fixation and pursuit, we adopted the Double-sigmoid function analysis in the present study. Thus, the dependence of the neuronal responses in area MSTd on the image motion on the screen remained regardless of pursuits or fixation when we used a CBP of 0.5 cycles/deg as the background stimulus and its speed was lower than 20 deg/s on the retina. However, when its speed was over 40 deg/s on the retina, the responses of MSTd neurons differed considerably depending on the tracking conditions, and their responses rapidly began to decrease. In addition, the responses of the MT neurons decreased irrespective of the tracking condition when the stimulus speed of the CBP of 0.5 cycles/deg exceed 40 deg/s on the retina, which is similar to that observed with MSTd neurons. The peak speeds of the speed-tuning curve of the mean responses of the MSTd and MT neurons on the retina were $\sim$30 and $\sim$25 deg/s, respectively. Moreover, the normalized responses in both areas between using RDP and CBPs of 0.5 and 0.25 cycles/deg among the tracking conditions decreased to $\sim$50% with its temporal frequency greater than 30 Hz on the retina (0.5 cycles/deg × 60 deg/s, 0.25 cycles/deg × 120 deg/s). Thus, the properties of the neuronal responses in both areas to visual motion on the retina at high temporal frequency were similar.

It has been reported that neuronal responses in area MST decreased rapidly when the temporal frequency of the background stimulus exceeded 20 Hz (Kawano et al. 1994). However, their study was mainly concerned with the relationship between the neuronal responses in area MST and ocular following responses elicited by a moving large-field textured background. In the present study, we used similar large-field visual stimuli and the results are consistent, although the monkeys performed the stationary fixation task. In addition, we tested the neuronal responses in areas MSTd and MT when the monkey fixated a stationary target or pursued a moving target. The neuronal responses in both areas decreased independent of eye movements when the speeds of the CBP exceeded a certain limit of the stimulus speed on the retina. The results of our study suggest that the decrease in neuronal responses in both areas when the visual stimuli moved at high speeds is due to characteristics of the visual system that depend on the limit of the temporal frequency of the visual motion on the retina. In areas MSTd and MT, we found that the characteristics of the neuronal responses to the visual motion with high temporal frequencies on the retina were similar. Thus, our findings are consistent with the idea that the source of retina-based signals on the MSTd neurons might be area MT.

However, when the temporal frequencies were lower than 20 Hz on the retina, there was a considerable difference between the responses of MSTd and MT neurons to image motion. It is possible that our results are biased in favor of neurons that prefer a large-field background image moving at high speeds, because we always used a large-field visual stimulus (80 deg × 80 deg) moving at 20–80 deg/s in 1 of the 8 directions for screening the neurons. We classified many neurons, even some of the MT neurons, as preferring high speeds. Most of the MSTd neurons showed their best response with the RDP (160 deg/s) at the highest background speed we tested. However, most of the MT neurons preferred to medium speeds ($49.0 \pm 43.4$ deg/s) when the RDP was used. According to the difference in preferences for stimulus speeds of the RDP, MSTd neurons might receive information of retinal image motion from the MT region in addition to other areas (Boussaoud et al. 1992). It has been reported that some V1 neurons (both simple and complex) have temporal-frequency response profiles that are low pass, whereas others tend to be band pass (Foster et al. 1985; Perrone and Thiele 2002). In addition, there is experimental evidence that the neurons in V1 provide direct and indirect inputs to the MT (Movshon and Newsome 1996; Ponce et al. 2008). Accordingly, it is conceivable that the area V1 may be source of the decrease in speed-tuning responses of MT and MSTd neurons.

It has been reported that both areas of MT and MST contain neurons that discharge during smooth pursuit of a small target in the dark. Some of these neurons, which are called visual-tracking neurons or pursuit cells (Sakata et al. 1983; Newsome et al. 1988), were also responsive to moving large-field stimuli. In the present study, we found that some MT and MSTd neurons exhibited the pursuit-related responses when the animal pursued a target moving against a dark background prior to the presentation of the image stimulus. Similar to our previous report (Inaba et al. 2007), such pursuit-related modulations were relatively weak, and no significant differences in the effects of the eye movements on the response shifts to the background motion were found between neurons with and without pursuit-related modulations. The lack of visual-tracking neurons strongly modulated during pursuing a small target against a dark background might be due to our
screening method using a moving large-field RDP. To understand what sort of relevant processing occurs in area MSTd for encoding the visual motion in space irrespective of eye movements, it is necessary to carry out further research on other types of MSTd neurons including the visual-tracking neurons.

Although we found that the retinal image motion due to pursuit eye movements was compensated to serve visual motion in space in MSTd neurons, the amount of the compensation revealed by the response shift of the speed-tuning curve differed depending on the direction of the pursuit, in the preferred or antipreferred directions. Consistent with our previous study (Inaba et al. 2007), the shift of the speed-tuning response during pursuit in the preferred direction was weaker than the shift during pursuit in the antipreferred direction (see Fig. 5A,B). This might be due to the characteristics of either the available visual inputs (e.g., speed tuning) or insufficient extraretinal information, or both. Further detailed experiments on the characteristics of the compensation with different pursuit directions or speeds might provide more information on this issue.

In summary, MSTd neurons were able to respond to visual motion in space when the moving stimulus on the retina did not exceed the limit of the temporal frequency of the visual motion processing system. Our results suggest that such response properties in area MSTd might be formed by receiving retina-based visual information from MT neurons during pursuit eye movements.

Funding
Ministry of Education, Culture, Sports, Science and Technology. KAKENHI (17022019).

Notes
We are grateful to Drs S. Shinomoto, K. Miura, and S. Hata for helpful advice and discussion; I. Kawamoto for technical assistance; and N. Yamamoto and M. Takeshita for secretarial assistance. Conflict of Interest: None declared.

Address correspondence to Naoko Inaba, PhD, Department of Integrative Brain Science, Graduate School of Medicine, Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan. Email: ninaba@brain.med.kyoto-u.ac.jp

References


