Gaze-Related Response Properties of DLPN and NRTP Neurons in the Rhesus Macaque

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INTRODUCTION

Combined eye-head movements are often required to track a moving target or to acquire a stationary target. Cooperation between visual and vestibular systems requires calculation of gaze to be able to accurately match eye velocity in space to target velocity. Such signals could have their origin, in part, from the frontal eye field cortex (FEF), where gaze velocity-related neurons were discovered during smooth pursuit or during motion of a large-field stimulus (4/43). A significant proportion of our rNRTP gaze velocity neurons (10/18) were also modulated during the VORd. We found that the majority of smooth pursuit-related neurons in rNRTP were best classified as gaze velocity (18/35) or gaze acceleration (11/35) sensitive. The remaining neurons were classified as eye position or eye/head related. We used multiple linear-regression modeling to determine the relative contributions of eye, head and visual inputs to the responses of DLPN and rNRTP neurons. Our results support the suggestion that both DLPN and rNRTP play significant roles not only in control of smooth pursuit but also in control of gaze.

Cortical neurons deliver signals to the vestibulo-cerebellum by way of the basilar pontine nuclei (Brodal 1980b; Gerrits and Voogd 1987). The dorsolateral pontine nucleus (DLPN) and rostral smooth pursuit region of the nucleus reticularis tegmenti pontis (rNRTP) are major components of the cortico-ponto-cerebellar pathway (Distler et al. 2002; Glickstein et al. 1994; May and Andersen 1986). While the DLPN and rNRTP are known to be essential for smooth pursuit (Mustari et al. 1988; Suzuki and Keller 1984; Suzuki et al. 1990, 1999; Thier et al. 1988; Yamada et al. 1996), their role in control of gaze is unclear.

The DLPN receives visual inputs from the extrastriate cortex (Distler et al. 2002; Glickstein et al. 1980, 1994; May and Andersen 1986), including areas MT/MST and sends mossy fiber projections to the contralateral ventral paraflocculus and dorsal paraflocculus (Glickstein et al. 1994; Nagao et al. 1997) and vermal lobules VI and VII (Brodal 1979, 1982; Langer et al. 1985). The rNRTP is known to receive inputs from the FEFs, supplementary eye fields (SFEs) (Brodal 1980a; Giorli et al. 2001; Huerta et al. 1986; Kunzle and Akert 1977; Shook et al. 1990), and to a lesser extent, from areas MT and MST (Distler et al. 2002). Projections of the rNRTP may differ from those of the DLPN in preferentially targeting vermal lobules VI and VII (Brodal 1980b, 1982). Therefore the neurons in the DLPN and rNRTP might carry different signals related to the control of gaze.

Aside from functional roles in control of gaze, it is also unknown whether DLPN and rNRTP smooth pursuit neurons have different roles in visual-vestibular behavior, even though the neurons in these two regions have similar responses during smooth pursuit tracking. Early studies found visually sensitive neurons in the rNRTP and DLPN. Such signals could play a role in supporting the vestibular ocular reflex (VOR) because residual retinal slip during the VOR engages optokinetic or smooth pursuit mechanisms to produce further compensation for head movements over a broad frequency range (Das et al. 1998; Raymond and Lisberger 1998). In this study, we attempt to characterize the role of the DLPN and rNRTP in relation to horizontal eye motion in space during behavior requiring interaction between visual and vestibular mechanisms. A preliminary report containing some of the findings described here has been published (Ono et al. 2003).

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METHODS

Surgical procedures

A detailed description of our surgical procedures can be found in earlier publications (Mustari et al. 1997, 1988, 2001). Behavioral and single unit data were collected from three normal juvenile rhesus monkeys (Macaca mulatta), weighing 3–5 kg. Sterile surgical procedures were carried out under aseptic conditions using isoflurane anesthesia (1.25–2.0%) to stereotactically implant a stainless steel head stabilization post (Crist Instruments, Hagerstown, MD) and chambers for recording. In the same surgery, a scleral search coil for measuring eye movements (Fuchs and Robinson 1966) was implanted underneath the conjunctiva of one eye using the technique of Judge et al. (1980). All surgical procedures were performed in strict compliance with National Institutes of Health guidelines, and the protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Emory University.

Behavioral paradigms

During all experiments, monkeys were seated in a chair with the head stabilized in the horizontal stereotaxic plane. Neurons in the DLPN and rNRTP were first classified as either large-field or parafoveal depending on the relative size of their visual fields and their response during smooth pursuit. Neurons that responded strongly for motion of a large-field (75° × 75°) stimulus, while the monkey fixated a centrally located stationary spot (~0.2° diam) were classified as large-field sensitive neurons. Neurons that responded during high-frequency oscillation of a small laser spot against a dark background and also during smooth pursuit of a small diameter (0.2°) target spot moving at low frequency (0.1–0.75 Hz; ±1°) were classified as smooth pursuit or parafoveal neurons (May et al. 1988; Mustari et al. 1988). We subjected smooth pursuit–related neurons to further testing to determine whether their responses were related to eye position or eye acceleration. For this testing, we required the monkey to fixate at static locations (~10, 0, +10), and we plotted a rate–position curve for each neuron. If a neuron showed no static rate–position sensitivity, modulation during sinusoidal smooth pursuit would most likely be related to eye velocity or eye acceleration. For all neurons modulated during horizontal smooth pursuit, we employed four vestibular testing conditions (typically 0.5 Hz; ±10°) including 1) sinusoidal whole-body rotation in darkness (VORD), 2) viewing an earth-stationary target during sinusoidal chair rotation (VORl), 3) viewing a target that moved exactly in-phase with the head to produce VOR cancellation (VORx0), and 4) viewing a target that moved equal and opposite to the head to produce VOR enhancement (VORx2). Large-field neurons were tested only in the VORD condition to examine vestibular (head movement–related) responses.

Data collection and analysis

Eye movements were detected and calibrated using standard electromagnetic methods (Fuchs and Robinson 1966) using precision hardware (CNC Electronics, Seattle, WA). Motion of the laser spot was controlled by a two-axis mirror galvanometer (General Scanning, Watertown, MA). Vestibular stimulation was provided by a servo-controlled 60 ft-lb DC torque motor (Neurokinetics, Pittsburgh, PA) that oscillated the chair sinusoidally about the vertical axis. All stimulus generation was computer controlled using custom Labview software and National Instruments hardware (Austin, TX). Eye, head, and target position feedback signals were processed with anti-aliasing filters at 200 Hz using 6-pole Bessel filters prior to digitization at 1 KHz with 16-bit precision. Velocity arrays were generated by digital differentiation of the position arrays using a central difference algorithm in Matlab (Mathworks, Natick, MA). Unit activity was recorded using custom made glass coated tungsten electrodes or commercial epoxy-coated tungsten (Frederick-Haer Corp., Brunswick, ME). The impedance of the electrodes was in the 1–3 MΩm range. Single unit action potentials were detected with either a window discriminator (Bak Electronics, Mount Airy, MD) or template matching algorithm (Alpha-Omega, Nazareth, Israel) and represented by a TTL level that was sampled at high precision as an event mark in our data acquisition system (CED Power1401, Cambridge, UK). During analysis, neuronal response was represented as a spike density function that was generated by convolving the spike times with a 5-ms Gaussian (Richmond et al. 1987).

Localization of NRTP and DLPN

We used both functional and anatomical criteria for localization of units in the rNRTP or DLPN. Recording chambers were stereotaxically implanted (anterior = 3; lateral = 1; 20° away from the midline) and aimed such that a track located in the center of the chamber intersected a point near the oculomotor nucleus (Fig. 1A). We first mapped the location of oculomotor neurons before running tracks to
deeper sites either in the NRTP or DLPN. Because we used a 20\degree angle for our tracks, we could reach both the NRTP and DLPN on each side of the brain using a single chamber. During recording, we mapped the saccade related region of the NRTP and the more rostral smooth pursuit–related region (rNRTP). Our studies were confined to the smooth pursuit region of the rNRTP and to the DLPN. We placed marking lesions (20 \mu A; 10s) on representative tracks at or near the depth of pursuit-related rNRTP (Fig. 1) or DLPN neurons (e.g., Mustari et al. 1988) to confirm the location of our recording sites. At the conclusion of our recording experiments, animals were deeply anesthetized and perfused with physiological saline followed by 4% paraformaldehyde. Frozen sections were cut at 50 \mu m, and every section was mounted on microscope slides and stained for Nissl substance to allow histological reconstruction of electrode tracks.

Model fitting and optimization

From previous studies and our quantitative characterization, it is clear that there are several different unit types with complex characteristics in the DLPN and NRTP. To provide a more objective method for unit classification and to consider possible combinations of signal types, we used a model estimation procedure to investigate potential information encoding within the individual response profiles of smooth pursuit–related units in the DLPN and rNRTP. We have already used a similar model estimation method to study information coding in parafoveal smooth pursuit-related cells in the pretectal nucleus of the optic tract (NOT) (Das et al. 2001). Eye, head, and retinal error velocity data were filtered using an 80-point finite impulse response (FIR) digital filter with a band-pass of 0–50 Hz. Saccades were marked with a cursor on eye velocity traces and were removed. After desaccading, the missing eye data were replaced with a linear fit connecting the pre- and post-saccadic regions of data using Matlab (Mathworks). Averaged data from at least 10 trials in which the eye was judged to be on target were used to identify coefficients in the following models. We applied our modeling procedure after pooling data obtained during smooth pursuit, VORx0, and VORx2. These conditions (VORx0 and VORx2) are most important for classifying neurons as gaze related. We excluded VORd and VOR1 conditions from our modeling studies, because gaze velocity is close to zero value in those two conditions

\[ FR(t) = A + BE(t) + CH(t) + DR(t) \]  
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In the equations shown above, \( FR(t) \) is the estimated value of the unit spike density function at time \( t \). \( E(t) \) denotes the eye motion at time \( t \), \( R(t) \) denotes head motion at time \( t \), \( H(t) \) denotes the retinal error motion at time \( t \), and \( A-G \) are constants that specify the coefficients in the models. Therefore model 1 relates unit response to eye, head, or retinal error velocity parameters. Model 2 relates unit response to eye, head, or retinal error acceleration parameters, and model 3 relates unit response to eye, head, or retinal error velocity and acceleration parameters, i.e., a combination of models 1 and 2. The goodness of fit was determined by calculating the coefficient of determination (CD). Since simply increasing the number of terms in the model could lead to improvement in CD, we also calculated a Bayesian information criteria (BIC) index between the experimentally observed unit data and the model estimated fit. The BIC measure served as a cost index that penalized adding new terms in the model (Angelaki and Dickman 2003; Cullen et al. 1996). BIC were calculated as following

\[ BIC(t) = log(1/N\sum[M(i) - dat(i)]^2) + P/2 log(log N/N) \]  

where \( dat(i) \) represents the firing rate modulation obtained experimentally during visual-vestibular behavior, \( M(i) \) is the corresponding value estimated from the model fit, \( N \) is the number of trials of sinusoidal smooth pursuit tracking or VOR task, and \( P \) is the number of the model parameters fit. For an increase in complexity of the model to be valid (e.g., model 3 compared with model 1), there must a relative increase in the CD and a relative decrease in the BIC index.

We also calculated coefficients of partial determination (partial \( r^2 \) values) as another indicator of the relative importance of each term (eye, head, and retinal error velocity and acceleration) to the firing rate of the neuron.

RESULTS

Response properties of DLPN neurons during visual, smooth pursuit, and vestibular testing

We recorded 51 neurons in the DLPN of three monkeys. Of these, 23 neurons responded to smooth pursuit of a small spot in dark and 28 neurons responded to motion of a large-field stimulus but not during smooth pursuit. Most smooth pursuit neurons (22/23) showed a monotonically increasing firing rate with eye velocity in a particular direction (Fig. 2). Only 1 of 23 pursuit neurons had apparent eye acceleration sensitivity. Of the 22 smooth pursuit eye velocity–related neurons, 13 neurons modulated during both VORx2 and VORx0 conditions but were not modulated during the VOR1 condition (Fig. 3A).

![Typical responses of smooth pursuit–related DLPN neuron during horizontal sinusoidal tracking at 3 different frequencies. Amplitude was ±10°. Solid and dashed lines indicate average target velocity and eye velocities, respectively, after desaccading the data. Positive eye velocity values indicate rightward eye velocity.](http://jn.physiology.org/lookup/doi/10.1152/jn.00534.2003)
Seven smooth pursuit–related neurons were modulated during gaze velocity Purkinje cells of the flocculus. Figure 3A shows a representative DLPN gaze neuron with a contralateral smooth pursuit preference. Peak unit firing rate was in-phase with stimulus velocity (Fig. 3A, row 1). This neuron also responded during ipsilateral head rotation in VORx2 condition (Fig. 3A, row 4). During VORx0 (Fig. 3A, row 5), the response reversed its phase compared with that during VORx2. During VORl condition (Fig. 3A, row 3), in which gaze was nearly stable, modulation was minimal. Therefore the activity of this neuron in these conditions defines a contralateral gaze velocity neuron. This gaze velocity neuron did not modulate significantly during VORd (Fig. 3A, row 2), indicating that its response was contingent on the presence of a visual target. We found 13/23 (57\%) smooth pursuit–related neurons in DLPN that could be classified as gaze velocity sensitive.

**Eye velocity sensitivity in DLPN smooth pursuit neurons**

We classified smooth pursuit–related neurons as eye velocity neurons if they were modulated during VORl and VORx2 in same direction as smooth pursuit, but were not well modulated during VORx0. Figure 3B shows neural activity of a representative neuron with an ipsilateral smooth pursuit modulation that was in-phase with peak eye velocity (Fig. 3B, row 1). This neuron also responded during contralateral head rotation in VORx2 (Fig. 3B, row 4) conditions. During VORx0 (Fig. 3B, row 5), in which eye velocity was negligible, the modulation was minimal. Moreover, during VORl (Fig. 3B, row 3), DLPN eye velocity neurons were well modulated, even though gaze was nearly stable. Therefore the activity of this neuron can be adequately characterized as an ipsilateral eye velocity neuron. This and other eye velocity neurons did not modulate significantly during VORd (Fig. 3B, row 2), indicating that the eye velocity sensitivity was contingent on the presence of a visual target. We found 7/23 (30\%) smooth pursuit–related neurons in DLPN that could be classified as eye velocity sensitive.

**Responses of DLPN neurons during VORd**

A total of 51 neurons in DLPN including smooth pursuit neurons (gaze or eye velocity) or large-field visual neurons, were examined using horizontal head rotation without a visual target in complete darkness (VORd). We found that only 3/51 neurons in DLPN were modulated during VORd. Of these three neurons, two had earlier been classified as smooth pursuit related and the third as a large-field neuron. The two smooth pursuit neurons responded to contralateral pursuit and also modulated during other VOR conditions including VORd, VORl, VORx2 and VORx0, when the head moved toward the contralateral. Thus their preferred directions in smooth pursuit and each VOR condition were not consistent with the criteria for classification as either gaze- or eye-velocity neurons. Therefore we classified these neurons as eye/head-velocity related (Fukushima et al. 1999; Scudder and Fuchs 1992). Only 1 of 28 large-field neurons was modulated during VORd. This large-field neuron responded during ipsilateral large-field motion and during VORd, when the head moved toward the ipsilateral. In summary, responses as tested during VORd appear rare in the DLPN.
Model testing during visual-vestibular behavior in DLPN

Although DLPN neurons often carry different contributions of eye, head, and retinal motion signals, additional analysis is required to determine the relative weighting of these signals. Therefore we decided to apply a modeling procedure employing multiple linear regressions to determine the relative strengths of eye, head, and visual motion signals present in each neuronal recording. Figure 4 shows the model estimation procedure on two typical neurons (same neurons as in Fig. 3) for model 1 (velocity model). Figure 4, A and C, shows the components that were used to make up the models. Figure 4, B and D, shows the experimentally observed neuron spike density function (solid line) and the corresponding model estimated fit (dotted line). Qualitative examination of unit characteristics showed that the neuron shown in Fig. 4, A and B, was most likely a gaze velocity neuron. Using the more rigorous multiple regression technique, we were able to confirm this classification. Thus the regression coefficients for eye (1.34) and head (1.39) velocity parameters are almost equal, indicating the neuron is really encoding a gaze signal. Note that even though the model yields separate coefficients for head and eye, it does not mean that the neuron has a frank head or eye sensitivity. Rather this type of smooth pursuit–related neuron seemed best classified as gaze velocity sensitive. Similarly, previous qualitative criteria suggested that the neuron shown in Fig. 4, C and D, was an eye velocity neuron. Regression coefficients using model 1 (same model as that used to analyze the previous neuron) for eye velocity sensitivity (1.21) was high, while the sensitivity for head velocity (0.08) was very low, indicating that this neuron was really encoding an eye signal.

We performed specific pair-wise comparisons on all of our DLPN neurons to determine whether model 3, which includes acceleration parameters, was superior to model 1, which considered only gaze or eye velocity signals. Figure 5A shows the results of comparing goodness of fit obtained from models 1 and 3. The majority (18 of 20) of data points are above the equality line, showing that fits obtained using both of velocity and acceleration parameters (model 3; CD = 0.77 ± 0.07; n = 20) are better than the fits obtained using velocity parameters (model 1; CD = 0.69 ± 0.11; n = 20; P < 0.01; paired t-test). To account for the increase in parameters in model 3 compared with model 1, we also calculated a BIC value. This served as a further check on the significance of improvement in goodness of fit. Figure 5B shows comparisons of BIC obtained from models 1 and 3. For all neurons, model 3 had smaller BIC values than model 1. Therefore this analysis shows that closest fits to the data are obtained when our model includes both velocity and acceleration parameters (model 3).

Using model 3 for gaze velocity neurons, average regression...
coefficients associated with eye velocity (1.12 ± 0.43; n = 13) and head velocity (1.09 ± 0.41; n = 13) are close, as are regression coefficients for eye acceleration (0.10 ± 0.07; n = 13) and head acceleration (0.09 ± 0.07; n = 13). The regression coefficients for eye velocity, head velocity, and acceleration of each gaze-related neuron are shown in Fig. 6, A and B (filled symbols). Gaze-related neurons are those where the sensitivities to eye velocity and head velocity are equal as are the sensitivities to eye acceleration and head acceleration. We also calculated the coefficients of partial determination (partial \( r^2 \) values) to determine the relative importance of each term. Average partial \( r^2 \) values for eye velocity (0.34 ± 0.20; n = 13) and head velocity (0.29 ± 0.12; n = 13) parameters are similar, as are partial \( r^2 \) values for eye acceleration (0.06 ± 0.07; n = 13) and head acceleration (0.05 ± 0.06; n = 13) parameters. The low partial \( r^2 \) values for acceleration terms

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**FIG. 5.** Pair-wise comparisons between models 1 and 3 of gaze and eye velocity neurons for DLPN. A: comparison of CDs obtained using model 1 vs. CDs obtained using model 3. B: comparison of Bayesian information criteria (BIC) values obtained with models that exclude or include acceleration parameters (model 1 vs. model 3). Fits for the sample group are better when an acceleration term is included in the model.

**FIG. 6.** Comparison of regression coefficient between eye, head, and retinal error velocity and acceleration parameters (total 6 parameters) for each gaze or eye velocity neurons in DLPN. A and B: comparison of regression coefficients for eye velocity vs. head velocity and eye acceleration vs. head acceleration, respectively. All the data points of gaze related neurons (filled symbols) for eye and head velocity and acceleration are around the equality line drawn on the plot, while all the data points of eye-related neurons (open symbols) for eye and head velocity and acceleration are below the equality line. Other scatter plots show comparison between all pairs of parameters for individual neurons.
suggest a small but significant contribution to the total neural response. The partial $r^2$ values for eye and head velocity and acceleration of each gaze velocity neuron are plotted in Fig. 7, A and B (filled symbols). The contributions of eye velocity and head velocity are large, indicating relative importance of both variables. These findings taken together are consistent with these neurons encoding a gaze signal (i.e., gaze velocity and gaze acceleration).

The regression coefficients for eye velocity neurons using model 3 showed that the sensitivity for eye velocity ($1.55 \pm 0.34$; $n = 7$) was significantly higher than the sensitivity for head velocity ($0.40 \pm 0.09$; $n = 7$), and the eye acceleration sensitivity ($0.12 \pm 0.08$; $n = 7$) was significantly higher than head acceleration sensitivity ($0.05 \pm 0.04$; $n = 7$; $P < 0.01$; paired $t$-test). The regression coefficients for eye and head velocity and acceleration of each eye velocity neuron are shown in Fig. 6, A and B (open symbols). Eye-related neurons are those where the sensitivity to eye velocity or eye acceleration was greater than the sensitivity to head velocity or head acceleration, respectively. Similarly, the partial $r^2$ values for eye velocity ($0.45 \pm 0.21$; $n = 7$) were significantly higher than head velocity ($0.05 \pm 0.05$; $n = 7$), and the partial $r^2$ values for eye acceleration ($0.09 \pm 0.07$; $n = 7$) were significantly higher than head acceleration ($0.01 \pm 0.02$; $n = 7$; $P < 0.01$; paired $t$-test). The partial $r^2$ values for eye and head velocity and acceleration of each eye velocity neuron are plotted in Fig. 7, A and B (open symbols). The partial $r^2$ values for eye velocity are large, while the partial $r^2$ values for head velocity and acceleration are close to zero, indicating relative importance of eye motion parameters. Therefore these neurons appear to be encoding an eye signal (i.e., eye velocity and eye acceleration). The model estimation procedure using regression coefficients and partial $r^2$ values on gaze or eye velocity neurons ($n = 20$) also showed a significant but small contribution from retinal image motion to unit spike density (Figs. 6 and 7). We therefore suggest that the main information encoded by these neurons is gaze or eye velocity rather than pure retinal image motion.

Response properties of rNRTP neurons

We recorded 43 neurons in the rNRTP of two monkeys that responded during smooth pursuit tracking of small target, motion of a large-field visual stimulus, or head rotation. Of these, 35 neurons responded to smooth pursuit, 4 neurons responded only to motion of a large-field stimulus, and 4 neurons were modulated during head rotation in darkness (VORd). The smooth pursuit neurons had a monotonically increasing discharge with eye velocity or acceleration. Of the 35 smooth pursuit neurons, 18 neurons were modulated in phase with eye velocity and responded to both VORx2 and VORx0 conditions, but were not well modulated during the VOR1 condition (Fig. 8A). Thirty-two percent (11/35) of our rNRTP smooth pursuit neurons had apparent eye acceleration.

FIG. 7. Comparison of coefficients of partial determination (partial $r^2$ values) between eye, head, and retinal error velocity and acceleration parameters for each gaze or eye velocity neuron in DLPN. A and B: comparison of partial $r^2$ values for eye velocity vs. head velocity and eye acceleration vs. head acceleration, respectively. All data points of gaze-related neurons (filled symbols) for eye and head velocity and acceleration are around the equality line drawn on the plot, while all the data points of eye-related neurons (open symbols) for eye and head velocity and acceleration are below the equality line. Compared with velocity terms, acceleration terms show smaller partial $r^2$ values, indicating lower relative importance of these variables. However, the relationship for gaze-related vs. eye-related neurons is maintained. Other scatter plots show comparison between all pairs of parameters for individual neurons.
sensitivity, which can be seen in several conditions, including VORx2 and VORx0 (gaze acceleration phase: Fig. 8B). A significant proportion (20/43) of our smooth pursuit–related neurons in rNRTP also responded during VORd. In contrast, only a small proportion (4/43) of NRTP neurons responded during motion of a large-field stimulus.

Gaze velocity neurons of rNRTP

We examined the characteristics of each rNRTP neuron using the same five tasks that used to assess DLPN neurons during visual-vestibular behavior. We classified horizontal smooth pursuit–related rNRTP neurons as gaze neurons when they evinced head and eye sensitivity as described above for DLPN neurons. Figure 8A shows neural activity of a representative gaze velocity rNRTP neuron with an ipsilateral preferred direction during smooth pursuit where peak firing rate is in-phase with stimulus velocity (Fig. 8A, row 1). This neuron also responded during contralateral head rotation in the VORx2 condition (Fig. 8A, row 4). During VORx0 (Fig. 8A, row 5), peak firing rate was opposite in phase compared with that during VORx2. During VORI condition (Fig. 8A, row 3), in which gaze was nearly stable, unit modulation was minimal.

FIG. 8. Response properties of a representative gaze velocity neuron (A) and gaze acceleration neuron (B) recorded from rNRTP during 5 different behavioral conditions (see METHODS). Each trace shows 10-cycle averages. Dashed lines indicate average eye velocities after desaccading the data.
Therefore the activity of this neuron in our standard test conditions is consistent with an ipsilateral gaze velocity neuron. This gaze velocity neuron was also modulated significantly during VORd (Fig. 8A, row 2). Of 35 smooth pursuit–related neurons in rNRTP, a large proportion of neurons (51%) could be classified as gaze velocity sensitive.

Gaze acceleration neurons of rNRTP

We classified smooth pursuit–related neurons in rNRTP as eye acceleration sensitive if they were modulated during smooth pursuit with peak firing rate in-phase with acceleration. To be classified as gaze acceleration sensitive, these neurons also must be modulated during VORx2 with the same phase and direction that was observed during smooth pursuit and in the opposite direction during VORx0 (Fig. 8B). During VORI, the phase relationship and direction of the gaze acceleration–related response was not the same for the smooth pursuit and VORx2 conditions. For some neurons, we tested sinusoidal tracking at different frequencies and with different initial positions to distinguish between acceleration and position sensitivity. Figure 8B shows an acceleration-sensitive neuron whose preferred direction during smooth pursuit was ipsilateral and in-phase with stimulus acceleration (Fig. 8B, row 1). This neuron also responded during VORx2 in-phase with acceleration and in the same direction as in smooth pursuit (Fig. 8B, row 4). During VORx0 (Fig. 8B, row 5), peak firing rate was opposite in-phase to that observed during VORx2. Figure 9 shows the same acceleration-sensitive neuron during sinusoidal tracking at different frequencies (0.25, 0.5, and 0.75 Hz) and with three different initial positions. This neuron shows a monotonically increasing firing rate with eye acceleration (Fig. 9A). Peak firing rate was in-phase with acceleration and independent of eye position over the tested range (−20° to +20°; Fig. 9B). Therefore the activity of this neuron under our test conditions defines an ipsilateral eye acceleration neuron. We did not always have an opportunity to employ multiple sinusoidal frequencies or sinusoids with different offsets to separate eye position and eye acceleration influences before losing unit isolation. If we found that unit firing rate during sinusoidal tracking was in-phase with position or acceleration, we tested neurons during fixation at different eccentricities, which allowed us to determine the potential relationship between static eye position and firing rate (Fig. 10). Neurons with a significant relationship between static eye position and firing rate could be classified as eye position sensitive (3.93 ± 1.34 spike/s/°; n = 5; Fig. 10A). In contrast, we found that many of our neurons, where firing was in-phase with position or acceleration, did not show significant sensitivity to static eye position (0.05 ± 0.06 spike/s/°; n = 11; Fig. 10B). Therefore such neurons could be classified as acceleration-related (e.g., Fig. 8B). Finally, for neurons where peak modulation was in-phase with eye velocity, sensitivity to static eye position was nonexistent or low in both rNRTP (i.e., 0.04 ± 0.05 spike/s/°; n = 18; Fig. 10C) and DLPN neurons (i.e., 0.03 ± 0.03 spike/s/°; n = 20; Fig. 10D). Of 35 smooth pursuit–related neurons in rNRTP, a significant proportion (32%) of neurons could be classified as gaze acceleration sensitive.

Responses of rNRTP neurons in VORd

A total of 43 neurons in rNRTP were examined using horizontal head rotation in complete darkness (VORd). A
significant proportion of our gaze velocity neurons (10/18) in rNRTP were modulated without any visual motion as shown during the VORd. One of these gaze velocity neurons is shown in Fig. 8A. This neuron responded during VORd when the head moved toward the ipsilateral (Fig. 8A, row 2). Similarly, four of our gaze acceleration neurons were modulated during VORd (Fig. 8B). The neuron was modulated when the head moved toward the ipsilateral (Fig. 8B, row 2).

Only one of our rNRTP smooth pursuit neurons that responded to ipsilateral pursuit was also modulated during VORd conditions, when the head moved toward the ipsilateral. Their preferred directions in smooth pursuit and each VOR condition were not consistent with the criteria for classification as either gaze or eye velocity neurons. Therefore we classified this type of neuron as eye/head velocity–related (cf. Fukushima et al. 1999; Scudder and Fuchs 1992). We also found an example of a large-field visual neuron that was modulated during VORd. This large-field neuron responded during ipsilateral large-field motion and modulated clearly during VORd, when the head moved toward the ipsilateral. Another four neurons were modulated during VOR conditions but not during smooth pursuit, and therefore were classified as head motion sensitive.

Model testing during visual-vestibular behavior in rNRTP

As was the case for DLPN neurons, we used multiple linear regressions to determine the relative contributions of eye, head, and visual motion inputs to the response modulation of rNRTP neurons. Figure 11 shows the model estimation procedure on two typical neurons that were qualitatively characterized as a gaze velocity neuron (Fig. 11, A and B) for model 1 and a gaze acceleration neuron (Fig. 11, C and D) for model 2. Figure 11, A and C, shows the components that were used to make up the models. Figure 11, B and D, shows the experimentally observed neuronal response, represented as a unit spike density function, and the corresponding model estimated fit. Qualitative examination of unit characteristics indicate that the neuron shown in Fig. 11, A and B, was most likely a gaze velocity neuron. Using multiple linear regression, we found that the regression coefficients for eye (1.56) and head velocity (1.63) parameters were almost equal, indicating that the neuron is really encoding a gaze signal. Similarly, previous qualitative criteria suggested that the neuron shown in Fig. 11, C and D, was a gaze acceleration neuron. Regression coefficients using model 2 (acceleration model) showed that the eye acceleration sensitivity (0.66) and head acceleration (0.65) parameters were almost equal, indicating that the neuron was really encoding a gaze acceleration signal.

Specific pair-wise comparisons were performed to determine whether 1) model 1 was superior to model 2 for velocity neurons; 2) model 2 was superior to model 1 for acceleration neurons; or 3) model 3 was superior to models 1 and 2. Figure 12A shows the results of comparing models 1 and 2 for all the rNRTP smooth pursuit–related neurons. The plot shows that there are two populations of neurons: one that clearly encodes acceleration (open symbols) and the other that preferentially encodes velocity (solid symbols). We also examined whether inclusion of both velocity- and acceleration-related terms (comparison of models 1 and 2 with model 3) yielded significant improvement in the fits. For example, Fig. 12B shows that our model for a typical velocity neuron had an improved CD following addition of an acceleration term (model 3; 0.78 ± 0.08; n = 18; P < 0.001; paired t-test). Similarly, Fig. 12C shows that our model for a typical acceleration unit had a higher CD following addition of a velocity term (model 3; 0.76 ± 0.07; n = 11) than the CD associated with model 2 (0.63 ± 0.06; n = 11; P < 0.001; paired t-test). We used the
BIC to check if increasing the number of terms in the more complex model was justified. Figure 12, D and E, shows comparisons of BIC obtained with models 1 and 2 with model 3. For all neurons, model 3 had smaller BIC values than models 1 and 2 for velocity and acceleration neurons. Therefore this analysis suggests that closest fits to the data were obtained when the model included both velocity and acceleration parameters (model 3), and addition of these parameters made significant contributions to the improved fits.

Using model 3 for gaze velocity neurons, average regression coefficients associated with eye velocity (1.22 ± 0.80; n = 18) and head velocity (1.32 ± 0.82; n = 18) are similar, as are the regression coefficients for eye acceleration (0.16 ± 0.18; n = 18) and head acceleration (0.20 ± 0.19; n = 18). The regression coefficients for eye and head velocity and acceleration of each gaze velocity neuron are shown in Fig. 13, A and B (filled symbols). Gaze velocity neurons are those where the sensitivities to eye-velocity and head velocity are equal, as is the sensitivity to eye acceleration and head acceleration. We also calculated the coefficients of partial determination (partial $r^2$ values) to determine the relative importance of each term. Average partial $r^2$ values for eye velocity (0.42 ± 0.20; n = 18) and head velocity (0.39 ± 0.20; n = 18) are similar, as are partial $r^2$ values for eye acceleration (0.08 ± 0.09; n = 18) and head acceleration (0.07 ± 0.09; n = 18) parameters. The low partial $r^2$ values for acceleration terms suggest a small but significant contribution to the total neural response. The partial $r^2$ values for eye and head velocity and acceleration of each gaze velocity neuron are plotted in Fig. 14, A and B (filled symbols). This analysis shows that eye velocity and head velocity are large, indicating relative importance of both variables. These findings taken together are consistent with these neurons encoding a “gaze velocity + gaze acceleration” signal.

The regression coefficients for gaze acceleration neurons using model 3 showed that eye acceleration (0.32 ± 0.23; n = 11) and head acceleration (0.45 ± 0.22; n = 11) contributions are not significantly different. The coefficients for eye velocity (1.04 ± 0.74; n = 11) and head velocity (1.07 ± 0.40; n = 11) indicate comparable contributions. The regression coefficients for eye and head acceleration and velocity of each gaze acceleration neuron are shown in Fig. 13, A and B (open symbols). Gaze acceleration neurons are those where the sensitivities to eye acceleration and head acceleration are equal, as are the sensitivities to eye velocity and head velocity. Partial $r^2$ values for eye acceleration (0.45 ± 0.22; n = 11) and head acceleration (0.40 ± 0.13; n = 11) parameters are similar, as are partial $r^2$ values for eye velocity (0.14 ± 0.13; n = 11) and head velocity (0.15 ± 0.13; n = 11) parameters. The partial $r^2$ values for eye and head acceleration and velocity of each gaze acceleration neuron are plotted in Fig. 14, A and B (open symbols). Clearly, eye acceleration and head acceleration make large contributions to neuronal firing. These findings, taken together, are consistent with these neurons encoding “gaze acceleration + gaze velocity.” Similar to DLPN neurons, our model estimation procedures using regression coefficients and partial $r^2$ values for these gaze velocity and gaze acceleration...
neurons indicate significant but small contributions of retinal error motion to unit spike density (Figs. 13 and 14). In summary, we suggest that these neurons are most sensitive to gaze movement rather than retinal error motion per se.

**DISCUSSION**

The basilar pontine nuclei are important components of the neural substrate involved in smooth eye movements (Mustari et al. 1988; Suzuki and Keller 1984; Suzuki et al. 1999; Thier et al. 1988; Yamada et al. 1996). The goal of our studies was to compare neurons in DLPN and rNRTP during combined visual-vestibular behavior. To achieve this goal, we tested DLPN and rNRTP neurons of three monkeys during visual motion of a large-field stimulus, smooth pursuit of a small target, and passive vestibular stimulation and found that neurons in the DLPN and rNRTP have different response properties during visual-vestibular behavior. Using statistical modeling, we found that DLPN and rNRTP neurons indeed have different balances of visual, eye, and head motion signals. As described in methods, we used the widely accepted practice of modeling data averaged over many trials. One of the disadvantages of using averaged data is that the influence of transient signals (e.g., retinal image slip due to a brief slowing down or speeding up of the eye) tends to be obscured. Therefore even though our modeling results appeared to show only a small but significant parametric modulation of neuronal firing with retinal slip, we may have underestimated this contribution. Our results clearly show that smooth pursuit–related neurons in DLPN and rNRTP seemed best classified as gaze velocity, eye velocity, and gaze acceleration sensitive using multiple linear regression modeling.

**Properties of smooth pursuit neurons in DLPN**

The majority of smooth pursuit neurons in DLPN were related to gaze or eye velocity (Fig. 15A). None of our gaze or eye velocity–sensitive DLPN neurons were modulated during VORd, and only a small proportion of all smooth pursuit–related DLPN neurons responded during VORd (Fig. 15C). More than one-half of DLPN neurons discharged in relation to sinusoidal smooth pursuit with peak discharge rate at peak eye velocity (Mustari et al. 1988; Suzuki et al. 1990; Thier et al. 1988). Our results obtained from DLPN smooth pursuit–related neurons are comparable to those reported in previous studies. However, earlier studies did not include vestibular testing. We found that some DLPN neurons related to eye velocity during smooth pursuit were also modulated during VORx2 and VORx0, when gaze moved toward same direction as smooth pursuit. How-
ever, other DLPN neurons were modulated during VOR1 and VORx2 in the same direction but had no significant modulation during VORx0. Our new results indicate that DLPN neurons, which were previously thought to be related to eye velocity during smooth pursuit (Mustari et al. 1988; Suzuki et al. 1990; Thier et al. 1988), might be best classified as gaze or eye velocity–sensitive neurons. One point to note is that these neurons generally did not respond during eye movements that occur during VORd. This indicates that neuronal modulation is related to gaze or smooth pursuit eye velocity associated with tracking a visual target.

As reviewed in the Introduction, the DLPN receives appropriate connections from MT/MST cortex and projects to regions of the cerebellum (e.g., flocculus and ventral paraflocculus) known to play a role in smooth pursuit or gaze control. The goal of our multiple linear regression modeling was to provide a more objective and quantitative method of determining what information is being encoded by a particular DLPN and rNRTP neurons. Therefore using the same model structure (model 1) for different neurons, we were able to objectively categorize neurons into gaze velocity or eye velocity types. A second finding was that adding an acceleration term improved the models fit significantly, suggesting that smooth pursuit–related neurons in DLPN encode not only velocity parameters but also acceleration parameters. We did not attempt to model large-field visual neurons. However, similar modeling studies have been performed by Kawano et al. (1992), demonstrating that DLPN neurons encode large-field visual motion for short-latency ocular following (Miles et al. 1986).

Properties of smooth pursuit neurons in rNRTP

We classified the majority of smooth pursuit–related neurons in rNRTP as gaze velocity or gaze acceleration sensitive (Fig. 15B). A large proportion of our rNRTP smooth pursuit neurons were also modulated during VORd (Fig. 15D). In contrast, only a small proportion of rNRTP neurons responded during motion of a large-field visual stimulus. Most rNRTP neurons were modulated during smooth pursuit of a small target spot (Fig. 15B). Previous studies have shown that the response of some rNRTP neurons encode pursuit eye velocity, whereas other neurons encode eye acceleration during sinusoidal smooth pursuit tracking (Suzuki et al. 2003). We found similar results in testing smooth pursuit responses of rNRTP neurons. However, we found that rNRTP smooth pursuit–related neurons were also modulated during VORx2 and VORx0, when gaze moved toward same direction as smooth pursuit. Our results indicate that rNRTP neurons previously thought to be related to smooth pursuit eye-velocity alone could actually encode gaze velocity during eye and head motion. Similarly, our smooth pursuit related acceleration neurons were also modulated during VORx2 and VORx0, when gaze acceleration
moved toward the same direction as smooth pursuit. Therefore we suggest that rNRTP neurons previously thought to be related to smooth pursuit eye acceleration actually might encode gaze acceleration. Many of the rNRTP neurons were modulated during head rotation in darkness, suggesting that their gaze signals were not simply due to retinal image motion.

As reviewed in the Introduction, the NRTP is known to receive strong inputs from the FEFs and to project primarily to vermal lobules VI and VII. Recent studies demonstrated that smooth pursuit neurons in the FEF were related to gaze velocity (Fukushima et al. 2000). Most of these FEF neurons also responded to chair rotation in complete darkness (Fukushima et al. 2000). Smooth pursuit–related Purkinje cells in dorsal vermis (lobule VI-VII) also have responses related to gaze velocity (Sato and Noda 1992; Suzuki and Keller 1988). We suggest that rNRTP receives gaze (head and eye)-related input from FEFs. The response properties and projections of the rNRTP support the suggestion that the rNRTP is a major source of the gaze velocity information for the dorsal vermis. Other properties that could play a role in gaze have been shown to be represented in the NRTP. For example, Gamlin and Clarke (1995) have reported that some neurons in the NRTP have responses related to vergence. Vergence state is known to modulate the gain of the VOR and could play a role in gaze. Vergence-related responses in the NRTP could be derived, at least in part, from the FEF and MST cortex, where Fukushima et al. (2002) demonstrated the existence of vergence-related responses. We did not examine our rNRTP or DLPN neurons during vergence.

Using multiple linear regression modeling, we were able to objectively and quantitatively verify that rNRTP neurons were indeed gaze velocity or gaze acceleration related. Separating actual eye position sensitivity from eye acceleration sensitivity is not possible when only a single sinusoidal frequency is employed. However, we were able to separate potential eye position and eye acceleration influences by testing for relationships between firing rate and static eye position (see METHODS). For the neurons we modeled, eye position made little of no contribution to the modulation of the neuron during smooth pursuit. Our models had the best fits when we included velocity and acceleration terms.

Comparison between response properties of DLPN and rNRTP neurons

There are several distinct differences between DLPN and rNRTP, even though the neurons in both regions respond during visual-vestibular behavior as well as smooth pursuit. We were able to classify smooth pursuit–related DLPN neurons as gaze or eye velocity related. In contrast, smooth pursuit neurons in the rNRTP were best classified as gaze velocity or gaze acceleration related. This suggests that rNRTP may play a more important role in gaze control than in smooth pursuit eye movement control per se. Recent findings in cerebellar
studies support this suggestion. For example, Shinmei et al. (2002) reported that pursuit-related Purkinje cells in the dorsal vermis responded during cancellation of the VOR (VOR\textsuperscript{x0}). However, the majority of pursuit-related Purkinje cells in the floccular lobe did not respond during this condition (Fukushima et al. 1999; Lisberger and Fuchs 1978; Miles et al. 1980). These findings indicate that the dorsal vermis plays a large role in gaze control than the floccular lobe. Our results support the suggestion that rNRTP and DLPN are major sources for gaze and smooth pursuit signals in the dorsal vermis and floccular lobe, respectively. Several lines of evidence support this suggestion. First, we found that about one-third of smooth pursuit neurons in rNRTP could be classified as gaze acceleration sensitive, while only a small number of DLPN neurons were related to acceleration. Second, a large number of rNRTP neurons responded during VOR\textsubscript{d}, in contrast to the small percentage of DLPN neurons that responded during VOR\textsubscript{d}. Even though a large proportion of rNRTP neurons were modulated during VOR\textsubscript{d}, the modulation was most likely due to the gaze movement rather than the head movement because the phase of activity related to head motion was not consistently placed across other vestibular paradigms (e.g., VOR\textsubscript{d}, VOR\textsubscript{r}, VOR\textsubscript{x0}, VOR\textsubscript{x2}). Finally, more than one-half of DLPN neurons responded during large-field visual motion, whereas only a small proportion of rNRTP neurons responded during such testing. These large-field visual neurons have been shown to play a role in visually elicited ocular following or optokinetic eye movements (Kawano et al. 1992; Miles and Kawano 1986). These different functional roles may in part reflect different balances of cortical-pontine inputs (Distler et al. 2002; Stanton et al. 1988) and pontine efferents to the vestibulo-cerebellum (Glickstein et al. 1994). Our results provide evidence to support the suggestion that a FEF/SEF-rNRTP-dorsal vermis pathway parallels an MT/MST-DLPN-floccular lobe pathway (Suzuki et al. 2003) for control of gaze and smooth pursuit.

Role of the basilar pontine nuclei in gaze control

It is important to define the essential role played by different centers contributing to the cortico-ponto-cerebellar pathway for gaze control. By defining the information carried at different stages in this pathway, we can determine how the transformation of initial sensory-motor signals occurs in the pontine nuclei and cerebellum to create a motor command for gaze. When we track a slowly moving object during head rotation, engaging smooth pursuit is often necessary. Neurons in area MST are known to be essential for initiation and maintenance of smooth pursuit (Newsome et al. 1988). Neurons in the FEFs appear to contain all the signal components needed to calculate
gaze velocity including retinal slip, eye, and head velocity (Bruce and Goldberg 1985; Fukushima et al. 2000; Gottlieb et al. 1994; Tian and Lynch 1996b). These two cortical areas have reciprocal connections (Stanton et al. 1993; Tian and Lynch 1996a; Tusia and Ungerleider 1988). Therefore it is likely that the FEF and other cortical areas effect gaze control, at least in part, through connections involving the DLPN and NRTP.

The vestibulo-cerebellum, including the flocculus and ventral paraflocculus, is known to play an essential role in the VOR. The vestibulo-cerebellum receives inputs from canal and otoliths neurons that play essential roles in the rotational and linear VOR, respectively. Additional inputs important for modification of the VOR and other visual-vestibular behavior reach the vestibulo-cerebellum by way of the pontine nuclei (DLPN and NRTP). Taken together, cortical areas (e.g., FEF and MST) might be necessary for the initial calculation of a gaze velocity command, while cerebellum may contribute to modulation of these signals to ensure appropriate adaptation to different behavioral contexts. Previous studies (Belton and McCrea 1999) suggested that the mechanisms for suppressing the VOR during active and passive head movements are quite different and that the flocculus and ventral paraflocculus are needed only when the movements are not self-generated. Therefore the basilar pontine nuclei might have different roles for gaze control during active and passive head movements. Further studies employing active head movements are needed to resolve this question.

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