

Methods

Data were collected from nine adolescent Japanese monkeys (*Macaca Fuscata*), weighting 5 - 9 kg. All animals had been previously trained to fixate a small target spot on a tangent screen for a liquid reward (Wurtz 1969). A cylinder for chronic recording of single neuron activity was implanted in each monkey under pentobarbital sodium anesthesia and in aseptic conditions. A fixture was attached that allowed the head to be fixed in the standard stereotaxic position during the experiments. Scleral search coils were implanted in both eyes to record eye movements, according to the technique of (Judge et al. 1980) and eye position was monitored via an electromagnetic induction technique (Fuchs and Robinson 1966). The coil output voltages were calibrated with respect to eye position by having the animal fixate small LED targets at known positions along the horizontal and vertical meridians. All experimental protocols were approved by the Electrotechnical Laboratory Animal Care and Use Committee.

Behavioral paradigms and visual stimuli

The behavioral paradigms and visual stimuli used in the present study were identical to those of (Shidara and Kawano 1993) and are described elsewhere (Kawano et al. 1992, 1994; Shidara and Kawano 1993). In brief, during the recording sessions the monkey sat in a primate chair with its head secured to the chair, and faced a translucent screen (85 deg x 85 deg)

located 235 or 500 mm in front of the animal. The visual stimulus was back-projected on the screen as a ramp movement of the random-dot pattern; each ramp started 50 ms (in some cases, 100 - 300 ms) after the end of a saccade directed to the central part of the screen. The stimulus lasted 250 - 300 ms, then the screen went blank for 0.5 - 2 s while the animal remained in the dark. The ramps were presented at five speeds (10, 20, 40, 80, and 160 deg/s) and in eight directions (right, left, up, down, and four diagonals). The monkeys were given an occasional drop of fruit juice to remain alert and facilitate fast saccades.

Recording technique

Single unit activities were recorded using tungsten microelectrodes in the MST, DLPN, or VPFL. A hydraulic microdrive (Narishige Mo-9) was mounted on the recording cylinder, and glass-coated tungsten microelectrodes were used for the initial identification and mapping of each area and the neighboring structures. A fixed grid system (Crist et al. 1988) was then used to introduce and fix in place a stainless steel guide tube through the dura. The tips of the guide tubes were positioned 3 - 5 mm above the MST, DLPN, or VPFL, and flexible tungsten electrodes were used to record through the tube.

Acquisition of behavioral and unit data

Our previous studies indicated that the neurons in the MST and DLPN, and the P cells in the VPFL, discharged during brief, sudden movements of a large-field visual stimulus, eliciting ocular following, and that in most cases they increased the firing rate before the eye movements (Kawano et al. 1992, 1994; Shidara and Kawano 1993). We sought to further characterize the response properties of the neurons that discharge in relation to ocular following by selecting neurons according to their discharge sensitivity to a moving visual scene that elicited ocular following. After isolating a single unit, we observed its responses to a visual scene moving at 80 deg/s in eight directions and selected those in which the activity was modulated by one of these stimuli (the preferred direction). We then moved the visual scene in the preferred direction and recorded the neuronal and ocular responses at the five speeds.

During the ocular following responses, the mirror velocity and the horizontal and vertical components of eye position and eye velocity (measured with the search coils and filtered with a 6-pole analog Bessel filter using a cut-off frequency of 100 Hz), were recorded at 500 Hz. The speed of the random dot pattern on the screen was proportional to the mirror velocity. The single cell activity of each area was isolated using a time-amplitude window discriminator and was simultaneously recorded at 1,000 Hz. Some of the data presented here were taken from prior studies (Kawano et al. 1992, 1994; Shidara and Kawano 1993) and were reanalyzed.

Data preparation

We selected neurons whose responses to each stimulus were recorded for more than 30 trials under each condition. The firing frequency of each neuron and eye movement during 30 trials under the same stimulus conditions were ensemble averaged for each cell after excluding the trials with saccadic intrusion (N = 20 P cells, 35 MST neurons, and 32 DLPN neurons). The responses were aligned with the stimulus onset (time 0) and the eye acceleration profiles were obtained by digital differentiation of eye velocity profiles after averaging. Retinal slip as a visual motion signal was obtained by subtracting eye movement from mirror movement. To align the filtering delays, the ensemble average firing pattern (i.e., firing frequency temporal pattern) was low-pass filtered with a 6-pole Bessel digital filter using the same cut-off frequency (100 Hz) as that of the analog filter for the eye movements. All data were low-pass filtered with the same cut-off frequency to avoid an estimation error (the Butterworth filter cut-off was 50 Hz).

Analysis method (linear-regression models of firing rate)

To quantitatively analyze the relationship between sensory information as input (retinal slip) and neural activity, and the relationship between motor information as output (eye movement) and neural activity, we used a linear time-series regression method (Gomi et al. 1998; Shidara et al. 1993) that could reconstruct temporal patterns of the ensemble average

firing frequencies of neurons in each area. Our analysis was conducted according to the method of (Gomi et al. 1998). To compare the results from different areas, we applied the same linear-regression model to the data from each area using acceleration, velocity, and position of (A) sensory information (retinal slip), or (B) motor information (eye movement). Figure 1 shows the temporal patterns of each component of retinal slip as input and eye movement as output. The equation used for the analysis is as follows:

$$\hat{f}(t - \delta) = a \cdot \ddot{x}(t) + b \cdot \dot{x}(t) + c \cdot x(t) + d \quad (1),$$

where $\hat{f}(t)$, $\ddot{x}(t)$, $\dot{x}(t)$, $x(t)$, and δ are the reconstructed firing frequency of a neuron; the acceleration, velocity, and position of eye movement or retinal slips at time t ; and the time delay, respectively (Fig. 1). Four coefficients (a, b, c, d) and the time delay (δ) were estimated in such a way as to minimize the squared estimation error. A linear-regression method was applied to the firing pattern, from 10 ms after stimulus onset (ramp onset) to 2 ms before the end of the stimulus (the duration was 238 ms or 288 ms), to estimate each coefficient at a particular δ . The search range for δ was limited from -20 to 20 ms using the eye movement components, and from -80 to -30 ms using the retinal slip components.

An index "coefficient of determination" (C.D.; (Gomi et al. 1998; Hines and Montgomery 1972) expressed in the following equation (2) was used to evaluate the performance of the model.

$$C.D.=1-\frac{\sum_t(\hat{f}(t)-f(t))^2}{\sum_t(f(t)-\bar{f})^2} \quad (2),$$

where $\hat{f}(t)$, $f(t)$, \bar{f} indicate the reconstructed firing frequency, the observed firing frequency at time t , and the averaged firing frequency during the observation period, respectively. The C.D. ranges from 0 to 1, and approaches 1 when the reconstructed firing frequency was close to the observed value. If the C.D. is close to 1, it suggests that the temporal patterns of the firing frequency encode sensory or motor information. If the firing frequency is not linearly correlated with acceleration, velocity, or position of eye movement or retinal slip, the index approaches 0. This index equals the correlation coefficient squared.

Local Fitting and Global Fitting

Previous studies demonstrated the magnitude of ocular following is strongly dependent on stimulus velocity (Miles et al. 1986), and more recent experiments demonstrated that different temporal patterns of firing frequency can be induced by different stimulus velocities, i.e. neural responses are also strongly dependent on stimulus velocity (Kawano et al. 1992, 1994; Shidara and Kawano 1993). To investigate the relationship

between firing pattern and sensory/motor information under the single stimulus condition, we used Local Fitting (Gomi et al. 1998). By applying the model to the firing pattern at one of the five speeds, a local relationship between the neuronal firing pattern and the ocular responses or retinal slip can be examined. The temporal firing patterns in response to the five stimulus velocities in the preferred direction of a neuron were reconstructed by each set of parameters yielding five sets of parameters calculated per neuron. To test whether the model can be applied independent of the stimulus speeds such that all five responses could be reconstructed together using a single set of parameters from eye movement or retinal slip, we used Global Fitting (Gomi et al. 1998).

Modeling check

We applied the combination model from the first-order model to the second-order model having acceleration, velocity, position, bias terms, and delay to reproduce the observed firing patterns. Each model's suitability was then evaluated using Cp-statistics (Gomi et al. 1998; Hines and Montgomery 1972) and the t-test. The equation used for finding the Cp-statistics value is as follows:

$$Cp = \frac{\sum (\hat{f}(t) - f(t))^2}{\sigma^2} - n + 2p \quad (3),$$

where n is the number of data points for the regression, p is the number of degrees of freedom for the model, σ^2 is the estimated population variance, $\hat{f}(t)$ is the reconstructed firing frequency, and $f(t)$ is the observed firing frequency. In this analysis we used the estimated variance of the full term model [Eq. (1)] as the estimated population variance, σ^2 . The Cp-statistics value indicates whether an increase in the number of parameters in a model is warranted considering the accompanying increase in the C.D. A decrease in the Cp-statistics value suggests that the increase in the number of parameters in a model is warranted; that is, for an ideal model, the Cp-statistics value is at a minimum.