Neuronal Substrates of Motor Learning in the Velocity Storage Generated During Optokinetic Stimulation in the Squirrel Monkey

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INTRODUCTION

The vestibular and optokinetic systems work synergistically to maintain image stability over a wide range of head movements (Paige 1983). The vestibulococular reflex (VOR) generates short-latency compensatory eye movements during head turns, whereas the optokinetic response (OKR) generates longer-latency compensatory eye movements in response to movements of the visual field. Both systems share a velocity storage element that builds up eye velocity that can persist even in the absence of stimulation (Raphan et al. 1979). In the OKR system, this velocity storage, called optokinetic nystagmus (OKN) velocity storage, is seen as an exponentially decaying optokinetic after nystagmus (OKAN) in the dark, which can be defined by its magnitude, direction, and long time constant of decay. It has been demonstrated in the horizontal system that the intimate coupling between the VOR and OKN velocity storage is maintained after VOR motor learning, as long-term adaptation of the horizontal VOR changes both the VOR gain and the velocity storage after optokinetic stimulation (OKS) (Demer 1981; Lisberger et al. 1981). Horizontal VOR gain changes, however, do not affect a short time constant optokinetic response, termed initial OKN (OKNi), leading these previous investigators to postulate a plastic site for VOR motor learning located before the pathways mediating OKN velocity storage and OKNi converge.

Such a link between VOR gain and OKN velocity storage has not previously been demonstrated for the vertical system, which exhibits several differences from its horizontal counterpart. First, vertical OKR is asymmetric: Upward OKS evokes vertical eye movements during OKAN that are comparable in magnitude to those of the horizontal (Lisberger et al. 1981); however, downward optokinetic stimulation evokes only a very small OKN response (Matsuo and Cohen 1984). Second, vestibularly driven vertical eye movements require the coordination of two semicircular canal planes and four extraocular muscle pairs, compared with the single canal plane and two extraocular muscle pairs required for pure horizontal eye movements. Third, horizontal and vertical OKAN are maximal at different head orientations with respect to the gravito-inertial axis (Dai et al. 1991; Raphan and Cohen 1985; Raphan and Sturm 1991). In an upright posture, horizontal OKAN is maximal and vertical OKAN is minimal (Cohen et al. 2002; Raphan and Cohen 1988). Conversely, vertical OKAN is maximal when the animal is tilted 90° in roll, indicating a differential effect of gravity. Therefore the link between VOR and OKN velocity storage needs to be tested independently in the vertical system.

In both the horizontal and vertical systems, the coupling between VOR and OKN velocity storage is reflected at the level of the vestibular nuclei, where many neurons contain both vestibular and visuooculomotor signals (Allum et al. 1976; Waespe and Henn 1977a). Indeed, lesions in the vestibular nuclei affect both VOR and OKN velocity storage (Cannon and Robinson 1987; Cohen et al. 1973; Holstein et al. 1999; Waespe and Henn 1977b; Waespe et al. 1985; Zee et al. 1976). Based on this evidence and their own findings regarding the link between VOR motor learning and OKN velocity storage,
Lisberger and colleagues (1981) postulated that a plastic site supporting long-term VOR gain changes is located in the brain stem. In later work, Lisberger (1994) showed that changes in the brain stem vestibular pathway, at the level of the flocculus target neurons (FTNs), contribute to VOR gain changes after VOR motor learning. However, a neural correlate for the change in OKN velocity storage has not yet been reported. Here, we report such a neural correlate for VOR gain increases, but not decreases, in dorsal Y-group FTNs and interpret this finding in terms of different mechanisms for VOR gain increases and decreases, and in terms of differences between the horizontal and vertical VOR and OKR systems.

Methods

For behavioral studies, we used a total of seven squirrel monkeys (062, 064, 065, 066, 070F, 404, and 408) between 4 and 7 yr and 700 and 1,200 g. Three monkeys (062, 065, and 064) were also used for neuronal recordings in the dorsal Y group nucleus.

Animal preparation

Animals underwent a first surgical procedure where we implanted an eye coil (11 mm diam) to record eye movements (Robinson 1963) and a head fixation system. In a second surgery, several weeks after the first, we implanted a recording chamber aimed at a midpoint between the abducens nucleus and the dorsal Y group nucleus (062, 065, and 064). Experiments started ~2 mo after the first surgery to allow adequate recovery. Amphetamine sulfate (0.05–0.1 mg/kg) was administrated orally during the experiments (mixed with milk or juice) to maintain a constant level of alertness. Experimental protocols were approved by the Washington University Committee on Animal Care and performed in accordance with the National Institute of Health guidelines.

Experimentally induced VOR motor learning

Our method of inducing long term VOR motor learning has been described in detail previously (Partsalis et al. 1995a). Briefly, animals wore a pair of customized magnifying or minimizing lenses secured to their head with dental acrylic. The animals wore lenses for ±2 wk before behavioral and neuronal recordings were performed to guarantee that the new VOR gain had reached a stable value (Kuki et al. 2004). Lenses were cleaned twice a day and the animals were closely monitored during the period they wore the lenses.

Experimental setup and recording procedures

Our experimental setup allowed us to test the horizontal and vertical components of the VOR and optokinetic responses (OKR). For horizontal stimulation, the animal was seated in an upright position in a primate chair atop a rotating table, whereas for vertical stimulation, the primate chair was placed at a 90° angle with the animal lying on its right side to allow maximum charging of the OKN velocity storage. Additionally, our system allowed us to move the primate chair toward or away from the center of the table to align the axis of rotation with the center of the head during both horizontal and vertical stimulation (see supplementary Fig. 1†). For optokinetic stimulation, we used a projection system placed above the animal that consists of an illuminated optokinetic drum with alternating transparent and black stripes. This pattern was projected on a white screen located in front of the animal such that rotation of the illuminated drum results in the corresponding movement of the pattern over the screen. The axis of rotation of the drum coincides with that of the rotating table. VOR in the light (VORL) or dark (VORD) was induced by rotation of the table while the drum remained still. For optokinetic stimulation, we rotated the illuminated optokinetic drum with no movement of the table. Vertical and horizontal eye data were calibrated assuming perfect compensatory eye movements during VORl (0.5 Hz, ±40°/s) in the nonadapted animal (Paige 1983).

Neuronal activity was recorded using tungsten microelectrodes of 1–4 MΩ impedance (FHC, Bowdoinham, ME) that were advanced remotely using a hydraulic microdrive (Trent Wells, South Gate, CA). Neuronal data were filtered (pass band: 100–5,000 Hz), amplified and digitalized on-line by means of an amplitude threshold set manually above the noise. Table velocity, drum velocity, horizontal and vertical eye position, and neuronal data (raw waveforms and spike events) were recorded on a PC computer using a Power 1401 acquisition system and Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Paradigms

Our paradigms included the following.

Step optokinetic stimulation, generated by presenting an optokinetic stimulus moving at a constant velocity (160°/s for 20–30 s) and followed by a period in complete darkness (10–20 s). In this paper, we use the direction of the slow phase eye movements to indicate the direction of the OKR (hence, the direction of OKR is the same as the direction of the optokinetic stimulus). This paradigm was used to study the velocity storage capabilities of the system. We used such a large optokinetic velocity lasting for several tens of seconds to permit a complete charging of the velocity storage (see RESULTS, Fig. 1) (see also Lisberger et al. 1981). The eye velocity that results immediately after this optokinetic stimulation (when lights are turned off) is called the saturation velocity and represents the maximum velocity that can be stored by an animal (capacity of the velocity storage).

Vertical and horizontal sinusoidal VOR in the dark (VORD), generated by sinusoidal rotation of the table (usually 0.5 Hz, ±40°/s). This paradigm was used to measure the gain of the VOR (no visual feedback) and to obtain the neuronal sensitivities to head parameters (see following text and Hirata and Highstein 2001).

Vertical sinusoidal optokinetic stimulation, generated by rotation of the illuminated optokinetic drum (usually 0.5 Hz, ±40°/s). This paradigm...
was used to obtain the neuronal sensitivities to eye and retinal slip parameters (see following text and Hirata and Highstein 2001).

Vertical sinusoidal VOR enhancement (VORe), VOR suppression (VORs), and VOR in the light (VORl), generated by sinusoidal rotation of the table (usually 0.5 Hz, ±40°/s) in the light with the optokinetic system fixed (VORl) or moving in the same (VORs) or opposite (VORe) direction of the table. These paradigms were used to test the reliability of our analysis (see following text and Hirata and Highstein 2001).

Analysis methods

Off-line analysis was performed using Matlab 6.5 (MathWorks, Natick, MA). The saccade periods were eliminated from the eye and neuronal data using an acceleration threshold and verified by manual inspection. The remaining portion of the data (desaccaded) was used for further analysis.

Analysis of the behavioral responses to steps of optokinetic stimulation

We divided the response to steps of optokinetic stimulation into three periods (Fig. 2A): 1) The time window between the first 150 ms to 2 s of optokinetic stimulation (light on and optokinetic drum moving at 160°/s). We called this period OKNi (initial velocity of optokinetic nystagmus); 2) a period that included the last 3 s of optokinetic stimulation (light on and optokinetic moving at 160°/s). We called this period OKNf (final velocity of optokinetic nystagmus); 3) the period that followed the end of the optokinetic stimulation (final light off period). This period was called OKAN (optokinetic after-nystagmus). The initial velocity of optokinetic after-nystagmus (OKAni) was considered equivalent to the saturation velocity (maximum capacity of velocity storage) because of the large stimulus velocity employed. In two animals, we verified that these stimulus conditions indeed saturate the velocity storage (see Fig. 1). Because the animals often make saccades during the initial period in which the
light is extinguished, OKANi was extrapolated using an exponential function that fits the eye velocity (average eye velocity of each intersaccadic period) during the OKAN period (Fig. 2B). This function can be expressed mathematically as

\[ F(t) = A + Be^{et} \]  

where \( F(t) \) is the estimated slow phase eye velocity, \( A \) and \( B \) are coefficients (\( A \) represents the asymptote of the exponential function), \( t \) represent time since the start of the OKAN, and \( e \) is the time constant. OKANi was obtained as the value of the equation at \( t = 0 \). Exponential fits were visually inspected to ensure that they accurately captured the OKAN slow phase velocity.

**Analysis of the neuronal responses to steps of optokinetic stimulation**

To quantitatively describe the response of Y neurons during the OKAN period, we compared the neuronal response to the eye movement (position, velocity, and acceleration). We do this because during the OKAN period the only measurable signal feeding the circuit is the eye movement. Hence the equation used to fit the neuronal discharge can be expressed as follows

\[ f(t) = \alpha_eE_{acc} - \beta_eE_{vel} + \gamma_fE_{pos} + \delta + \epsilon \]  

where \( f(t) \) denotes the instantaneous firing rate, \( \alpha_e \), \( \beta_e \), and \( \gamma_f \) denote sensitivities to eye acceleration, eye velocity, and eye position, respectively. \( E_{acc} \), \( E_{vel} \), and \( E_{pos} \) are the actual eye acceleration, eye velocity, and eye position, and \( \delta \) and \( \epsilon \) denote the baseline firing rate and an error term or a residual component. To estimate the values of \( \alpha_e \), \( \beta_e \), and \( \gamma_f \), we performed a multiple linear regression over the desaccaded data that considered the interpolated values of eye acceleration, velocity, and position with respect to the instantaneous firing rate of the neuron (see Hirata and Highstein 2001). The goodness of fit was evaluated by comparing the residuals of the estimation with the neuronal discharge during spontaneous eye movements.

**Analysis of the neuronal response during sinusoidal visual and vestibular stimulation**

To fully characterize the Y neuron responses and ultimately obtain the head-velocity sensitivities, we used a multiple linear regression approach to extract information from the neuronal response during sinusoidal stimulation. This method has been previously described in detail (Blazquez et al. 2003, 2006; Hirata and Highstein 2001). Briefly, the neuronal response of Y neurons at 0.5-Hz stimulation can be explained by the linear addition of signals related to the following pathways: efferent copy pathway (including eye position, velocity, and acceleration), retinal slip pathway (including retinal slip velocity and acceleration) and head-velocity pathway (including head velocity and acceleration). We obtained the neuronal sensitivity to eye parameters and retinal slip parameters during sinusoidal optokinetic stimulation (0.5 Hz). The residuals of the estimations are compared with those during spontaneous eye movements in the light. Following this, we obtained the neuronal sensitivity to head parameters during VORd. VORs, VORe, and/or VORl were later used to validate the reliability of the estimation. The analysis of residuals was done using an Ansari-Bradley test that compared the residuals with the intrinsic neuronal noise during spontaneous eye movements in the light (sinusoidal optokinetic stimulation, VORe, VORl, and VORs) or in the dark (VORd) (Hirata and Highstein 2001). The goal of this lengthy signal extraction process is to obtain the head-velocity sensitivity (\( \beta_h \)) of the Y group neuron, and is necessary because Y group neurons respond to both head- and eye-related parameters during rotation in the dark (VORd).

Further signal extraction from Y neurons is possible because the overall head-velocity sensitivity (\( \beta_h \)) of Y neurons results from vestibular signals arriving via two pathways, indirectly via the cerebellar flocculus (\( \beta_{hF} \)) and directly from the brain stem (\( \beta_{hnon-FL} \)) (Blazquez et al. 2000; Partsalis et al. 1995b; Sato and Kawasaki 1987). This can be mathematically expressed as

\[ \beta_h = \beta_{hFL} + \beta_{hnon-FL} \]  

To separate the floccular and nonfloccular components from the head-velocity sensitivity of Y neurons, we employed the method described in our previous report (Blazquez et al. 2006) that utilizes the overall eye and head-velocity sensitivities of Y neurons and can be explained as follows. The portion of the head-velocity sensitivity of individual Y neurons that is due to its floccular input is directly related with their eye-velocity sensitivity and we have previously shown that this relation can be expressed as

\[ \beta_{hFL} = \beta_s/(0.94VORg^{0.86}) \]  

Thus we can extract the value of \( \beta_{hnon-FL} \) using Eq. 3 and 4 as follows

\[ \beta_{hnon-FL} = \beta_s - \beta_s/(0.94VORg^{0.86}) \]  

Once this nonfloccular head-velocity sensitivity is obtained, we can compare it with the eye-velocity sensitivity during OKAN (the velocity storage sensitivity) to see how the vestibular and visuo-oculomotor neural representations change with VOR motor learning.

**Notations**

In this report we use a similar prefix (OK-) to define characteristic features of the behavioral response. This terminology is explained throughout the text but for clarity we have also provided definitions in the preceding text.

**OKR: OPTOKINETIC RESPONSE.** Behavioral response caused by the optokinetic stimulation. The direction of the response is defined by the slow phase eye movement; thus the direction will coincide with that of the optokinetic stimulation. “OKR” alone refers to the behavioral responses to step velocity of optokinetic stimulation (160°/s), whereas “sinusoidal OKR” refers to the behavioral response to sinusoidal optokinetic stimulation (0.5 Hz). OKR is further divided into three periods (OKNi, OKNf and OKANi) that are defined in the following text.

**OKAN.** Phase of the OKR that occurs in the dark, immediately after the optokinetic stimulation. This is the velocity storage component.

**OKANi.** Corresponds to the response at the beginning of the OKAN period. It is calculated by the exponential fitting or the average slow phase eye velocity during the first second of OKAN.

**OKN VELOCITY STORAGE.** Corresponds to the indirect visual pathway, which has a long time constant and remains active for several seconds in the absence of visual stimulation (Cohen et al. 1977).

**OKNi.** Corresponds to the average response at the beginning of the OKR (150 ms to 2 s), primarily the short time constant OKN.

**OKNF.** Corresponds to the average response at the end of optokinetic stimulation (last 3 s before lights are turned off) and is thought to be the sum of the short time constant OKN and OKN velocity storage.

**RESULTS**

Monkeys were trained to increase or decrease their VOR gains by the chronic wearing of magnifying or minimizing lenses. Average vertical VOR gain values were 0.5 ± 0.07, 0.85 ± 0.06, and 1.48 ± 0.14 for the low, normal, and high gain states.
Behavioral results

CHARACTERIZATION OF OKR IN THE NORMAL GAIN ANIMAL. Figure 2A illustrates that OKR consists of an initial phase wherein eye velocity increases rapidly (OKNi), followed by a second phase where eye velocity increases slowly to a plateau (OKNf). After this, if the illumination is turned off, eye velocity does not fall immediately to zero; rather it decays slowly toward zero. This continuing eye movement in the dark is a form of velocity storage called optokinetic after nystagmus (OKAN) (Cohen et al. 1977). The capability of the velocity storage system can be quantified by the maximum OKAN velocity achieved once the system has fully saturated (OKANi). Unlike the horizontal OKR, vertical OKR is asymmetrical and depends on the direction of the moving visual stimulus. Thus upward OKR reaches large plateau values (OKNf, 98 ± 21.7°/s) and large OKAN initial velocities (OKANi, 85.9 ± 11.6°/s), whereas downward OKR reaches small and variable plateau eye velocities (OKNf, −36.42 ± 31.0°/s) and small OKAN initial velocities (OKANi, −23.88 ± 12.°/s; see, Table 1). Figure 2B illustrates eye movements evoked after upward and downward optokinetic stimulation in the normal gain state. The OKAN evoked after downward optokinetic stimulation occasionally manifests several phases wherein the direction of eye velocity alternates (Himi et al. 1988). These additional phases of OKAN (not illustrated), called OKANII, III and subsequent, are thought to be generated by different mechanisms than those involved in OKAN I and will not be quantified in this study (Himi et al. 1988; Igarashi et al. 1990). Figure 2C and Table 1 (normal gain animal) illustrate the OKR evoked by left- and rightward stimulation in the same monkey to demonstrate that one key difference between horizontal and vertical OKR is the symmetry of the horizontal response.

OKR AFTER CHRONIC VOR MOTOR LEARNING. The effect of chronic VOR motor learning on the vertical component of OKR depends on the direction of adaptation. High gain learning caused significant increases in OKNf for upward OKR as well as significant increases in OKANi for up- and downward OKR (Table 1). However, low gain learning did not cause significant changes in any of these variables (P values were >0.99, >0.27, >0.68, and >0.13 for up- and downward OKNf and OKANi). Thus the OKR was enhanced after high gain adaptation but showed no significant change after low gain adaptation (Fig. 3, A–C, and Table 1). In agreement with previous reports (Demer 1981; Lisberger 1981), the horizontal OKR shows significant changes following both high and low gain adaptation, namely increases (high gain) or decreases (low gain) in OKNf and OKANi (Table 1 and Fig. 3, D–F). Average horizontal VOR gain values were 0.55 ± 0.05, 0.87 ± 0.08, and 1.36 ± 0.13 for the low, normal, and high gain states.

Y neuron response during OKR in the normal gain animal

The activity of 16 Y neurons was recorded in the normal gain animal during step optokinetic stimulation. Y neurons were identified by their location and their response during sinusoidal stimulation at 0.5 Hz (Fig. 4, A and B). In the normal gain animal, Y neurons increase their firing rate for upward eye movements during sinusoidal OKR (0.5 Hz) and show little or no modulation during head rotation in the dark. Thus Y neurons show upward head- and eye-velocity signals, indicating that they are gaze velocity neurons (see Partsalis et al. 1995 for further description).

In the normal gain animal all Y neurons responded during the short time constant OKAN (OKNi), but under visual inspection, only 50% (n = 8) of the neurons show responses during OKAN. Qualitatively, these Y group neuron responses during the OKAN period were generally small and were always positively related with the behavior. Figure 4 illustrates the response of a Y neuron. During OKNi, the firing rate increases for upward optokinetic stimulation and decreases for downward optokinetic stimulation (Fig. 4, C and D). A similar phenomenon is seen during OKNf. However, during OKANi the firing rate decreases immediately to values close to the baseline value (Fig. 4, C and D). Table 2 (middle row) and Fig. 4 (E and F) illustrate the average neuronal responses for the population of Y neurons during OKNi, OKNf, and OKANi in the normal gain animal. During upward OKR (Fig. 4E), Y neurons exhibited an initial sharp increase in their firing rate that drove overall neuronal firing to a level above the baseline rate (OKNi, 52.2 ± 24.1 spikes/s above the baseline firing rate). This increased firing was maintained until the end of the optokinetic stimulation (OKNf, 47.2 ± 16.2 spike/s above the baseline firing rate). When the lights were turned off (OKANi), Y neuron firing decreased sharply (8.2 ± 14.1 spike/s above the baseline firing rate). For some Y neurons, the firing rate decreased to a level slightly above the baseline discharge during OKANi followed by a slow decay that continued during the OKAN period, indicating that some Y neurons may have a small positive sensitivity to OKN velocity storage in the normal gain animal. During downward OKR, Y neurons showed an initial sharp decrease in firing rate (OKNi, 27.5 ± 34.8 spike/s below the baseline firing rate), that was maintained at a level below the baseline firing rate while stimulation continued (OKNf, 26.4 ± 30.8 spike/s below the baseline firing rate).

<p>| Table 1. Behavioral responses to step optokinetic stimulation (OKS) before and after vestibuloocular reflex (VOR) motor learning |
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<tr>
<th>Gain</th>
<th>VOR Gain</th>
<th>Uniform OKS</th>
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For each column, significant different values than those corresponding to the normal gain animal are indicated (*P<0.05 or **P<0.01). OKNi and OKNf, initial and final; respectively; optokinetic nystagmus; OKANi, initial velocity of optokinetic after nystagmus. A P<0.01 is indicated with bold numbers.
Immediately after the light was extinguished (OKANi) the firing rate of Y neurons increased abruptly (OKANi, 9.4 ± 10.5 spike/s above the baseline firing rate). At the population level, changes in the mean firing rate were significant (P < 0.01) for all phases except for OKANi.

To more quantitatively describe the response of Y neurons during the OKAN period, we used a multiple linear regression approach that calculates the firing rate during OKAN as a function of eye acceleration, eye velocity, and eye position (see METHODS). Results were different for up- and downward OKR. For upward OKR, 87% of the neurons (14 of 16) showed positive eye-velocity sensitivities (increased their firing rate for upward eye velocity) during OKAN with a mean sensitivity of 0.21 ± 0.22 spike/s per °/s. The eye position sensitivity was equally distributed (7 neurons were up eye position and 9 were down eye position) with a mean of 0.15 ± 1.62 spike/s per °. For downward OKR, most cells (81.3%) showed negative eye-velocity sensitivities (decreased their firing rate for upward eye velocity during OKAN) with a mean sensitivity of −0.39 ± 0.44 spike/s per °. Eye position sensitivities during downward OKR were close to 0 (mean 0.22 ± 1.7 spike/s per °, with 37% downward and 63% upward). These results agree with our qualitative assessment of the responses.

The present results suggest that the population response of Y neurons during step OKR in the normal animal is dominated by the short time constant OKN component and shows a small but not significant OKAN component, and Y neurons have an upward directional preference during OKAN following upward optokinetic stimulation and a downward directional preference following downward optokinetic stimulation. This difference in directional preference, while not necessarily originating in the dorsal y group, could help maintain the asymmetries in the vertical OKR.

Changes in the response of Y neurons after chronic VOR adaptation

Y neurons are known to change their modulation during VORd after chronic VOR motor learning (Blazquez et al. 2006; Partsalis et al. 1995a). In the low gain animal, Y neurons increase their firing rate during upward head movements (neuronal gain: 0.66 ± 0.39 spike/s per °/s and phase: 30.2 ± 22°), whereas the opposite is observed in the high gain animal (neuronal gain: 0.55 ± 0.37 spike/s per °/s and phase: 181 ± 12.3°; Table 2, Figs. 5, A and B, and 6, A and B) (see Partsalis et al. 1995a for details). The baseline firing rate in the dark was 84.4 ± 18.7, 103.7 ± 31.1, and 118.6 ± 29.9 spike/s for the low, normal, and high gain states, respectively. Here, we show
that VOR motor learning is accompanied by additional changes in the response of Y neurons during OKR.

RESPONSE OF Y NEURONS DURING UPWARD OPTOKINETIC STIMULATION IN ADAPTED ANIMALS. In the high gain adapted animal, visual inspection indicated that all recorded Y neurons showed a clear OKAN period response with a similar time constant to the eye velocity (Fig. 5C). Changes in the mean firing rate of Y neurons during each period of the OKR (OKNi, OKNf, and OKANi) can be seen in Table 2. During upward optokinetic stimulation, Y neurons responded by sharply increasing their firing rate during OKNi. This firing

<table>
<thead>
<tr>
<th>Gain</th>
<th>VOR Gain</th>
<th>Meanfr OKNi</th>
<th>Meanfr OKNf</th>
<th>Meanfr OKANi</th>
<th>αc</th>
<th>βc</th>
<th>γc</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>1.45 ± 0.14</td>
<td>65.5 ± 18.9</td>
<td>86.6 ± 20.7</td>
<td>9.5 ± 13.5**</td>
<td>0.007 ± 0.007</td>
<td>0.16** ± 0.16</td>
<td>1.81 ± 1.81</td>
</tr>
<tr>
<td>Normal</td>
<td>0.84 ± 0.06</td>
<td>52.2 ± 24.1</td>
<td>46.2 ± 16.2</td>
<td>8.2 ± 14.1</td>
<td>0.003 ± 0.002</td>
<td>0.22 ± 0.22</td>
<td>1.62 ± 1.62</td>
</tr>
<tr>
<td>Low</td>
<td>0.5 ± 0.07</td>
<td>36.9 ± 13.2</td>
<td>38.2 ± 12.5</td>
<td>10.8 ± 15.3**</td>
<td>0.036 ± 0.007</td>
<td>-0.23 ± 0.19**</td>
<td>2.6 ± 2.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gain</th>
<th>Meanfr OKNi</th>
<th>Meanfr OKNf</th>
<th>Meanfr OKANi</th>
<th>αc</th>
<th>βc</th>
<th>γc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upward OKS</td>
<td>-39.8 ± 24.78</td>
<td>-30.9 ± 29.2</td>
<td>12.3 ± 17.6</td>
<td>0.0042 ± 0.005*</td>
<td>0.03 ± 0.03</td>
<td>-0.81 ± 0.49</td>
</tr>
<tr>
<td>Downward OKS</td>
<td>-27.5 ± 34.8</td>
<td>-26.4 ± 30.8</td>
<td>9.5 ± 10.5</td>
<td>0.0007 ± 0.002</td>
<td>0.39 ± 0.44</td>
<td>0.25 ± 0.74</td>
</tr>
</tbody>
</table>

Columns labeled as Meanfr OKNi, Meanfr OKNf, and Meanfr OKANi indicate the change in the mean firing rate with respect to the baseline discharge (measured before OKS). Thus positive values in these columns indicate increases in mean firing rate and negative values decreases. For each column, significant different values than those corresponding to the normal gain animal are indicated (*P < 0.05 or **P < 0.01). A P < 0.01 is indicated with bold numbers.

FIG. 4. Response of Y neurons in the normal animal during vestibular and visual stimulation. A–D: response of the same neuron during different behavioral tasks and E and F, the population data. Neuronal response is presented at the top of each panel in A–D and behavioral response at the bottom. Additionally in A and B the stimulus, head velocity (A) or optokinetic stimulation velocity (B), is shown as dashed lines. In the normal gain animal, Y neurons show little, if any, modulation during dark VOR (VORd; A) but show a clear modulation during sinusoidal optokinetic stimulation (B). Upward optokinetic stimulation evokes an initial increase in the firing rate that is maintained through the optokinetic stimulation period. During the OKAN period, the discharge of Y neuron returns immediately to resting rate values. The contrary happens during downward optokinetic stimulation (neuronal firing decreases during optokinetic stimulation period). White lines shown over the behavioral response in the OKAN period in C and D represent the exponential fitting. Dark lines shown over the neuronal response during the OKAN period in C and D represent the estimated neuronal response using Eq. 2 (see METHODS). E and F: average and SD of the change in the neuronal firing rate during the OKNi, OKNf, and OKANi periods for upward (E) and downward (F) optokinetic stimulation (see also Table 2). Statistical significance is indicated by a single (P < 0.05) or double (P < 0.01) asterisk. Note that most Y neurons show a response during saccades, but these portions are removed prior to analysis.
rate was further increased during OKNf. During OKANi Y neurons sharply decreased their firing rate, although it remained largely above the resting rate (48.98 ± 13.5 spike/s above resting level). The population response during OKANi in the high gain animal was significantly larger (\(P < 0.01\)) than in the normal gain animal. After low gain VOR learning, the average firing rate of Y neurons during OKNi and OKNf were similar to those observed in the normal gain animal; however, cells tended to decrease their firing rate below the baseline rate during OKANi (−10.77 ± 15.3, Fig. 6C). Thus a comparison between the mean firing rate during OKANi in the adapted versus the normal gain animals showed that the discharge of Y neurons during OKANi increases after high gain adaptation and decreases and becomes negative (below the baseline firing rate) after low gain adaptation (Table 2).

Identical methods as described for the normal gain animals were used to quantitatively describe the OKAN eye-velocity sensitivities of Y neurons after VOR motor learning. Results suggest that Y neurons have upward eye-velocity sensitivities during OKAN in the high gain adapted animal and downward eye-velocity sensitivities during OKAN in the low gain adapted animal, in agreement with the increases and decreases in firing rate noted above. Figure 7, A and B, shows that the eye-velocity sensitivities of Y neurons after upward optokinetic stimulation were positively correlated with the VOR gain (increasing sensitivity with increasing VOR gain). Changes in eye position and eye acceleration sensitivity during OKAN were not significant (Table 2).

RESPONSE OF Y NEURONS DURING DOWNWARD OPTOKINETIC STIMULATION IN ADAPTED ANIMALS. Changes in the mean response of Y neurons during OKNi, OKNf, and OKANi after downward optokinetic stimulation were not as clear as for upward optokinetic stimulation (see Table 2). Neuronal responses during OKNi and OKNf were variable. Y neuron responses during OKAN for downward optokinetic stimulation showed no significant changes after VOR motor learning.

Neuronal sensitivities during the OKAN period for downward optokinetic stimulation were also different from those observed for upward optokinetic stimulation (Fig. 7, C and D). In general, Y neurons showed downward eye-velocity sensitivities in the low gain adapted and normal animals. In the high gain adapted animal, however, most Y neurons (8 of 12) showed upward eye-velocity sensitivities. There was no significant change in eye-position sensitivity during OKAN for downward optokinetic stimulation, and sensitivity to eye acceleration showed a small change (ANOVA single factor \(0.05 < P < 0.01\); Table 2). We do not have an explanation for this change in eye acceleration sensitivity. Although the eye-movement-related sensitivities (position, velocity, and acceleration) in Y neurons were different for upward and downward optokinetic stimulation, VOR motor learning evoked similar directional changes in these components for both upward and
downward optokinetic stimulation. For example eye-velocity sensitivities tended to increase with increasing gain.

In summary, while Y neurons in the normal gain animal on the whole showed mixed directional preferences for OKAN eye velocity for both up- and downward OKR, Y neurons in the adapted animals showed clear deviations from this pattern with high gain animals showing positive eye-velocity sensitivities and low gain animals showing negative eye-velocity sensitivities (see Table 2). Interestingly, the behavior only showed significant changes after high gain adaptation.

Because the eye-velocity sensitivity during OKAN is the only Y neuron eye-related parameter that changes consistently with VOR motor learning and this change mirrors the qualitative firing rate changes observed in the OKAN period often considered the OKN velocity storage component, we argue that the eye-velocity sensitivity serves as a measure of the OKN velocity storage representation of Y neurons.

**Relationship between vestibular and OKN velocity storage pathways at the level of Y neurons**

A relationship between vestibular and OKN velocity storage signals has been previously established at the behavioral level (Cohen et al. 1973; Demer 1981; Lisberger 1981) and was observed in some vestibular neurons (Waespe and Henn 1977b). In this section, we ask whether vestibular and OKN velocity storage signals are also related at the level of Y neurons.

Vestibular signals arrive at Y neurons via two pathways, a brain stem pathway, disynaptic from the vestibular nerve, and a cerebellar pathway (Fig. 8A). Both pathways add at the level of Y neurons resulting in an overall upward head-velocity signal regardless of the VOR gain. We were able to estimate the overall head-velocity sensitivity of 24 Y neurons (7, 11, and 6 in low, normal, and high gain adapted animal respectively) (see METHODS section and Hirata and Highstein 2001). Estimation of the head-velocity sensitivity of the remaining Y neurons (21) was not possible because cells were lost before sufficient data were collected (18 neurons) or because the fitting of the data were not reliable (3). Our results showed no significant changes in the head-velocity sensitivity of Y neurons after VOR motor learning (1.17 ± 0.17, 0.71 ± 0.40, 0.91 ± 0.44, for the low, normal, and high gain adapted animal). In agreement with a previous report (Blazquez et al. 2006), we found that the vestibular signals arriving to Y neurons from the cerebellar flocculus (P_h_FL) always provide the neurons with an upward head-velocity signal that increases monotonically with VOR gain changes (0.30 ± 0.16, 1.00 ± 0.48, 1.59 ± 0.51, for the low, normal, and high gain adapted animal). In agreement with a previous report (Blazquez et al. 2006), we found that the vestibular signals arriving to Y neurons from the cerebellar flocculus (P_h_FL) always provide the neurons with an upward head-velocity signal that increases monotonically with VOR gain changes (0.30 ± 0.16, 1.00 ± 0.48, 1.59 ± 0.51, for the low, normal, and high gain adapted animal).
activity for upward and downward optokinetic stimulation. This relationship shows similar slopes for both directions of optokinetic stimulation with $r^2$ values of 0.59 and 0.55 for up- and downward, respectively. A relationship between $\beta_h$ and OKAN eye-velocity sensitivity was also observed ($r^2$ values of 0.45 and 0.39 for up- and downward optokinetic stimulation); however, because $\beta_h$ does not switch its directional preference like $\beta_{\text{non-FL}}$ does (see DISCUSSION) (see also Blazquez et al. 2006) we believe that the changes in the OKAN eye-velocity sensitivity of Y neurons are most likely due to changes in $\beta_{\text{non-FL}}$. Thus we show a correlation between changes in brain stem head velocity and OKN velocity storage pathways at the neuronal level.

**DISCUSSION**

**Summary**

We designed this study to obtain further information on the sites of plasticity involved in VOR motor learning within the cerebellum brain stem loop. We were primarily interested in determining whether the vertical OKR changes after VOR motor learning and, if so, whether Y group neurons show plasticity that supports this change. To test this, we used a standard experimental method to induce long-term (chronic) VOR gain adaptation and compared behavioral and neuronal responses to optokinetic stimulation in different gain states. We found that 1) chronic VOR motor learning differentially affects the vertical and horizontal OKAN: horizontal OKAN changes in the same direction as VOR gain, and vertical OKAN changes only after VOR gain increases. 2) Y neurons as a population change their response during the OKAN period after chronic VOR motor learning: Y neurons show downward eye-velocity sensitivities in the low gain animal and upward eye-velocity sensitivities in the high gain animal. We argue that these changes are likely due to changes in the OKN velocity storage representation of Y neurons and are associated with changes in their direct brain stem (nonfloccular) vestibular input. Our results support and extend previous models that propose the existence of shared modifiable elements located in the brainstem that can account for changes in the VOR and OKAN after chronic VOR motor learning (see last section of DISCUSSION).

**Differences between vertical and horizontal OKAN before and after chronic VOR motor learning**

In agreement with previous reports, we documented differences between the vertical and the horizontal components of OKAN (Matsuo and Cohen 1984). Horizontal OKAN showed symmetrical responses for left- and rightward optokinetic stimulation, whereas the vertical OKAN was asymmetric, exhibiting a large response for upward optokinetic stimulation (comparable in magnitude to the horizontal counterpart), and a small response for downward optokinetic stimulation. Our neuronal data show a representation of this asymmetry; namely, we
found different neuronal sensitivities to eye velocity during up- and downward OKAN in Y neurons, suggesting that these neurons do not contribute equally in the generation of eye movements during up- and downward OKAN. Our experiments cannot say whether or not this asymmetry arises in the Y group. However, as premotor neurons, Y neurons do maintain the asymmetry in the behavior.

Horizontal OKANi changes with chronic VOR motor learning, and this change parallels changes in VOR gain (Demer 1981; Lisberger et al. 1981). However, we show that in the vertical system OKANi changes only after chronic VOR gain increases, suggesting different mechanisms for chronic VOR gain increases and decreases. There is growing evidence to support this view. We have previously shown nonmonotonic changes in Purkinje cells with VOR motor learning (Blazquez et al. 2003) and recently suggested that the head-velocity sensitivity of vertical PVPs, or some other group of neurons in the brain stem pathway, changes after only low gain training (Blazquez et al. 2006). Additionally, we have found that the Purkinje cell-Y neuron synapses undergo an LTD-like form of plasticity after low, but not high gain VOR motor learning (Blazquez et al. 2006; see also Gittis and du Lac 2006). Raymond’s group showed that VOR gain can be rapidly restored to its original values after VOR gain increases but requires longer training time to revert after VOR gain decreases (Boyden and Raymond 2003), which agrees with our finding of a longer time constant of VOR gain decay after low gain than after high gain training (Kuki et al. 2004).

An alternative hypothesis to explain our results is that VOR motor learning causes changes in the retinal slip information driving the OKN velocity storage, which could lead to a slower build-up of eye velocity during the stimulation period due to nonlinearities in the visual system. Waespe and colleagues (1983) showed that flocculectomized monkeys require more time to completely charge the velocity storage and argue that this effect is due to an increase in retinal slip during the initial phase of the OKR. This might suggest that in our adapted animals, the 160°/s stimulus we employed may not have fully charged the OKN velocity storage. However, we did not find any significant change in the OKNi after VOR motor learning (Table 1), suggesting that there are no significant changes in the retinal slip. Additionally, our changes consisted of increases in the velocity storage after high gain adaptation and no decrease after low gain adaptation, which cannot be explained by this rationale. Further, we allowed a minimum charging time of 20 s, which, as our control experiments indicate (see Fig. 1), should be sufficient to fully charge the OKN velocity storage even if the charging dynamics have slightly changed. However, more experiments with a larger variety of stimuli are required to further resolve this issue.

**Y neurons receive inputs from both the short time constant OKN and OKN velocity storage visual pathways**

The response of Y neurons during sinusoidal optokinetic stimulation, velocity steps of optokinetic stimulation, and OKAN suggest that they carry signals related to both the short time constant OKN and the OKN velocity storage pathways. The short time constant OKN pathway reaches vestibular neurons (such as, Y neurons) through the cerebellar flocculus (Langer et al. 1985; Partsalis et al. 1995b; Rambold et al. 2002). Lesions of the cerebellar flocculus severely affect pursuit behavior (Rambold et al. 2002), which is related to the short time constant OKN, and completely eliminate the response of Y neurons during sinusoidal optokinetic stimulation (Partsalis et al. 1995b). The OKN velocity storage pathway travels through the brain stem and is intimately associated with pathways carrying head-velocity information (Waespe and
Lesions in the vestibular nuclei and vestibular nerve reduce or completely abolish the OKAN (Cannon and Robinson 1987), whereas floccular lesions do not have a significant effect on OKAN behavior (Weaase et al. 1985). Thus it is likely that the OKN velocity storage pathway reaches Y neurons via their brain stem vestibular pathway.

The brain stem vestibular pathway to Y neurons consists of the VIIIth nerve and interneurons located in the superior vestibular nucleus. The interneurons have either anterior or posterior canal-related signals, and in the normal gain animal, their head-velocity signals cancel each other at the level of Y neurons, resulting in a negligible or small head-velocity signal of nonfloccular origin (Blazquez et al. 2000; Partsalis et al. 1995b). Our results demonstrate that Y neurons as a population show a small sensitivity to upward eye velocity during OKAN in the normal gain animal, which likely reflects the presence of a small OKN velocity storage sensitivity. The notion that Y neurons contain an OKN velocity storage signal is supported by the similar time constants of decay in the eye velocity and neuronal response during the OKAN period (Fig. 7E) and by visual inspection of the raw behavioral and neuronal data (Figs. 4–6). A similar relationship between OKAN eye velocity and neuronal activity has been previously shown for neurons in the vestibular nuclei (Waespe and Henn 1977b). We suggest that the small OKN velocity storage representation, or sensitivity, observed in Y neurons in the normal gain animal is the result of a small net head-velocity input arriving from the brain stem vestibular pathway. However, our experiments cannot determine whether the OKN velocity storage sensitivity in Y neurons is generated there or arrives via the vestibular nucleus interneurons.

**Y neurons show different directional tuning for OKN velocity storage in the low and high gain animal**

We propose that the changes in Y neuron OKN velocity storage sensitivity after VOR motor learning result from changes in pathways that carry vestibular information. This is supported by evidence indicating that OKN velocity storage is intimately related with VOR (Demer 1981; Lisberger et al. 1981; Raphan et al. 1979). Thus changes in the strength of a vestibular pathway to Y could result in changes in the head-velocity signal of Y neurons and consequently changes in the OKN velocity storage signal.

There are two main vestibular pathways that can carry the modified OKN velocity storage signal to Y neurons: floccular inputs and brain stem (disynaptic) vestibular inputs (see Fig. 8A). Floccular inputs to Y neurons are not a likely candidate to carry the modified OKN velocity storage signal because floccular Purkinje cells do not change their vestibular sensitivities in the direction necessary to explain the changes in OKN velocity storage sensitivity of Y neurons. The floccular Purkinje cells projecting to Y neurons always provide an up-head-velocity input, whereas the OKN velocity storage sensitivity of Y neurons is negative for low and positive for high VOR gains (see Results and Blazquez et al. 2003, 2660). A more plausible candidate is the direct vestibular pathway to Y neurons, which appears to be inversely related with the OKN velocity storage signal (see Fig. 8, B and C), switching its directional preference in the low and high gain adapted animal. In agreement, Waespe and Henn (1977a) showed that vestibular nucleus neurons have opposite directional preferences for OKN velocity storage and head velocity. We observed a similar relationship in vertical head velocity only neurons in the squirrel monkey (in preparation). Nevertheless, further studies using inactivation or neuronal recording of the flocculus before and after VOR motor learning are necessary to test our hypothesis.

**Unified conceptual model of motor learning for the VOR and OKN velocity storage**

Our results during the last few years (Blazquez et al. 2003, 2006) have led us to extend previous models of the substrates for motor learning in the VOR. We have proposed two brain stem-level modifiable elements for the vertical system, one at the Y neurons and another in a parallel pathway to Y neurons, possibly the PVPs. Chronic increases in the VOR gain affect only the modifiable element located at the level of Y neurons, whereas chronic decreases in VOR gain affect both modifiable elements.

In this paper, we show that vertical VOR gain changes are accompanied by changes in the vertical OKAN. Further, we show that the Y group neuronal sensitivities to OKN velocity storage are correlated with their neuronal sensitivities to the brain stem vestibular pathway. Because the vestibular and OKN velocity storage systems are so tightly coupled, any model of VOR motor learning should also be able to explain the changes observed in OKN velocity storage. We propose that Y group neurons change their OKN velocity storage sensitivity in parallel with changes in their head-velocity sensitivity for increases in VOR gain, whereas additional changes in the OKN velocity storage and head-velocity sensitivities of a second pathway are utilized for decreases in VOR gain. The existence of an additional modifiable pathway for the vertical system has not been experimentally verified yet and thus remains speculative; however, plasticity in a second pathway is required to explain the behavior.

This proposal deviates from that of a previous model developed for the horizontal system, which proposed a single modifiable element in the brain stem (Lisberger 1994; Lisberger et al. 1981). Our behavioral data on the horizontal OKN support this previous model, but our behavioral and neuronal data on the vertical OKN suggest that the vertical system requires a second modifiable element to support the behavior after VOR motor learning. This highlights a major difference between the horizontal and vertical pathways for visuo- and vestibulooculomotor behavior, which will be an important consideration for future research.

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