1. INTRODUCTION

It has been shown that human subjects can perform smooth-pursuit eye movements directed by first- and second-order motion stimuli. This type of eye movement represents an important behavioral probe for visual motion processing. The analysis of the initiation of these eye movements revealed specific differences for different types of motion stimuli. This indicates that first- and second-order motion may be processed by different mechanisms. In support of this notion, there is evidence from psychophysical, clinical, and neuroimaging studies indicating that first- and second-order motion are indeed processed separately in different areas of striate and extrastriate cortex.

It must be noted that the term second-order motion is used for a highly heterogeneous class of motion stimuli that includes motion defined by disparity, spatial phase shift of periodic luminance changes, relative motion, and texture orientation (for an overview see Ref. 8). Obviously, the segmentation cues between object and background are different in all of these stimuli.

It is well accepted that the processing of visual motion in primates is a cortical feature. In area V1, where the first stage of cortical processing takes place, roughly 20% of the neurons responded directionally to the movement of a visual stimulus. Most likely, these directionally selective neurons constitute the input to the motion area within the superior temporal sulcus (STS); the middle temporal area (MT) or area V5 (owl monkey, and rhesus monkey). Neurons in area MT project to the next motion area in STS, the middle superior temporal area (MST). The contribution of areas MT and MST to the processing of visual motion was convincingly revealed by single-unit recordings in monkeys performing specific visual tasks. It was possible to correlate single-unit activity with motion perception (see, e.g., Ref. 16; for review, Ref. 17). Even in the absence of a moving stimulus, some neurons in these areas responded to inferred motion. Finally, when second-order motion was used, it was proposed that neurons in area MT may code for motion irrespective of specific form parameters of the stimulus. In addition, it must be stressed that neurons in area MST do respond selectively to complex visual stimulus properties such as expansion/contraction and rotation (e.g., Ref. 20), whereas neurons in area MT lack this specificity.

However, the neuronal substrate underlying the processing of second-order motion has so far been addressed only by experiments consisting of single-unit recordings in either anesthetized animals or monkeys that were trained to simply fixate a stationary spot. Some neurons in the cortical area V1 responded selectively to orientation of drift-balanced bars. When the response to moving stimuli was addressed, no direction-selective response from area V1 was reported for second-order stimuli. Neurons recorded from cat area 18 were shown to respond to drift-balanced motion stimuli. Several studies addressed the responses of primate area MT, an area specialized for motion processing as explained above.
Specifically, neurons in area MT were shown to respond to stimuli defined by temporal structure, texture, and contrast envelopes.

The majority of second-order stimuli contain differences in temporal structure (flicker) between figure and background. In our study, we applied two types of second-order motion that differ in segmentation cues. The first type was a drift-balanced second-order motion stimulus defined by a difference in temporal structure. The other type of second-order motion stimulus was defined by opposed direction of dot and object motion (theta motion). In this stimulus, temporal structure differences between object and background can be eliminated.

Three important questions are addressed by our study. First, none of the above-mentioned studies examined whether the animals could perceive the moving second-order stimuli at all. Obviously, in the context of a behavioral study, it must be demonstrated that monkeys are able to perceive the stimuli. Second, whether the neuronal responses to the motion stimuli are affected by the segmentation cues between figure and background should be examined. Third, since there is some evidence that the responses of neurons in area MST are able to encode more-complex stimulus properties than those of area MT, the responses of neurons recorded from areas MT and MST should be compared. Preliminary results have been published.

2. MATERIALS AND METHODS

A. Visual Stimuli

Four different motion stimuli were used in the experiments: one first-order motion stimulus and three second-order motion stimuli. The background consisted of a random pattern of white dots with a density of 2% on a dark screen (mean luminance 1.9 cd/m²). The motion stimulus was a rectangular object made up of a random dot pattern with the same dot density as the background. The relationship between the motion of the object and the motion of the dot pattern was varied for the different stimuli (see Fig. 1):

1. Fine Fourier (fF): The moving bar was made up of an unchanging pattern of random dots moving coherently across the dynamic background.
2. Drift-balanced (Db): The bar acted as a moving window in the dynamic background, through which a second layer of unchanging dots was seen. The bar pattern was stationary in space. With respect to the temporal structure of figure and background, this stimulus is an inverse version of an earlier described drift-balanced stimulus.
3. Theta motion (Th-dyn): The bar consisted of coherently moving dots. However, the motion of the dots was in the direction opposite to that of the bar, again leading to the impression of a moving window through which a second layer of moving dots could be seen.

These three stimuli were presented in front of a dynamic background, with the random dot frames changing every 16.7 ms (60 Hz).

4. Theta motion (Th-stat): The theta-motion stimulus is presented on a stationary background.

Table 1 shows which segmentation cues, namely, flicker (temporal changes in luminance) and motion between the moving stimuli and the background, are present in each stimulus.

B. Chronic Single-Unit Recordings

Under sterile conditions and intubation anesthesia, the monkeys received a classical dental cement implant including head holder and recording chamber, as well as a subconjunctival search coil to precisely monitor the eye position. All animal procedures were carried out in accordance with the guidelines laid down by the National Institutes of Health and the German law and were approved by the local ethics committee. The center of each recording chamber was aimed at the MST (lateral 19, posterior 3.5, and dorsal 16 mm) tilted 30° upward in a parasagittal plane. The single-unit activity was recorded
with use of self-made glass-insulated tungsten electrodes whose high stability and stiffness allowed transdural tracks without a guiding tube. The microelectrode signal was preamplified, low-pass filtered at 10 kHz, and fed to a multispike detector (Alpha Omega, Model MSD). The temporal resolution of the sampling of the neuronal activity was 4 kHz. Horizontal and vertical eye position were fitted to the responses to leftward and rightward motion with a t-test. Only neurons with a significant difference (p < 0.01) were accepted. The tuning width was determined as the standard deviation of a Gaussian function fitted to the responses elicited by the different directions. The speed tuning of a given neuron was determined by the response to different dot velocities (range 1 to 40°/s).

3. Direction-Discrimination Task
During the direction-discrimination trials, the monkey fixated a small red stationary target (diameter 20 arc min) placed in center of the visual field during the stimulus presentation. The size of the gaze control window was 2° in each dimension. At the onset of each trial, either a static or a dynamic random dot background was presented for 500 ms. The base activity of a neuron was determined in a 300-ms time interval during this period. A randomly selected motion stimulus was then presented for 1000–1500 ms. The neuronal response to the stimulus was defined as the activity in the middle 500 ms of this period. To quantify the strength of this response, we calculated a modulation index (MI) defined as

\[
MI = \frac{activity \, during \, stimulus \, presentation}{base \, activity}.
\]

The stimulus trajectory was adjusted to ensure that the bar crossed the center of the receptive field halfway through the presentation time. The height of the stimulus was fixed to 10°, and the width was adjusted between 2.5° and 5° so that the stimulus width was always smaller than the width of the receptive field.

The speed of the moving stimulus was adjusted according to the preferred speed of each neuron, in the range between 8 and 15°/s. The lower limit was determined by the difficulties the animals had in performing the task. The upper limit resulted from the decay of the first-order motion component of the theta stimulus at high stimulus speed that was due to the short lifetimes of the dots inside the stimulus bar.

After the stimulus disappeared, the monkeys had to keep fixation for another 500 ms. They then had to report, within 2000 ms, the perceived direction of stimulus motion by making a saccade to one of two alternative targets (diameter 20 arc min, green color) which were presented 20° left and right of the fixation point. For correct behavior the monkeys were rewarded with a small portion of water or apple juice. The sequence of events of
the direction-discrimination task is shown in Fig. 2. During mapping of the receptive fields and determination of a neuron’s tuning, the monkeys had to direct their attention toward the fixation spot. In the discrimination task, they had to attend to the moving object.

3. RESULTS

A. Performance in the Behavioral Task

After a training period of several weeks, both monkeys were able to correctly discriminate the motion direction of all four stimuli. However, the reliability of the answers was slightly different between the stimuli (Fig. 3). A two-factorial analysis of variance revealed significant effects of monkey ($p < 0.001$), stimulus type ($p < 0.001$), and the combination of the two factors ($p < 0.001, n = 64$). This analysis shows that the performance varied from one stimulus to the next as well as between individual monkeys. However, the significant interaction of the two factors indicates that each monkey had an individual pattern of performance for judging the direction of the individual stimuli.

B. Single-Unit Recordings

1. Localization of Recorded Neurons

The neurons whose activity is included in this report had to meet two criteria. First, the neurons had to respond directionally ($p < 0.01$) to the horizontal movement of at least one of our motion stimuli. Second, at least five correct trials for each stimulus type and motion direction had to be collected. Our resulting data sample consisted of 38 MT and 68 MST neurons (see Fig. 4).

The anatomy of only one monkey is available at the present time; the other monkey is still in the experiment. Drawings of parasagittal sections with the recording sites of MT and MST neurons are shown in Fig. 5 for monkey F. For monkey G, the differentiation was performed by the coordinates of the recording location, the recording depth, the ratio of eccentricity, and the size of the neuronal receptive fields (see Fig. 4(d)) and the presence of an extraretinal signal during the execution of smooth-pursuit eye movements.$^29$

![Fig. 2. Sketch of a single trial. Each trial was subdivided into four phases. The monkey had to fixate a red dot in the first three phases, and in the fourth phase he reported the perceived direction of stimulus motion by a saccade toward the target located in the direction of the movement of the previously displayed stimulus. The base activity of a neuron was obtained during the last 300-ms interval of fixation 1. The neuronal response to the stimulus was obtained during the stimulus presentation in a time window of 1000 ms.](image)

![Fig. 3. Percentages of correct responses of monkeys F and G for the four different motion stimuli, as indicated. Effects of monkey, stimulus type, and combination of the factors are significant (two-factorial analysis of variance, $p < 0.001, n = 64$).](image)

![Fig. 4. Description of the data sample. (a) Histogram of preferred directions of all recorded neurons. Since the values for neurons recorded from areas MT and MST were not statistically different, we pooled the values obtained from the two areas. 0° represents rightward motion, 90° upward motion. (b) Histogram of tuning widths of all recorded neurons. (c) Histogram of directional selectivity to horizontal stimulus movement expressed as DI. (d) Ratios of area to eccentricity of receptive fields of neurons recorded from areas MT and MST in monkey G. Differences between the two areas were significant ($t$-test).](image)
2. Response to First-Order Motion Stimulus

Figure 6 gives the trajectory of a fF stimulus, the behavior of the monkey, and the responses of a neuron during the different parts of the discrimination trials as explained in Section 2. Note that the monkey correctly reported every presentation of the fF stimulus, as indicated by the saccades at the end of every trial.

As expected, most of the neurons in both areas responded well to first-order motion stimuli (Fig. 6). However, the directional selectivity is usually lower than in the direction-tuning paradigm [see Fig. 7, compare Fig. 4(c)]. This reduced selectivity can be explained by the fact that the fF stimulus is not as strong as the stimulus used in the direction-tuning paradigm (dots moving in a circular aperture). No difference in directional selectivity was seen between the responses from areas MT and MST, so we present only pooled data here.

3. Response to Drift-Balanced Motion Stimuli

A subset of neurons in both areas showed a directional response to Db stimuli similar to that of the neuron shown in Fig. 8. Forty-one percent (14/38) of the MT and 50% (34/68) of the MST neurons showed a significant direc-

Fig. 5. Drawings of four parasagittal sections of monkey F. The borders of area MT were plotted on the basis of dense myelination. The sites where MT and MST neurons were recorded are labeled. The direction of microelectrode penetrations is also shown.

Fig. 6. Response of a typical MT neuron during discrimination of a first-order stimulus. From top to bottom, the position of stimulus and response targets, eye position, and neuronal activity as raster and peri-stimulus-time-histogram (PSTH) are shown. The time of onset of saccades, which is taken as the reaction time, is marked with an x in the raster display. (a) The stimulus moved in the preferred direction; (b) the stimulus moved in the nonpreferred direction. The thick horizontal line in the PSTH marks the time interval in which the response to the stimulus was determined. gh37-2 is the designation of the individual neuron.

Fig. 7. Distribution of directional selectivity of the responses elicited by the fF stimulus expressed as DI ($n = 106$).

tional selectivity elicited by Db stimuli. The responses of most neurons revealed a decrease in the directional selectivity to drift-balanced motion compared with first-order fine Fourier motion, as shown in Fig. 9. For all recorded neurons \((n = 106)\), the average reduction in DI was 53%. Again, we found no differences between the responses of neurons in areas MT and MST. Neither the average directional selectivity nor the percentage of neurons responding to the Db stimulus was significantly different.

Eight percent (9/106) of the recorded neurons showed no significant directional response to the fF stimulus but responded significantly to the Db stimulus. However, the strength of the directionality of these neurons was rather low (average DI = 0.28).

4. Responses to Theta-Motion Stimulus

Figure 10 shows the responses of a typical neuron in area MT to the movements of fF, Th-stat, and Th-dyn stimuli. The preferred motion of most neurons recorded was different for the theta-motion stimulus than it was for the fine Fourier motion (see top and bottom rows in Fig. 10). This apparent inversion is not surprising since the theta-motion stimulus consisted of single dots moving in the direction opposite to the motion of the object. The change of preferred direction in this case indicates that the neuron encoded the motion of the individual dots only and did not respond to the motion of the object. When the theta-motion stimulus was presented on a static background (Th-stat), the neurons responded to the movement in the preferred as well as in the nonpreferred direction (see Fig. 10, middle row). However, we did not find a single neu-
that gave a response that signaled the direction of object motion in the case of the theta stimulus either on the static or on the dynamic background.

In Fig. 11, the directional selectivity obtained from 76 neurons by ff and Th-dyn stimuli are compared. No differences in responses to Th-stat and Th-dyn stimuli were found between area MT and MST. The directional selectivity and the modulation in preferred and nonpreferred directions were not significantly different between the two areas. Therefore we do not separate the two areas in Figs. 11 and 12. To illustrate the inversion in preferred direction, the DI values obtained by Th-dyn were multiplied by −1 if the preferred direction was different between ff and Th-dyn stimuli. The DI values of the two stimuli correlate ($r = -0.64$, $p < 0.001$). The legend shows which DI values were significant ($t$ test of the responses in preferred and the nonpreferred directions).

Forty-seven percent (36/76) of the neurons responded significantly to the Th-stat stimulus moving in the preferred direction, and 29% (22/76) of the neurons responded significantly to the same stimulus moving in the nonpreferred direction. However, with the Th-dyn stimulus, the response to the direction of object motion vanished almost completely. As shown in Fig. 12(b), only 5% (4/76) of the neurons showed a significant modulation in the preferred direction. The response to the movement of the individual dots remained unchanged by the temporal structure of the background; 57% (43/76) responded significantly in the nonpreferred direction. As shown in Table 1, when theta motion was presented in
clusively those neurons (lus and receptive field and analyzed the responses of ex-
we addressed the issue of directional matching of stimu-
movement in our sample of neurons was high. However,
data-collection periods possible.
keep the paradigm as simple as possible to make long
pixel arrangement of the display. Second, we tried to
the theta-motion stimuli is barely visible owing to the
stimulus movement in our study for two reasons. First,
by our statistical analysis. We used only horizontal
motion only.
The absence of a response of Th-dyn in the preferred di-
rection might alternatively be explained by a mismatch of
the preferred direction of the neuron and the direction of
stimulus motion. The response to the direction of object
motion in theta motion might be weak when the stimulus
direction is not well matched to the preferred direction of
the neuron. The weak responses might not be detected
by our statistical analysis. We used only horizontal
stimulus movement in our study for two reasons. First,
for oblique directions the first-order motion component in
the theta-motion stimuli is barely visible owing to the
pixel arrangement of the display. Second, we tried to
keep the paradigm as simple as possible to make long
data-collection periods possible.
As shown in Fig. 4(c), the selectivity for horizontal
movement in our sample of neurons was high. However,
we addressed the issue of directional matching of stimu-
lus and receptive field and analyzed the responses of ex-
cursively those neurons (n = 26 out of 76) that had pre-
ferred directions within the range of ±20° from the
horizontal. Results of this analysis are shown in the in-
sert of Fig. 12(b). The mean value of the MI was not
changed (p = 0.83, t-test) by this selection. This indi-
cates that the mismatch in preferred direction cannot ac-
count for the absence of response to direction of object
motion.

5. Differences in Responses of MT and MST Neurons
Besides the significant difference in size of receptive fields
at a given eccentricity, no other significant differences be-
tween MT and MST neurons were found in the course of
this study (Fig. 13), either in directional selectivity of IF
and Db stimuli or in the responses to the different types
of theta-motion stimuli.

4. DISCUSSION
Our monkeys performed the psychophysical task cor-
rectly, which indicates that they were able to perceive the
movement of each type of motion stimulus. Whereas all
106 neurons recorded from areas MT and MST responded
directionally to the motion of a fF stimulus, only roughly
half responded directionally to a moving stimulus defined
by Db motion. We did not find a single neuron coding the
movement of a theta-motion-defined stimulus indepen-
dent of its first-order motion component.

A. Performance in the Direction-Discrimination Task
The performances of the two monkeys in the direction-
discrimination task differed significantly (expressed as a
percentage of correct responses). These differences
might emerge from differences in training history, atten-
tional abilities, and motivation of the animals. Perfor-
ance was also significantly affected by the stimulus
type. This might be explained by a difference in percep-
tual demands of these stimuli. However, the significant
interaction between the factors monkey and stimulus
type shows that each monkey had individual stimulus
preferences. This suggests that the observed differences
are most likely not based on different processing of the
stimuli but rather on different training and abilities of
the individual monkeys.
In general, the movement of an object can be detected
by two different mechanisms: either by the change in po-
sition or by low-level motion detection. By means of a
psychophysical study, it was shown that human subjects
use motion signals to detect first-order motion, whereas
the detection of second-order motion stimuli depends on
the change in position. It is impossible to determine
which cue was used by our monkeys. However, our pre-
viously published study on smooth-pursuit eye move-
ments showed that human subjects are able to perform
these eye movements to second-order motion stimuli.1
This suggests that in the direction-discrimination task,
the object motion can also be used.

B. Comparison of Neuronal Responses and Pursuit
Initiation
An important question addresses the issue of whether the
neuronal responses can be predicted solely from the
movements of the individual dots or whether the re-
sponses are affected by higher-order processing such as
flow-field analysis or even extraretinal signals such as
eye-movement-related signals.29
As shown in Fig. 11, the absolute value of the direc-
tional selectivity obtained by the first-order component of
the Th-dyn stimulus was significantly reduced compared
with that obtained by the fF stimulus. The number and
velocity of moving dots were identical in the two stimuli;
only the direction was inverted. This difference in neu-
ronal responses parallels the observed difference in pur-
suit initiation elicited by these stimuli.2 Neither the
neuronal responses nor the initiation of pursuit eye move-
ments can be explained solely by the movement of the in-
dividual dots.
C. Relevance of Segmentation Cues

A subset of neurons in areas MT and MST responded to second-order motion stimuli defined by temporal structure (Db), as reported by Albright. The percentage of responsive neurons was lower in our study (54%) than in the previous report (87%). This can be explained by the differences in the stimuli used in the two investigations. The stimulus used by Albright was a dynamic bar moving over a static background, whereas in our experiment this stimulus was a static area moving on a dynamic background. In the Db stimulus, the segmentation cue between figure and background was a difference in temporal structure, which is processed early in the visual system. This is supported by the finding that some neurons in area V1 gave orientation-selective responses to dynamic-noise bars presented on a static background.

In the case of the Th-stat stimulus, some neurons in areas MT and MST showed a significant activation by the motion of the individual dots as well as by the motion of the object. The fact that we did not find neurons responding to the Th-dyn stimulus moving in the preferred direction indicates that these neurons responded only to the flicker component of the object motion, which was present only in the Th-stat and the Db but not in the Th-dyn stimulus (see Table 1). So the segmentation cue “opposed motion” is not encoded explicitly in the neuronal activity of MT and MST neurons.

It was previously suggested that the processing of the theta motion independent of its temporal structure component was achieved by a two-layer model of elementary motion detectors. As our results suggest, the first layer, responding to the first-order motion components, might be equivalent to the neurons in areas MT and MST. The second layer of elementary motion detectors seems not to be implemented by short-range connections within these areas. On the basis of our negative results, two possibilities for implementation of the second layer within the primate brain remain. First, neurons in highermotion areas, such as more rostral areas within the STS or area VIP, a special motion area within the intraparietal sulcus, might respond to the movement of the object independent of the movement of individual dots. Alternatively, the movement of the theta-motion stimulus might be encoded in the population activity of areas MT and MST.

In summary, our results suggest not only that first- and second-order motion stimuli are processed separately but also that second-order motion stimuli based on different segmentation cues between figure and background are processed by different mechanisms. The hypothesis of form–cue-invariant motion processing in areas MT and MST is not supported by our results.

D. Methodological Limitations

Since we report a negative result, i.e., that neurons in areas MT and MST were not able to code for the movement of a stimulus independent of the specific stimulus parameters, we have to examine whether this negative result is a possible effect of the specific conditions in the stimulus presentation.

The ratio of the width of receptive field and stimulus is of special importance since only at the stimulus border can the segmentation cues between figure and background be used to detect the stimulus motion. Responses would be lost if the stimulus were substantially larger than the receptive field. Therefore we adjusted the width of the stimuli in our experiment in a way such that they were always smaller than the width of the receptive field. This possibility can therefore be excluded by our experimental procedure.

A mismatch between speed and directional tuning of a neuron and the properties of the presented stimulus could also hide weak directional responses. Even though there was some mismatch caused by limitations in presentation of second-order stimuli (as described above), the directional responses in most neurons in our sample remained strong. This indicates that the tuning width of the neurons was large enough to guarantee clear responses even when the stimulus was not optimally fitted to the preferences of the neuron. Our analysis also revealed that the average directionality of responses to Th-dyn were not affected if we restricted the analysis to neurons with the horizontal preferred direction. It therefore cannot be presumed that responses to the Th-dyn stimulus were extinguished by choice of the stimulus parameters.

E. Absence of Differences between Areas MT and MST

It is well established that area MST receives its major input from area MT, so area MST is the station subsequent to area MT in the processing of motion that underlies goal-directed behavior. Some neurons in area MST, especially those in the dorsal part (MSTD) are able to respond selectively to flow field properties. Other neurons, especially those in the lateral part (MSTl) included extraretinal signals such as those related to eye movement or head movement.

The anatomical reconstruction of our recording sites shows that the majority of MST recordings were located within the floor of STS, where subarea MSTl is located. The visual properties of areas MT and MSTl neurons are quite similar; the major difference consists in the extraretinal response property of area MSTl neurons.

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