Motor Coding in Floccular Climbing Fibers

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Submitted 9 November 2005; accepted in final form 10 December 2005

Winkelman, Beerend and Maarten Frens. Motor coding in floccular climbing fibers. J Neurophysiol 95: 2342–2351, 2006. First published December 14, 2005; doi:10.1152/jn.01191.2005. The climbing fibers (CFs) that project from the dorsal cap of the inferior olive (IO) to the flocculus of the cerebellar cortex have been reported to be purely sensory, encoding "retinal slip." However, a clear oculomotor projection from the nucleus prepositus hypoglossi (NPH) to the IO has been shown. We therefore studied the sensorimotor information that is present in the CF signal. We presented rabbits with visual motion noise stimuli to break up the tight relation between instantaneous retinal slip and eye movement. Strikingly, the information about the motor behavior in the CF signal more than doubled that of the sensory component and was time-locked more tightly. The contribution of oculomotor signals was independently confirmed by analysis of spontaneous eye movements in the absence of visual input. The motor component of the CF code is essential to distinguish unexpected slip from self-generated slip, which is a prerequisite for proper oculomotor learning.

INTRODUCTION

The flocculus is a compartment of the cerebellar cortex that traditionally serves as an exemplary model for cerebellar learning and motor control. It is well accepted that the flocculus computes a correction signal for the vestibular nucleus from various signals about vestibular, retinal, and eye movement dynamics that are received from many brain regions through the mossy fiber system (Blazquez et al. 2004). In this way, the flocculus is part of the eye reflex circuit involved in the control of ocular reflexes such as the vestibulo-ocular reflex (VOR) and the optokinetic reflex (OKR). These reflexes aid vision by maintaining a stationary projection of a visual scene on the retina on the basis of vestibular and visual motion signals, respectively.

Like in all other regions of the cerebellar cortex, all signals converge on Purkinje cells that deliver the whole output of the flocculus. Each Purkinje cell also receives prominent excitatory input from a single climbing fiber, originating from the contralateral inferior olive (IO). Activity of the climbing fiber (CF) elicits characteristic multipeaked complex spikes (CSs). This distinct input system has received much attention in the last decades for its potential teaching function in the formation of a cerebellar motor memory or its function in real-time motor control (Simpson et al. 1996).

The CSs of the flocculus are known to respond to visual stimulation, more specifically slip of the retinal image. This has been shown in the anesthetized rabbit using optokinetic stimulation of a large part of the visual field (Graf et al. 1988). Direct projections from motion sensitive visual nuclei such as the Accessory Optic System (AOS) and the Nucleus of the Optic Tract (NOT) to the dorsal cap (Giolli et al. 1985; Maekawa and Takeda 1977, 1979; Takeda and Maekawa 1976), which is the part of the IO that projects to the flocculus, can explain this. However, the dorsal cap does not only receive visual input. For instance the prepositus hypoglossi nucleus (PrH) is the main source of inhibitory projections to the dorsal cap of the IO (De Zeeuw et al. 1993, 1995; Frens et al. 2001). The PrH is thought to carry an efference copy of oculomotor commands (McCrea 1988; McFarland and Fuchs 1992). Unilateral and bilateral lesions of the PrH showed significant effects on the average floccular CS firing rate but had no apparent effect on CS modulation to constant velocity optokinetic stimulation (Arts et al. 2000). However, because these experiments were performed in anesthetized animals, it is very possible that no oculomotor signals could be relayed to the IO. Such projections make it unlikely that the CS input to the flocculus contains only sensory (i.e., retinal slip) information. It is the purpose of this study to specifically characterize an extraretinal motor component in the CF code.

Some indications that extraretinal signals related to self-motion influence CS modulation have been reported. Several groups showed that robust sinusoidal rotation of rabbits in complete darkness could produce residual CS modulation in a large fraction of floccular Purkinje cells (De Zeeuw et al. 1995; Ghelarducci et al. 1975; Simpson et al. 2002). In these experiments, it is impossible to relate this modulation exclusively to vestibular or oculomotor signals. Using transparent optokinetic stimulation, our laboratory recently showed that modulation of floccular CS under comparable retinal slip conditions differs with the oculomotor behavior of the rabbit (Frens et al. 2001). This strongly suggests an extraretinal influence, but the nature of such a modulation is presently unknown.

In monkey, (para-)floccular climbing fiber activity correlates better and fits more linearly with eye movement than with retinal slip during an ocular following task (Kobayashi et al. 1998). However, these authors chose to correlate the CS activity to the mean eye movement at 10 ms after the spike. Therefore their data may provide insight in a putative efferent motor consequence of the CS. Our study takes the opposite approach by asking what a Purkinje cell receiving a CS could infer about the past, present, or future oculomotor behavior of the animal.

METHODS

Animal preparation

Experiments were conducted using two female Dutch belted rabbits. Both animals were equipped for chronic experimentation with an
acrylic head fixation pedestal and implanted search coils in both eyes. Recording chambers were positioned above the paramedian lobule of the cerebellum. All surgical procedures have been published elsewhere (Frens et al. 2000; Mathoera et al. 1999). Surgical procedures and experimental protocols are in accordance with the guidelines set by the Animal Welfare Committee of the Erasmus University as well as with the Principles of Laboratory Animal Care (National Institutes of Health).

Neuronal recording and spike sorting

CF activity was recorded in both Purkinje cell layer and molecular layer using standard extracellular recording techniques. Recordings were considered to be single units when a simple spike pause was present or, for molecular layer recordings, when the CS magnitude was prominent and constant and the interspike interval distribution characteristic for a single CF. The electrode signal was preamplified, band-pass filtered (100–3,000 Hz; CyberAmp 380, Axon Instruments) and sampled (25 kHz; Power 1401, Cambridge Electronic Design). The raw electrode signal was further processed off-line. Fifty Hertz hum and harmonics was removed off-line from the electrode signal. Possible spikes were identified by level detection and sorted using the first four principal components of the total spike wave set (Goossens et al. 2001). All spike waves in the set were aligned onto the first positive directed peak, and corrections were made to account for different numbers of complex and simple spikes when relevant. The spike times of identified CS were stored for further analysis.

Neuronal characterization and selection

Well-isolated units in the flocculus were initially tested for preferred OKS direction using a handheld pattern followed by sinusoidal vertical axis (VA) OKS that served as an extra control. Climbing fibers projecting to zones 2 and 4 of the flocculus respond best to rotation of the visual field around the vertical axis (De Zeeuw et al. 1994). Units showing this preferred direction were selectively used for our analysis. The visual input to VA units was further analyzed using 1-ms light pulses delivered to each eye separately in a random manner (200–1,224-ms interflash time). The light-pulse stimulus induced an (often bimodal) CS transient that peaked at 38 ± 1 ms, followed by a short inhibition. This latency toward the CS peak was considered the minimal visual delay. From a total of 168 recorded units, 91 were classified as VA neurons. For a subset of 32 cells, the quality and duration of the recordings was sufficient for further analysis. Recording duration ranged from 253.9 to 3,243.9 s (mean = 848.2 s), with CS numbers ranging from 313 to 5,982 (mean = 1,075.7).

Visual stimulation and eye movement recording

After the initial cell characterization, trials of 10 min optokinetic stimulation were presented to the rabbit. The stimulus consisted of vertical axis translation of a random dot pattern projected on a cone-shaped translucent screen that was placed over the animal. The velocity of rotation was driven by colored noise with a Gaussian distribution of stimulus velocities. Four different noise stimuli were used (Fig. 2A) with identical power spectra, except for a scaling factor. For all stimuli the mean velocity was 0°/s, and the variance was 1, 2, 4, and 8.5°/s. All these stimuli contained velocities that are well within the velocity tuning range of the accessory optic system (Soodak and Simpson 1988), which is the prime source for retinal slip signals in the vestibulo-cerebellum. After the optokinetic paradigm, background CF activity in the dark was recorded for as long as the isolation permitted. Complete darkness was secured by placing two black hemispheres over the eyes in addition to switching off the room lights.

Eye position was recorded using the scleral search coil technique described elsewhere (Van der Steen and Collewijn 1984). The noise level of the eye position recording was ~50° (SD). Eye and stimulus position signals were low-pass filtered (300 Hz; Axon CyberAmp 380) and sampled at 1 kHz. Eye and stimulus velocity were computed off-line by differentiation and application of a Gaussian smoothing filter (τ = 15 ms).

Data analysis

BEHAVIOR. All data analysis was performed using the Matlab software package (The Mathworks). Power spectra, transfer, and coherence function estimates of the OKS and oculomotor behavior were computed with Welch’s averaged periodogram method using 75% overlapping 1,000 point sections and Hamming windows. Sections containing saccades rarely occurred and were automatically excluded using a manually adjustable velocity threshold. A window of 250 ms before and after a saccade was additionally excluded from analysis.

The optimal delay between the stimulus and the ensuing eye movement was detected by finding the time lag that resulted in the maximal cross-correlation between these two signals. Stimulus/eye velocity relationships were determined by first aligning the eye and stimulus velocity signal in time using the optimal delay, followed by calculation of mean eye velocities coinciding with stimulus velocities that were binned to 0.1°/s steps. The gain was computed as the ratio between these values. The gains to ipsilateral and contralateral movement were averaged to get the absolute gain.

SPIKE SIGNALS. Spike-triggered averages were made by aligning retinal slip and eye velocity signals about the arrival times of all N complex spikes in a recording. Missing values caused by exclusion of saccades were ignored. Because of the randomness (Keating and Thach 1995) and ultra-low firing rate (~1 Hz) of the CS signal, each CS was considered to encode an independent event. As a consequence, the CS signal was defined as a one-symbol code, leading us to calculate how informative a CS occurrence is for the Purkinje cell using the CS-conditional transmitted information. We used a method for estimating the transmitted information similar to that used by Optican and others (Optican et al. 1991; Optican and Richmond 1987; Richmond and Optican 1990), except that they used the stimulus-conditional information. In short: CS-conditional transmitted information \( T(\text{cs};X) \) is defined as the Kullback-Leibler divergence (Kullback and Leibler 1951) between the CS-conditional probability density function \( P(X|\text{cs}) \) and the expected stimulus distribution \( P(X) \)

\[
T(\text{cs};X) = \sum_{i=1}^{n} p(x_i|\text{cs}) \log \left( \frac{p(x_i|\text{cs})}{p(x)} \right)
\]

where \( n \) is the number of all stimulus values on which the probability densities are estimated. If \( P(X) \) and \( P(X|\text{cs}) \) are identical, the transmitted information is 0. If a CS encodes only a single stimulus value \( x \), the gained information equals \(- \log_2 P(x)\). Probability density functions (pdf) were estimated using convolution of all \( n \) data points with a Gaussian kernel

\[
\hat{p}(x) = n^{-1} \sum_{j=1}^{n} K(u)
\]

where \( u = \frac{x - x_j}{\hat{\sigma}_x} \cdot h \), \( \hat{\sigma}_x \) is the SD and \( h \) is the bandwidth. Because eye movement distributions tend to be bimodal rather than a unimodal normal (e.g., Fig. 3B), the SD of the stimulus distributions \( \hat{\sigma}_x \) was replaced by the sample interquartile range divided by 1.35. For normal distributions, this equals the SD. \( K(u) \) is the Gaussian kernel function

\[
K(u) = (2\pi)^{-1/2} \exp(-u^2/2)
\]

We used the optimal bandwidth that minimizes the mean integrated squared error in \( \hat{p}(x) \) when the underlying distribution is normal (Silverman 1986)
where $m$ is the number of CS used to estimate the CS-conditional stimulus pdf.

The probability densities were determined for stimulus values that were 0.1°/s apart. Higher resolutions only slightly improved the information estimates. Because of the sparseness of data points in the tails of the distributions, probability density estimates of especially the CS-conditional stimulus distributions would be unreliable in the tails. Therefore we truncated the distributions at the 2.5 and 97.5% percentiles. We used a bootstrapping procedure to estimate the mean ($T_b$) and variance ($\sigma^2_b$) of the transmitted information when the stimulus and CS response are independent. For these estimates we used $10^3$ artificial CS trains composed of randomly shuffled inter-CS intervals. The variance of the estimate of the transmitted information ($\sigma^2_b$) was bootstrapped from the CS-conditional stimulus values using $10^3$ iterations. The bias-corrected information values were calculated as

$$\hat{I} = T - \frac{T^2}{T_b}$$

and information variances became

$$\sigma^2_b = G^2 \left( 1 + \frac{T^2}{T_b} \right)^2 + \sigma^2_s \left( -2 \frac{T_s}{T} \right)^2$$

The variance of the information peak times was estimated from the peak times of a bootstrapped set of $10^3$ spike-triggered information estimates. Each of these estimates was based on a randomly resampled set (with replacement) of CS firing times.

**Results**

In this study, we specifically set out to distinguish the putative oculomotor component in the floccular CS code from a (sensory) retinal slip component. To that means, we took advantage of the delay between slip and ensuing eye movement to break up the tight relation between the two. This was accomplished using a constantly changing OKS (Fig. 1C). The advantage of this approach over the classical sinusoidal OKS is that such a latter stimulus evokes a sinusoidal eye response with the same frequency, and consequently, sinusoidal retinal slip, which is per definition the difference between the stimulus and the response velocity. It is impossible to relate CS activity to either one of these signals without making assumptions about latencies. By using (colored) noise as OKS, we can assess how CS activity relates temporally to retinal slip and eye movement.

The full-field OKS, rotating about the vertical axis, was presented to two alert rabbits (Fig. 1A), whereas the CS activity of floccular VA Purkinje cells was monitored extracellularly (Fig. 1B). The velocity of the OKS rotation (Fig. 1C) was driven by colored noise with Gaussian distributions of stimulus velocities ($\sigma^2_v = 1, 2, 4,$ and $8.5°/s$ about an average velocity of $0°/s$; Fig. 2A). These stimuli could induce eye movements through the OKR (Fig. 1D), and the frequency range extended high enough to effectively dissociate eye movement from instantaneous retinal slip (Fig. 1E).

**Behavior**

Figure 2 shows the response of the animals to the stimuli (Fig. 2A). The gain of the oculomotor response, as estimated by the transfer function, was highest for the lower frequencies in all stimulus conditions (Fig. 2B). However, increased OKS power within a frequency bin had a negative effect on the oculomotor gain, which underlines the nonlinear nature of the OKR. The coherence function (Fig. 2C) shows what fraction of the oculomotor response is linearly related to the OKS. The coherence functions are similar for all stimuli, decreasing almost linearly with increasing frequency. Note that frequency components $>12$ Hz in the oculomotor response are unrelated to the OKS. The phase shift of the response was such that it could be best described by a group delay of $~80$ ms for all stimuli (Fig. 2D and E). At this delay, the OKS that caused the

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**FIG. 1.** Experimental setup and measured signals. A: experimental setup. The rabbit was placed in a panoramic visual stimulus that rotated about the vertical axis with a noisy velocity profile. Movement into the ipsilateral direction, i.e., temporally from recording site, was defined as positive. A small time sample of measured signals is shown at the right. B: single unit activity of floccular Purkinje cells was recorded, along with the velocity of (C) optokinetic stimulus (OKS) and (D) eye movement. E: retinal slip velocity equals difference between OKS and ensuing eye movement. In B, complex spikes are indicated with an asterisk.
eye movement, accounted for only 1% of the variation in the actual OKS, and consequently the ensuing slip. Therefore our stimulus accomplished an effective dissociation between instantaneous slip and eye movement. When adjusted for the delay, the eye movement response appeared as a nonlinear function of the OKS velocity (Fig. 2F). The average velocity gain of the animals (Fig. 2G) ranged from 0.3 for 3°/s OKS velocity or higher to 0.75 at the lowest OKS velocities.

If one compares the velocity gain functions of the four stimuli, it is important to note that the stimuli have different velocity distributions. However, in the range that is present in all stimuli (<4°/s), the gain functions are practically identical, although at low velocities (<1°/s), the stimuli with a high variance (4 and 8.5°/s) induce apparently lower gains. Most likely this is related to the nonlinear response of the optokinetic reflex to retinal slip and the integration time that it requires. Increasing the variance will decrease the average duration that a certain velocity is presented.

Spike-triggered average versus spike-triggered information

To explore the visuo-motor context in which CS occur, one can compute the cross-correlation between CS firing and slip and or eye velocity for different time lags using the classical spike-triggered average (STA). However, a correlation only describes the linear dependence between two variables, whereas the CS signal is tuned nonmonotonically to retinal slip with a peak response for the majority of CF at slip velocities ±1°/s (Barmack and Hess 1980; Kusunoki et al. 1990; Leonard et al. 1988; Simpson and Alley 1974), and the putative CS tuning to eye movement is unknown. It is important to notice that the tuning of the CS signal to a particular stimulus velocity x (i.e., slip or eye movement) can linearly be attributed to OKS input. D: phase spectrum of oculomotor response, with respect to stimulus. E: group delay. Same phase spectrum expressed as delays [delay = phase/(360 × frequency)]. F: eye velocity as a function of OKS velocity. Signals have been aligned in time. Note higher eye velocity response in ipsilateral direction. G: mean oculomotor gain function. This graph gives eye movement response gain to OKS velocity, averaged for ipsi- and contralateral direction.
range of stimulus values, we can use it to directly compare the CS tuning to slip and eye movement.

STAs as well as spike-triggered information graphs are summarized in Fig. 4. The slip signal averages of nearly all units showed a similar profile within each OKS condition (Fig. 4A). Qualitatively, for all stimuli, the average slip around a CS is directed toward the contralateral (nasal) direction before the spike and is followed by a peak directed toward the ipsilateral side at about the time of the spike. Because the delay between this second peak and the CS is shorter than the minimal visual delay [38 ± 1 (SE) ms; gray dashed line; see METHODS], only the contralateral directed slip can be responsible for the CS generation.

The eye movement velocity is clearly present in the STA as well (Fig. 4B): the average eye movement peaks toward the contralateral direction at about the time of the spike. This eye movement correlation to the CS explains the second peak in the slip STA. The eye rotates on average contralaterally at the time of the CS, whereas the OKS velocity at that time is on average 0°/s. Therefore the slip will be on average ipsilaterally directed. However, this is not directly related to the generation of the CS, but a mere consequence of the definition of retinal slip (slip = OKS − eye velocity). It is important to note that the STA shows no sign of eye movement lagging the CS, which suggests that the CS activity itself did not result in motor output.

From the STA analysis it became clear that a CS is not only correlated with slip but also with eye movement. In Fig. 4, C and D, we compare the mean transmitted information curves for the different stimulus conditions. As one can see, the average curves of both the slip information and the eye velocity information are surprisingly independent of stimulus condition. This means that these profiles describe the behavior of the IO over a large range of OKS conditions.

When we compare the maximum information of the individual CF signals, we find that the information about the eye

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**FIG. 3.** Tuning of the climbing fiber (CF) to retinal slip (left) and eye velocity (right). These panels show data from a representative Purkinje cell during stimulation with a noise stimulus (σ = 8.5°/s). A: distribution of retinal slip velocities. Solid curve shows distribution of retinal slip as it occurred throughout recording: \( P(x_{slip}) \). Dashed curve is distribution of retinal slip at 84 ms before occurrence of a complex spike (CS): \( P(x_{slip|cs}) \). This latency was chosen in such a way that both curves deviate maximally. B: same graph for eye velocity. Latency in this graph is 6 ms leading a CS. C: CS tuning curve for retinal slip. This graph can be constructed from A by dividing \( P(x_{slip|cs}) \) by \( P(x_{slip}) \) and multiplication with average CS firing rate (dotted line). D: same graph as C for eye velocity. Note that tuning for eye velocity is steeper and more linear. Dotted line shows average CS firing rate.

**FIG. 4.** Mean spike-triggered average (STA) and information plots averaged over all cells. A: slip STA averaged for each stimulus. Minimal visual delay counted back in time from occurrence of a CS is indicated by a vertical dotted line. B: same graph as A for eye velocity. C: slip information plot averaged for each stimulus. D: same graph as C for eye velocity. Color code in this figure is the same as Fig. 2. Shaded areas represent SE.
movement exceeds the slip information in all neurons (Fig. 5A). On average the eye movement information (in the order of stimuli with increasing variance: $0.61 \pm 0.03$, $0.75 \pm 0.05$, $0.65 \pm 0.06$, and $0.74 \pm 0.06$ bits, respectively) was more than twice the information of the slip ($0.26 \pm 0.02$, $0.20 \pm 0.03$, $0.27 \pm 0.01$, and $0.21 \pm 0.04$ bits), irrespective of the stimulus used. There was no significant correlation between these two parameters ($r = 0.14; P = 0.37$). As one can see from Fig. 5A, it is impossible to qualify a CF as solely “sensory” or “motor,” because there seems to be a continuum of possible sensorimotor combinations.

The timing of the eye movement information peaks (Fig. 5B) was tightly coupled to the CS ($-17 \pm 2$, $-12 \pm 2$, $-14 \pm 2$, and $-7 \pm 1$ ms, respectively), whereas the timing of the slip of individual neurons could vary considerably ($-128 \pm 6$, $-154 \pm 17$, $-104 \pm 5$, and $-114 \pm 26$ ms, respectively). Furthermore, the bootstrapped variance estimate for the timing of the individual information peaks was much larger for slip ($0.704 \pm 0.0265$) than for eye movement ($0.010 \pm 0.002$). Together this indicates a weaker temporal coupling both within the cell and between cells. The lower peaks and the higher temporal variability within a cell may have a common cause. If a signal (slip) is subject to increased temporal jitter in respect to the CS signal, the average mutual information given a fixed delay declines. In this case, the CS signal becomes less informative from the perspective of a Purkinje cell. Indeed, we find a significant ($P < 0.05$) negative correlation between the SD of the peak timing and the peak height for both slip ($r = -0.41$) and eye movement ($r = -0.34$). Within each OKS condition, all cells had surprisingly uniform tuning curves for eye velocity (Fig. 5D, low SE), whereas the tuning for slip (Fig. 5C) varied considerably. Over the range of eye movements that were present, the tuning of all cells was rather linear compared with the tuning to the total range of slip, but were on average similar when the same velocity ranges were compared.

Spontaneous activity in the dark

To test the notion of a motor component in the CF code independently, we performed an additional experiment where eye movements were recorded in the absence of sensory stimulation.

In total darkness, eye position drifts spontaneously at low velocities. This type of drift is incorporated by the oculomotor integrator (Frens and Van Opstal 1994) and therefore accounted for by the oculomotor system. If there is indeed a direct contribution of oculomotor input to the CS generation, we expect that a significant information peak can still be observed if the eye movement is related to “spontaneous” CS activity while the animal sits in the dark. Obviously CS cannot provide information about retinal slip under these circumstances. Nor can an indirect correlation between the CS and the

![FIG. 5. Comparison between slip and eye movement information. A: comparison between maximal retinal slip information and eye movement information. Each dot represents a neuron. Note that for every cell, eye movement information is larger than slip information. B: timing of maximal slip information compared with maximal eye movement information. Note that timing of eye movement is tighter both within and between cells. C and D: mean tuning curve for slip and eye velocity, respectively. Color code is the same as Fig. 2. Error bars and shaded areas represent SE.](http://jn.physiology.org/doi/fig/10.1152/jn.00208.2005)
eye movement that is caused by a correlation of slip with both the CS and the eye movement play a role (see DISCUSSION). However, because of this latter fact and the suboptimal velocities of the drifting movements \( (\mu \sim 0^/s; \sigma \sim 0.25^/s; \text{cf. Fig. 5D}) \), the amount of eye movement information is expected to be smaller than during oculomotor behavior in the light.

Because there is not much movement of the eyes in darkness, and because of the low firing rate of the CF, one requires substantial recording times to obtain sufficient data. Nonetheless, we could do this in nine different cells (mean recording time about 15 min). The results are shown in Fig. 6. The average drift of the eye in darkness was into the ipsilateral direction (Fig. 6, A and B, dashed line). In eight of nine cells, the STA shows a deviation from this drift velocity peaking toward the contralateral direction at about the time of the CS. Consequently, the occurrence of a CS increases the chance that the eye was moving contralaterally. The transmitted information that is associated with this deviation (Fig. 6C) resembles the eye velocity graphs that were obtained during OKS, with a highly similar timing of information \( (-7 \pm 2 \, \text{ms in darkness vs. } -14 \pm 1 \, \text{on average during OKS}) \), but—as expected—with a lower peak value \( (0.08 \pm 0.01 \, \text{bits}) \). On the basis of these data, we conclude that part of “spontaneous” CS activity in the dark can be attributed to oculomotor activity.

**DISCUSSION**

Traditionally, the optokinetic reflex is assessed by stimulating animals with sinusoidal stimuli, which is an approach that stems from linear systems analysis. However, the OKR is a nonlinear reflex. The nonlinear nature of the OKR is most clearly shown in Fig. 2. Here it is shown that the gain of the OKR to a certain frequency component in the stimulus is highly dependent on the power of that component (Fig. 2B). Increasing the power decreases the response, whereas the quality of the gain estimate in a frequency bin is similar for each OKS condition (Fig. 2C). The phase relation between a broadband stimulus and the ensuing OKR response can be best described by a delay of roughly 80 ms that is constant over all frequencies. This is in good agreement with findings in the rabbit optokinetic system with sinusoidal stimuli, where the gain of the response is a function of peak velocity (rather than frequency), and the phase difference can be best explained by a fixed delay of 75 ms, irrespective of the stimulation frequency (Collewijn 1969). The dependence on stimulus amplitude and frequency content are both incompatible with a linear system. A second disadvantage of sinusoidal stimulation is that it causes temporal correlation between instantaneous optokinetic stimulus velocity, retinal slip, eye velocity, and CS modulation. Without knowing the proper neural gains and delays of each signal, it is impossible to properly relate them to each other, without scanning a large range of frequencies. We set out to overcome this problem by presenting stimuli with lesser temporal structure, i.e., colored noise.

Using these stimuli, we showed that floccular CS firing is influenced by an oculomotor signal in addition to the retinal slip signal that was already recognized (Graf et al. 1988). As previously reported, the direction of the slip that elicits CS activity of VA Purkinje cells was toward the nasal side. The latency between the slip information peak and the CS was on average \( -131 \pm 7 \, \text{ms} \), which was slightly larger than the delay of 100 ms described in the monkey (Stone and Lisberger 1990). However the CS is not well time-locked to retinal slip (Fig. 5B). For the oculomotor signals, the optimal direction for evoking CS activity is in the same direction as the slip, but the timing is different, i.e., only a few milliseconds before the CS. In our paradigm, the height of the information peak about the ongoing eye movement was higher than the slip peak and more tightly coupled in time (Fig. 4, E and F). Also the CS tuning curves to eye velocity were considerably less variable (Fig. 5, C and D). Therefore it seems that the occurrence of a CS tells a Purkinje cell more about the oculomotor behavior of the animal than about the retinal slip. Note that this higher information can be partially caused by the cross-correlation with the visual input and partially because of direct motor efference copy, but that for a Purkinje cell the source of information is irrelevant.

Even in the absence of vision, an oculomotor signal was detectable in the CS activity, which independently shows a motor component in floccular CS (Fig. 6). Because the velocity of the spontaneous drifting movements is well below the optimal velocity to trigger a CS, the amount of information in darkness substantially lower (by a factor 10) than during OKS. However, the timing of the information is in complete agreement in both paradigms.

Neither during OKS nor in the dark did we observe systematic oculomotor activity after the CS. This shows that CS do not cause any oculomotor behavior. This is in contrast with previously reported data on the ocular following behavior (combined optokinetic and smooth pursuit responses) in the ventral paraflocculus of the monkey (Kobayashi et al. 1998).
These authors used a stimulus that was highly correlated in time (i.e., constant speed), and could therefore not directly dissociate the slip and the eye velocity component. Their argument is based on a more linear relation between CS activity and eye velocity at 10 ms after the spike than between CS activity and slip velocity at 40 ms before the spike. However, if one compares these relations within the same velocity range (between 0 and ~40°/s), the linearity seems equal (their Fig. 6, C and D). Only at higher slip velocities does the relation saturate. These higher velocities were not tested in the eye movement domain. Whether CSs result in eye movements in the monkey therefore remains an open question.

Although it is widely accepted that floccular complex spikes report the occurrence of retinal slip, we now show that, for a floccular VA Purkinje cell, a CS also provides information about a simultaneous eye movement. However, a correlation between eye movement and CS firing does not necessarily mean that this is caused by a direct causal relation. If an efference copy of the motor command is relayed to the IO, the motor command is the common ancestor that forms the causal efference copy of the motor command is relayed to the IO, the eye movement domain. Whether CSs result in eye movements in the monkey therefore remains an open question.

In this study, we have limited the analysis to retinal slip (Fig. 7, route R1). However, other routes that lead to a correlation between these two signals coexist. As mentioned above (Fig. 4, A and B), an instantaneous correlation between instantaneous slip and eye movement is merely caused by the definition of retinal slip (Fig. 7, route R2). For a temporal correlation between the CS and eye velocity, an alternative (although not mutually exclusive) explanation is that retinal slip is the common cause of both the CS and the compensatory eye movement (Fig. 7, route R3). Because of the correlation between slip and ensuing eye movement, a CS can provide information about both signals even if the eye movement is not causal to CS generation. However, it is highly unlikely that this latter option explains our results completely. Such a notion seems incompatible with the stronger relation that we find between eye velocity and CS, both in peak information (Fig. 5A) and timing (Fig. 5B). It would also predict a positive correlation between the amount of information that a CS encodes in the slip and the eye velocity domain. This is not the case (Fig. 5A). Finally, such a scheme could not explain the finding that also eye movements in the dark induce CF activity (Fig. 6). Our experiments cannot show the type (excitatory or inhibitory) of the anatomical projections that relay oculomotor information. It is impossible to distinguish excitation from lack of inhibition (or vice versa) in a combined signal.

The use of adding oculomotor signals to the sensory slip signal can be explained in several ways. Climbing fibers are generally considered to have a teaching function toward the cerebellar cortex. The induction of long-term depression (LTD) in parallel fiber-Purkinje cell synapses is well established (Bear and Linden 2000; Ito 2001), and evidence is accumulating that other cerebellar plastic processes are also affected by climbing fiber activity (Hansel et al. 2001; Jorntell and Ekerot 2003). Retinal slip has been proposed as the teaching signal for floccular Purkinje cells, because it has been thought to represent visual error (Ito 1982). Through LTD, the synaptic input that can be associated with this error is gradually eliminated. However, situations exist where the flocculus should not interpret retinal slip as an error signal. Self-generated eye rotations over a stable visual background induce contralateral retinal slip, which in turn would induce CS firing. The oculomotor contribution that we find may serve to ensure that retinal slip is not relayed to the flocculus if extraretinal visual signals can predict the occurrence of that slip, because nothing needs to be learned. In this way the complex spike signal is refined to report visual perturbations that could not be anticipated and shows a remarkable analogy with blocking phenomena that have been shown in other experimental paradigms such as classical eye blink conditioning (Kim et al. 1998; Medina et al. 2002) or with gating of cutaneous sensory CF activity (Apps 1999). It should be noted that these results are in line with Marr-Albus-Ito models that require “motor error” for oculomotor learning. We propose a more sophisticated error signal, but the essentials of the learning mechanisms remain.

The concept that a sensory expectation inferred from a motor command is subtracted from actual sensory feedback dates back half a century (von Holst and Mittelstaedt 1950). The application to CF signals, qualifying the CSs as “unexpected event” or “error” messages, was thoroughly researched and debated during the last two decades (see for extensive review: Simpson et al. 1996). However, the retinal slip signal conveyed by floccular CF afferents was itself regarded as an error signal, and no distinction was made between expected and unexpected slip. The particular proposition that unexpected retinal slip could be actively gated by the IO has been hypothesized recently (Devor 2000, 2002). Here the term “expected” is still faintly associated with “voluntary,” so that hypothetically only smooth pursuit and saccadic eye movements qualify to induce expected slip. We generalize the concept of expected slip to slip that could be inferred from head and eye movement signals.

Qualitatively, the oculomotor contribution as we measure it can indeed serve to create an “unexpected” slip signal. Eye movements induce slip in the direction opposite to the movement. The fact that the slip peak and the oculomotor peak have the same sign (both nasally directed) therefore fits the notion of “unexpected.” A complicating factor may be that the slip and the oculomotor peak do not coincide temporally but are roughly 75 ms apart (Fig. 4F). However, it should be noted that we correlate the real slip and eye velocity to the CS (Fig. 7, left), whereas the IO receives neural correlates of these signals (Fig. 7, right). Consequently, the actual signals that arrive in the olive may be more synchronous.

In this study, we have limited the analysis to retinal slip velocity and eye movement velocity. This does not mean that other modalities, or other orders of the same modalities, could be present in the CF code. For instance Simpson et al. (2002)

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**FIG. 7.** Simplified graph showing causal paths that relate CS activity to slip and eye movement. Three routes that connect eye movement to CS generation are indicated with R1, R2, and R3. Last 2 routes constitute concept that CS firing is modulated by visual signals, whereas route R1 represents a true oculomotor component influencing CS firing.
reported a vestibular component, and Kobayashi et al. (1998) reported a small but significant correlation with acceleration.

The oculomotor signal makes CS firing more likely when the eyes rotate toward the contralateral direction. This is different from results found previously (Simpson et al. 2002), where a residual CS modulation to vestibular stimulation in the absence of vision (VOR dark) was shown. The oculomotor signal described in our study is unlikely to be the same signal that causes the modulation under VOR dark conditions, because the CS facilitating eye movement directions are opposite. During VOR in the dark, complex spikes tend to occur when the eye moves toward the ipsilateral side and the head toward the contralateral side. This suggests that vestibular signals also play a role in CS modulation and counteract the influence of the oculomotor component. Strikingly, this is exactly what one would expect, because expected slip caused by self-motion can be estimated by the difference between head velocity (vestibular) and eye velocity (oculomotor).

Because of the 100% efficiency of the CF-Purkinje cell synapse, the source of extraretinal signals present in floccular CS activity lies at the level of the IO or further upstream and remains to be established. Given the timing of the oculomotor signal (at about 14 ms before the CS), we expect the source of the signal to be an effective copy of the oculomotor command rather than a sensory registration of the actual movement. The latency is simply too short to allow for a sensory involvement. The aforementioned projection from the NPH to the IO seems a likely candidate, but further research is necessary to establish such a role.

ACKNOWLEDGMENTS
We thank C. de Zeeuw for carefully reading the manuscript.

GRANTS
This project was funded by the Netherlands Organisation for Scientific Research-VIDI.

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