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We analyzed data from n=72 Purkinje cells in the oculomotor vermis of 5 rhesus monkeys as they made saccades to visual targets.

1. The data set

The data set included Purkinje-cell (P-cell) discharge from the oculomotor vermis (OMV) in 5 rhesus monkeys (*Macaca mulatta;* males; 5.0-7.4kg; monkeys B, F, W, K, and KO). These data were collected during two previous studies[8,9]. A scleral search coil was surgically implanted into one eye of each monkey, allowing measurement of eye kinematics via standard techniques[26]. Following recovery from surgery, the monkeys were trained to make saccades to visual targets (less than 0.4° in diameter) in a dimly lit room. The targets appeared within 25° of center. Monkeys were rewarded with applesauce for keeping their eyes within a virtual window which extended ±3° in both the horizontal and vertical directions about the target. After the monkeys were trained to saccade to a single target, they were trained to make saccades between successively presented targets.

Once the monkey could reliably track the targets for an extended period of time, a recording chamber was implanted on the midline of the cerebellum (14.5mm posterior of the interaural axis and directed straight down), providing access to the OMV. Single-unit activity was recorded with homemade tungsten electrodes with an iron-particle coating (100kΩ impedance at 1kHz). The position of the electrode for each recording was measured with respect to the center of the recording chamber, providing approximate coordinates of each P-cell within OMV. We recognized OMV by observing saccade-related changes in background activity. An isolated unit was classified as a P-cell if it produced a complex-spike (CS), which was identified online as a positive action potential with multiple wavelets. We focused our recordings on units which showed a saccade-related change in simple-spike activity in at least one

direction (a burst, pause, or a combination of the two). Saccade activity was assessed while the monkey made 15° saccades from the central fixation point to one of eight targets spaced at 45° intervals.

Neurophysiology data was sampled at 50kHz by a Power 1401 digitizer (Cambridge Electronic Design, Cambridge, UK) and subsequently band-pass filtered between 30Hz and 10kHz. The location of the eye, as measured by the scleral search coil, was sampled at 1kHz. Data were displayed in real-time on a computer monitor running Spike2 and saved for offline analysis[29].

We performed spike-sorting to isolate the simple-spike activity of each P-cell. The timing of each simple-spike was identified and subsequently down-sampled to 1kHz to coincide with the timing of the behavioral recordings. We identified saccades via an absolute velocity threshold, marking onset of the saccade as the time when the speed of the eye exceeded 20°/s. The end of the saccade was similarly defined as the time when speed fell below 20°/s. Trials in which the monkey moved its eyes in the wrong direction (> 90° with respect to the target), or trials in which the error at the endpoint exceeded 15°, were removed from the analysis (4.6% of all trials). The peak speed for each saccade was determined as the maximum magnitude of the velocity vector in the direction of the presented target.

To convert the simple-spikes of the P-cells into firing rates, we computed the inter-spike interval between two consecutive spikes, and then replaced the period between these two spikes with a box-car of magnitude equal to the reciprocal of the interval. We smoothed the resulting time-sequence with a normalized Gaussian of 2.5ms standard deviation (integral one), and then used this rate function to compute the mean and peak firing rate of the cell during the saccade period. The peak firing rate is the mathematical maximum of the firing rates during the saccade period, for both pause and burst cells. We use the term “firing rate” to refer to this instantaneous rate, and quantify it using units of Hertz (Hz).

Statistical analyses were conducted in R (R-project, Vienna, Austria). To assess the relationship between two variables we report Pearson’s R^2 coefficient as well as the results of a repeated measures analysis of variance (RM-ANOVA). If the data used for an RM-ANOVA failed the test for sphericity, we report the Greenhouse-Geisser corrected statistics. Paired sample t-tests were used to assess differences in response characteristics as a function of direction (CS-on/off). In cases where we used independent samples t-tests, we also assumed unequal variances between the two groups. All tests were two-sided with a significance level of 0.05, unless otherwise noted.

2. Complex-spikes

After we had identified a P-cell that exhibited phasic changes in simple-spike activity during a saccade, we identified the cell’s preferred complex-spike direction, termed CS-on. To do so we induced errors at the end of a saccade and recorded the resulting complex spikes in the P-cell. To induce errors, we used the

intra-saccadic step paradigm[27], blanking the original target during execution of the saccade and replacing it with another target so that at saccade termination the eyes appeared to miss the target by approximately 5°, as illustrated in Fig. 2a. The monkey began by fixating the central point. The central point disappeared and a target appeared at either 12° or 15° in one of 8 directions (spaced at 45° intervals). During the execution of the saccade to the target (when the eye velocity exceed 70°), the target was back-stepped by 5° relative to the original target. As a result the saccade over-shot the target, resulting in an error of approximately 5°. A second presentation of this intra-saccadic step paradigm in the opposite direction brought the monkey's eyes back to the central fixation point. For each monkey, we collected more than 30 trials in each direction. We counted the number of complex-spikes in the 50-200ms period following termination of the first (primary) saccade. The back-step direction (error direction) which elicited the highest probability of complex-spikes was classified as the CS-on direction (Fig. 2b).

The paradigm that we used for identifying the error vector that produced the highest probability of complex-spikes (i.e., CS-on direction) suffered from the short-coming that saccade direction and error direction were 180^{o} apart. Therefore, we examined whether probability of CS was dependent only on the direction of error, or whether it was also affected by the saccade direction that preceded that error[29]. To dissociate these two variables, for n=39 cells we systematically varied both error and saccade directions. An example of this is shown for a cell in Fig. 2c. This cell (N1) had a high probability of complex-spikes when the error vector was -45^{o}, regardless of whether the primary saccade was at direction of error$+0^{o}$, or direction of error$+180^{o}$. Similarly, the cell had a low probability of complex-spikes when the error vector was $+135^{o}$, regardless of whether the primary saccade was at direction of error$+0^{o}$, or direction of error$+180^{o}$. Across the population of n=39 cells probability of complex-spikes was modulated by direction of the error vector, but not the direction of the saccade (Fig. 2d): two-way RM-ANOVA showed a main effect of error direction ($p < 10^{-4}$) but no effect of saccade direction ($p > 0.5$).

For n=39 cells we were able to maintain excellent isolation of the P-cell throughout the recording period. For these cells, we manually identified a subset of complex-spike waveforms which served as a template for matching against all other complex-spikes during the recording. Using a Gaussian mixture model, we determined the presence/absence of a complex-spike in 30ms overlapping windows, providing us with the onset of a complex-spike with millisecond resolution. Looking at the same period (50-200ms following the primary saccade), we were able to construct an estimate of the probability of observing a complex-spike as a function of the angle between the endpoint of the primary saccade and the back-stepped target with better than 45° resolution (Fig. 4d, brown). For the remaining n=33 cells, we used the manually identified CS-on direction for all analyses.

The data sets that we analyzed were collected under two slightly different experimental protocols[8,9]. In all cases, we focused our analysis on the simple-spike related activity of the primary saccade rather than changes in activity due to adaptation or complex-spikes. For n=16 units, we presented primary targets in either the CS-on or CS-off direction and then pseudo-randomly modified the magnitude of the intra-saccadic step (ranging from -9° to +9°). Because the direction of intra-saccadic step (backwards, forwards, or no back-step) was randomized, the saccade to the presented target did not undergo adaptation. For the remaining cells, we asked the monkey to make primary saccades whose magnitude was either approximately 15° or 25°. Approximately 20 trials in both the CS-on and CS-off directions were presented without an intra-saccadic step (0°). After this block, we induced saccade adaptation in both the CS-on and CS-off directions by consistently stepping the target inwards (5-11°). We did not observe a qualitative difference between the population response for those saccades during the adaptation period and those during the stationary period (changes in eye velocity during adaptation coincided with changes in the magnitude of the population response). We therefore elected to use data from all saccades.

3. Population response

About 50 P-cells synapse onto a single neuron in the caudal fastigial nucleus[17]. The P-cells that project onto a single cFN neuron may be organized by their inputs from the inferior olive[18,30]. In this scenario, the olive projections divide the P-cells into clusters where each cluster of P-cells projects onto one cFN neuron. We therefore made the assumption that P-cells that projected onto a cFN neuron shared a critical feature: they had the same CS-on direction (Fig. 3A). We computed what a typical cFN neuron would receive from this population of P-cells by estimating a population response.

A recent study had shown that each simple-spike induced by the presynaptic firing of a P-cell influenced the post-synaptic cell in the deep cerebellar nucleus by producing an inhibition that had a 2.5ms time constant[17]. We therefore convolved the simple-spike train of each recorded P-cell with a normalized (i.e., integral of one) Gaussian of 2.5ms standard deviation. In all figures, we dissociate this quantity from the measurement of firing rate computed via the inverse of the inter-spike interval by using units of Hz to quantify firing rates, and units of spikes/second to quantify population response.

We calculated the population response by a bootstrapping procedure in which we sampled at random n=50 P-cells (with replacement) from our population of recorded neurons. In our recorded sample, we had roughly equal numbers of bursting (n=39) and pausing (n=33) cells. Therefore, in our bootstrapped population of 50 cells, we also had roughly equal numbers of bursting and pausing cells (27.1±1.9 bursting cells, mean±SD). As the bursting and pausing populations had different saccade-related

response profiles, standard error of the mean would result in a significant over-estimation of the variance of the population response that would be experienced at a nuclear neuron. We therefore elected to bootstrap 50 different populations of 50 randomly chosen P-cells. The standard error bars in these plots represent mean±SEM across bootstrapped samples.

4. Real-time prediction of motion of the eye

We organized the P-cells into clusters in which each cluster was composed of bursting and pausing cells (54% burst cells), all with a common CS-on direction (Fig. 3a), and then computed the population response, i.e., an estimate of the rate of simple spikes that converged onto a single cFN neuron during a saccadic eye movement. We found that when the saccade was in direction CS-off, the peak population response correlated near perfectly with peak saccade speed at R^2=0.98 (Fig. 3e). When the saccade was in direction CS-on, the peak population response also correlated near perfectly with peak saccade speed at R^2=0.99 ($p < 10^{-8}$) (Fig. 4b). A change in saccade direction altered the gain relating the population response to saccade speed (Fig. 4d): the gain was lowest when the saccade was in direction CS-on, and highest when the saccade was in direction CS-off. These results suggested that the P-cells, clustered by their CS-on direction, as a population produced simple spikes that were related to the real-time motion of the eye via a gain-field:

$$s(t) = \left|\dot{\mathbf{x}}(t+\Delta)\right|\left(a\cos(\theta-\theta_{CS})+b\right)+c \qquad \text{(S1)}$$

In this equation, $s(t)$ is the rate of simple spikes, $\left|\dot{\mathbf{x}}(t+\Delta)\right|$ is the magnitude of the eye velocity vector at time $t+\Delta$, a is a scaling factor, θ is saccade direction, θ_{CS} is direction of CS-off for that cluster of P-cells, and b and c are baseline offsets. The fit of this equation to the measured data (real-time motion of the eye vs. real-time population response, t = -100ms to +150ms with respect to peak saccade speed, for saccades in directions CS-on and CS-off, and peak speeds of 400-650 deg/s, binned by 25 deg/s) was highly significant (R^2=0.80, $p<10^{-5}$), resulting in the following parameter values: $a = 0.37$ spk/deg, $b = 1.88$ spk/deg, $c = 2588$ spk/s with a time lead of $\Delta = 19$ ms.

In contrast to organizing the P-cells into clusters and computing a population response, we also attempted to relate activity of single cells to motion of the eye. With this approach we found that the mean activity of the individual pause cells did not vary with saccade speed (Fig. 1d, p>0.20), and mean activity of the individual pause cells did not vary with saccade direction (Fig. E3a, p>0.4). Mean activity of the burst cells increased with saccade speed (Fig. 1d, $p<10^{-10}$), but mean activity of the burst cells did not

vary with direction (Fig. E3a, p>0.4). Therefore, consistent with previous reports[4,7], we could not detect an encoding of direction in the peak or mean activity of individual cells.

If the mean or peak response of the individual cells did not vary significantly with direction, how did the population response produce an encoding of direction (i.e., the cosine tuning)? We found that a change in saccade direction produced a subtle shift in the timing of the discharge in the pause cells (Fig. 4f). The pause in these cells occurred earlier when the saccade was in the CS-on direction, and later when the saccade was in the CS-off direction. In contrast, the burst timing was not dependent on saccade direction. As a result, the population response exhibited a smaller gain when the saccade was in direction CS-on, and a larger gain when the saccade was in direction CS-off (Fig. 4b).

We next asked how well the population of P-cells, clustered by their complex spike properties, predicted the real-time speed of the eye. According to Eq. (S1), if we consider the data across all directions, using the measured population response we should be able to predict the actual motion of the eye:

$$\left|\hat{\dot{\mathbf{x}}}(t+\Delta)\right| = \frac{1}{b}\left(s(t)-c\right) \qquad \text{(S2)}$$

In the above equation, $\left|\hat{\dot{\mathbf{x}}}(t+\Delta)\right|$ is the predicted real-time speed of the eye. The parameters *b* and *c* were identical to those determined in Eq. (S1).

Using Eq. (S2) we plotted the predicted real-time motion of the eye (Fig. E4A, saccades with peak speeds of 400, 525, and 650 deg/s). The predicted motion led the actual motion by +19ms, and was highly correlated with the actual motion (data for all saccades ranging from 400-650 deg/s, binned by 25 deg/s, R^2=0.74, $p < 10^{-5}$). Of particular importance was the fact that the predicted speed not only rose above baseline before saccade onset, it returned to below baseline before the end of the saccade. That is, the population response predicted in real-time the motion of the eye, and therefore could play a central role in controlling that motion, particularly in terminating the saccade.

We next asked whether individual neurons could predict the real-time speed of the eye. For each recorded cell, we fitted the parameters in Eq. (S2) for all saccades from 400-650 deg/s in 25 deg/s bins, leading to three parameter estimates for each neuron (*b, c*, and Δ). We found that after finding the best fit for each neuron, the predicted eye speed did not return to baseline until long after saccade termination (Fig. E4b). Across the neurons, the average delay was not significantly different than zero (two-sided t-test, p > 0.5), indicating that when we used individual cells, rather than the population, the predicted motion did not lead or lag the real-time actual motion of the eye. The mean squared error (MSE) for individual neurons was 245% (+/-10%) of the MSE achieved by the population response. That is,

the real-time motion of the eye predicted by individual neurons was much poorer than the population estimate.

We next considered whether a population composed entirely of either burst or pause cells could predict the real-time motion of the eye. We fitted Eq. (S2) to the mean activities of the burst and pause cells, and plotted the results in Figs. E4c and E4d. For the population composed entirely of burst cells, the predicted speed led the actual speed by +11ms, but critically did not return to baseline until long after saccade termination (Fig. E4c). The mean squared error for this bursting population was 146% of the MSE achieved by the population response composed of both bursting and pausing cells. A population consisting solely of pausing cells predicted that speed followed the actual speed by 9ms (that is, the best fit was a lag, not a lead). Similar to the exclusively bursting population, the predicted speed did not return to baseline until long after saccade termination (Fig. E4d). The mean squared error for pausing population was 162% of the MSE achieved by the population response which included all cells.

In summary, the population of P-cells, clustered by their CS-on direction, produced simple spikes that predicted the real-time motion of the eye. In this encoding, the speed and direction of the motion of the eye were multiplicatively encoded via a gain field, with the gain being largest for when the eye moved toward CS-off, and lowest for when the eye moved toward CS-on. Neither individual neurons, nor populations that exclusively included burst or pause cells, predicted the real-time speed of the eye with the accuracy of a population which combined these two cell types.

5. The importance of bursting and pausing cells in the population

The proportion of bursting and pausing cells in our data set were approximately equal (n = 39 bursting, n=33 pausing), resulting in 54% burst cells. However, two previous reports suggested that there may be a higher concentration of bursting cells in the OMV[4,5]. Both reports found that bursting neurons comprised approximately 70% of all cells in OMV. Therefore we asked if changes in the ratio of bursting to pausing P-cells would significantly alter our primary result that the population activity of OMV predicted eye velocity.

We performed the same analysis as in Fig. 3d, in which we estimated the population response, but rather than choosing 50 neurons randomly from our pool of 72 neurons, we always chose 35 bursting cells and 15 pausing cells (corresponding to 70% bursting in the population). We found that our primary result was robust to this modest change in ratio of bursting to pausing cells. That is, when 70% of the population was chosen to be bursting neurons, the peak response scaled linearly with the peak velocity of the saccade (R^2=0.94, $p<10^{-6}$). Importantly, the timing of the population response waveform remained

tightly coupled with saccade kinematics in that the response returned to near zero at saccade termination (Extended Data, Fig. 8).

However, this tight coupling of the population response and saccade speed disappeared if the population of P-cells was composed of a super majority of bursting or pausing cells. To illustrate this, we estimated what the population response by simulating populations that had differing proportions of burst and pause cells. The resulting population responses under the assumption that the population is composed entirely of bursting cells (100%), or 90% bursting, or 50% bursting, or 0% bursting (i.e., entirely pausing) are plotted in Extended Data Fig. 8. We found that as the membership within the population became highly skewed toward bursting or pausing, the population response no longer returned to zero at saccade termination. Therefore, the ability of the population to predict in real-time the speed of the eye was present only if the composition of the P-cells that converged onto a single cFN neuron (i.e., a P-cell cluster) included roughly equal number of pausing and bursting cells.

6. The importance of organizing the P-cells via their complex-spike properties

How critical is our assumption that each cluster is composed of P-cells that all share a similar complex-spike field? The traditional approach to measure population activity is to organize the cells based on their simple-spike activity, where for each cell the "preferred direction" is computed as the direction of action for which the cell shows the greatest simple-spike firing rate. This kind of analysis effectively assumes that the P-cells organize into clusters in which the cells share the same preferred direction of saccade, where preferred direction is measured via the rate of simple-spikes (Extended Data, Fig. 9a). Let us consider the consequences of this hypothesis.

We estimated the preferred direction of P-cells in two ways. First, we defined the preferred direction of each neuron as the direction which elicited the largest mean simple-spike firing rate during the saccade (max response). We then computed the population response for saccades of various speed (Extended Data, Fig. 9b). We found that the population response was no longer modulated by saccade speed ($p>0.3$).

Next, we calculated the preferred direction as the saccade direction in which there was the largest change in the magnitude of the simple-spike firing rate (max modulation). For bursting cells, this was the direction which elicited the largest mean simple-spike firing rate during the saccade. For pausing cells, this was the direction which featured the lowest simple-spike firing rate response (i.e., the direction of the largest pause). We then computed the population response for saccades of various speeds in this preferred direction (Extended Data, Fig. 9c). Again we found that the population response was not modulated by the speed of the saccade ($p>0.7$). Therefore, when we organized clusters of P-cells based on

their simple-spike responses rather than their complex-spike responses, the population response no longer encoded the speed of the saccade.

An important limitation is that our cells were recorded one at a time, yet in computing the population response we analyzed the data as if the cells were recorded simultaneously. Future work is needed to confirm our observations with simultaneous multi-cellular recordings.

Our results have broad implications regarding function and organization of the cerebellum. During saccades, the transformation of efference copy (via mossy fibers) into prediction of kinematic state, a concept termed a forward model, does not occur in the individual P-cells, but via combined activity of burst and pause P-cells that produce inhibition at the deep cerebellar nucleus. It is this inhibition produced by the combined activity of both groups of P-cells that predicts the motion of the eye during a saccade via a gain field, multiplicatively encoding speed and direction of movement. Therefore, our results demonstrate that the forward model, a theoretical concept central to conductance views of motor control, and often hypothesized to depend on the cerebellum[1,31,32], is represented during a saccade not in the activity of individual P-cells, but in the population activity that converges onto the cells in the deep cerebellar nuclei.

This encoding of movements is present only if there is a specific anatomical organization in the cerebellum: the projections from P-cells to nucleus neurons are not random, but likely organized by the complex-spike properties of the P-cells. That is, a nucleus cell receives projections from P-cells that share the same complex-spike field.

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