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Elimination of climbing fiber instructive signals during motor learning

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Abstract

The climbing fiber input to the cerebellum from the inferior olive is thought to act as a teacher whose activity controls the induction of motor learning. We designed training conditions that did not elicit instructive signals in the climbing fibers, but nevertheless induced robust and consistent motor learning in the vestibulo-ocular reflex of rhesus monkeys. Our results indicate that instructive signals in the climbing fibers are not necessary for cerebellum-dependent learning. Instead, instructive signals carried by either the climbing fibers or Purkinje cell simple spikes may be sufficient to induce motor learning, with additive effects occurring when both instructive signals are present during training.

To understand the algorithm a neural circuit uses to learn, one must determine how its patterns of activity during the induction of learning are translated into the cellular changes that encode memory. The cerebellum, which supports motor learning, is one brain region for which there is a well-developed theory about the neural events that induce plasticity during learning. The dominant theory over the last several decades has postulated that the climbing fiber input to the cerebellum from the inferior olive provides the neural instructive signals guiding cerebellum-dependent learning[1–3]. In support of this view, *in vivo* recordings have shown that climbing fiber activity signals errors during a number of different motor learning tasks[4,5]. Classic theory attributed cerebellum-dependent learning to a single form of climbing fiber–triggered plasticity in the cerebellar cortex[3,6], namely long-term depression at the synapses from parallel fibers onto Purkinje cells (cerebellar LTD; Fig. 1). More recent evidence suggests that multiple, distributed plasticity mechanisms contribute to cerebellum-dependent learning[7–15]. Nevertheless, climbing fiber–triggered plasticity is still widely viewed as being central to cerebellum-dependent learning[7,14,16].

Climbing fibers are positioned to control multiple plasticity mechanisms in the cerebellum and related circuitry. At the parallel fiber–to–Purkinje cell synapses, climbing fibers appear to control the induction of long-term potentiation (LTP) as well as LTD, as climbing fiber activity inhibits the induction of LTP[17,18]. Plasticity mechanisms at several additional types of synapses in the cerebellar cortex are also controlled by climbing fiber activity *in vitro* and *in vivo*[12,19–22]. Some investigators have suggested that motor learning requires changes in the deep cerebellar nuclei or vestibular nuclei[23], but such changes are generally viewed as being secondary to or dependent on climbing fiber–triggered changes in the cerebellar

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AUTHOR CONTRIBUTIONS

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cortex[7,14]. Thus, although cerebellar LTD is no longer considered to be the sole mechanism of cerebellum-dependent learning, instructive signals in the climbing fibers are still widely viewed as being central to learning. Here, we tested the necessity of the instructive signals carried by climbing fibers for motor learning and evaluated the contribution of other potential neural instructive signals.

Previous tests of the necessity of the climbing fibers for motor learning have been inconclusive. Lesion or pharmacological inactivation of the source of the climbing fibers, the inferior olive, abolishes cerebellum-dependent learning[24,25]. However, such manipulations abolish spontaneous activity in the climbing fibers as well as the task-related signals carried by changes in firing rate above and below the spontaneous level of activity. The elimination of spontaneous climbing fiber activity has the effect of producing abnormal neural activity at multiple sites in the cerebellar circuit[26,27]. Thus, the inability to learn after such manipulations cannot be directly attributed to the loss of instructive signals in the climbing fibers, but could simply reflect the gross cerebellar dysfunction associated with disrupted basal activity. To avoid this confound, we developed a behavioral approach to selectively eliminate instructive signals in the climbing fibers without affecting their baseline level of activity.

The behavioral task we used was the adaptive modification of the vestibulo-ocular reflex (VOR) by motor learning. The VOR is a reflexive eye movement that functions to stabilize images on the retina by generating eye movements in the opposite direction from head motion. If the VOR fails to stabilize images during head movements, motor learning can adjust the amplitude, or gain, of the VOR (that is, the ratio of eye velocity to head velocity) to restore image stability[8]. This form of motor learning requires the floccular complex of the cerebellum[28–32]. Signaling in the VOR circuit is understood at a level that enabled us to design training stimuli that would abolish instructive signals in the climbing fibers, while leaving intact most of the other aspects of the training conditions known to induce VOR learning.

Our results suggest that learning can be induced in the absence of instructive signals in the climbing fibers and that plasticity mechanisms controlled by other neural instructive signals make a substantial and independent contribution to motor learning. This climbing fiber–independent component of learning was correlated with the signals carried by the Purkinje cell simple spikes during training.

RESULTS

We analyzed the neural instructive signals available in the VOR circuit with standard visual-vestibular training stimuli used to increase or decrease VOR gain and variations of these stimuli designed to selectively eliminate the instructive signals. In the floccular complex of two rhesus monkeys, 102 Purkinje cells with task-related activity (head and/or eye movement sensitivity) were recorded. Our analysis focused on 58 of these cells, which were identified as horizontal gaze-velocity Purkinje cells (HGVPs), a subclass of neurons that have been implicated in VOR learning[33,34]. Spikes in a climbing fiber reliably trigger calcium spikes, called complex spikes, in its Purkinje cell targets in a one-to-one manner[35]; therefore, we used complex spike activity in a Purkinje cell as a measure of activity in its climbing fiber input, and we refer to it as a climbing fiber response. Complex spikes were well isolated in 48 of 58 HGVPs and 20 of 44 non-HGVPs. In each individual neuron recorded, we compared the responses to many different visual-vestibular training stimuli.

Climbing fiber instructive signals

In the laboratory, motor learning in the VOR is typically induced by pairing head movements with the motion of a single, large, coherently moving visual stimulus (Fig. 1b). Consistent with previous studies in primates and other species, the timing of peak climbing fiber activity relative to head motion discriminated between a '×2' visual-vestibular stimulus (see Online Methods), which induces an increase in VOR gain, versus a '×0' training stimulus, which induces a decrease in VOR gain[4,15,36], and thus carried information about whether the VOR gain needed to increase or decrease ($P < 0.05$; Fig. 2 and Table 1). During standard ×2 training, climbing fiber firing peaked during ipsiversive head movement; whereas climbing fiber firing peaked during contraversive head movement during standard ×0 training.

The climbing fiber responses were uniform across the population and reflected both an increased probability of firing during much of the 'preferred' half-cycle of the stimulus and a decreased probability of firing during much of the 'nonpreferred' half-cycle of the stimulus relative to the baseline spontaneous activity measured in the absence of head movement or visual stimulus movement (Fig. 2a–c). On the basis of the known physiology of the circuit, it has been proposed that these differently timed climbing fiber responses induce LTD and/or LTP in the appropriate vestibular parallel fiber–to–Purkinje cell synapses to support the observed changes in VOR gain[3,37] (Fig. 1; see Discussion).

To eliminate the climbing fiber responses during VOR training and thereby test their necessity for motor learning, we paired head movements with oppositely directed motion of a visual target and background. The origin of floccular climbing fibers is the dorsal cap of the inferior olive, which in turn receives its major input from the nucleus of the optic tract[38]. From what is known about the responses in the nucleus of the optic tract to visual motion[39,40], we expected that the effects of oppositely directed motion of the target and background might tend to cancel at the level of the inferior olive and this was confirmed by our recordings from the climbing fibers. When head movements were paired with target (T) motion and oppositely directed background (BG) motion (×0T/×2BG, ×2T/×0BG; see Online Methods for a more detailed description of the stimuli), the responses of the climbing fibers were greatly reduced compared with the responses in the same climbing fibers when the target and background moved together (Fig. 2 and Supplementary Fig. 1).

The reduction in climbing fiber response was greater for the ×0T/×2BG training stimulus than for the ×2T/×0BG stimulus. Each climbing fiber response was summarized by the amplitude of the overall firing-rate modulation during the stimulus cycle and the phase of the peak firing relative to head movement, calculated using a vector analysis (Fig. 2d). At the population level, the climbing fiber response to the ×2T/×0BG stimulus was significantly different from zero ($P < 0.05$, one sample *t* test; Table 1 and Supplementary Fig. 1). Therefore, this stimulus did not provide a good test of the necessity of climbing fiber instructive signals for motor learning. In contrast, the population response to the ×0T/×2BG stimulus was not significantly different from zero ($P > 0.05$, one sample *t* test; Table 1). Therefore, we conducted additional analyses to evaluate whether the climbing fiber response was truly eliminated during the ×0T/×2BG stimulus.

When the cycle-by-cycle variability of individual climbing fiber responses was considered, only 4 of 15 (monkey L) and 1 of 30 (monkey E) climbing fibers had significant responses to the ×0T/×2BG stimulus ($P < 0.05$; Fig. 2d). Moreover, the timing of peak firing was not consistent in these five climbing fibers with significant responses. Three climbing fibers increased their firing during ipsiversive head movement, as observed during ×2T/×2BG training, which increases VOR gain, and two climbing fibers increased their firing during

contraversive head movement, as observed during ×0T/×0BG training, which decreases VOR gain.

We considered the possibility that climbing fiber responses may be restricted to a very specific time in the stimulus cycle, but found no evidence for a temporally specific climbing fiber response to the ×0T/×2BG training stimulus. We compared the probability of climbing fiber firing during each 200-ms segment of the ×0T/×2BG stimulus cycle with spontaneous activity and with spike trains obtained by randomly shuffling the interspike intervals measured during the ×0T/32BG training stimulus to remove any potential signal (Fig. 2b,**c**). Because of natural variability in the climbing fiber interspike intervals, when spontaneous or shuffled activity was averaged across 2,000-ms 'stimulus cycles' (see Online Methods), there was, in each 200-ms time bin, a small percentage of the climbing fibers firing with a probability 2 s.d. above or below the mean (Fig. 2c). However, in each time bin, a similar percentage of climbing fibers had increased versus decreased firing, indicating that there was no signal carried by the population. In contrast, at any given time point during the ×0T/×0BG and ×2T/×2BG training stimuli, a larger percentage of climbing fibers fired 2 s.d. above or below baseline, and the percentage of climbing fibers with increased versus decreased firing was highly asymmetric, reflecting the signals carried by the population of climbing fibers during these standard stimuli. During the ×0T/×2BG training stimulus, climbing fiber activity was indistinguishable from spontaneous climbing fiber activity and shuffled spike trains (Fig. 2c). Furthermore, inspection of the raw climbing fiber spike trains revealed no evidence for a temporally specific response to the ×0T/×2BG training stimulus on a finer timescale or in a subset of trials (Supplementary Fig. 2).

Although the conflicting background motion during the ×0T/×2BG stimulus eliminated the climbing fiber responses, it did not affect the overall average firing rate. The average firing rate in the climbing fibers during the ×0T/×2BG training stimulus was the same as the average firing rate during spontaneous activity and during ×2T/×2BG and ×0T/×0BG training stimuli ($P > 0.05$, ANOVA; Supplementary Fig. 3). Thus, the ×0T/×2BG stimulus achieved selective abolition of instructive signals in the climbing fibers without affecting the baseline firing rate, as required to test the necessity of the instructive signals in the climbing fibers for learning.

Purkinje cell simple spike instructive signals

One candidate instructive signal that was previously proposed to guide motor learning in the VOR is the simple spike output of the Purkinje cells[23], and during the novel ×0T/×2BG stimulus, the Purkinje cell simple spikes carried robust signals that could potentially guide the induction of learning. As with the climbing fibers, the timing of peak simple spike activity relative to the head movement discriminated between the standard ×0T/×0BG and ×2T/×2BG training stimuli. Unlike the climbing fibers, the Purkinje cell simple spike activity also carried large, potentially useful instructive signals during ×0T/×2BG training (Fig. 3).

The simple spike activity of the Purkinje cells encodes both the vestibular input and the eye movements that the monkey makes to track the visual target[34]. The vestibular stimulus was the same across the experiments. The tracking eye movements were also similar during ×0T/×0BG and ×0T/×2BG training, as the motion of the visual target was the same. Therefore, the Purkinje cell simple spike responses that occurred during ×0T/×2BG training were indistinguishable from those that occurred during ×0T/×0BG training ($P > 0.19$, paired *t* test; Fig. 3 and Table 1), thus providing a way to assess the potential contribution of Purkinje cell simple spikes to the induction of learning in the absence of instructive signals in the climbing fibers.

Learning in the absence of climbing fiber signals

Despite the elimination of instructive signals in the climbing fibers, the ×0T/×2BG training stimulus induced consistent motor learning. In the two monkeys used for neural recordings, motor learning in the VOR was induced by presenting one of the training stimuli for 1 h (monkey E) or 2 h (monkey L). VOR learning was assessed by comparing the eye movement response to head movements in complete darkness before and after training. The standard ×2T/×2BG and ×0T/×0BG training stimuli induced increases and decreases in VOR gain, respectively (Fig. 4c). The ×0T/×2BG training stimulus also induced a significant decrease in VOR gain ($P < 0.001$, one sample t test; 4 of 4 training sessions in monkey L, 5 of 5 training sessions in monkey E; Fig. 4c), consistent with the instructive signals carried by the Purkinje cell simple spikes (Fig. 4b).

We recorded the neural responses and behavioral changes induced by additional training stimuli and found that stimuli that elicited similar responses in the climbing fibers could induce different changes in behavior, which were correlated with the simple spike responses that they elicited. For example, in monkey E, three training stimuli, ×0T/×2BG, ×0.5T/×1BG and ×1.5T/×0.5BG, each elicited no significant climbing fiber response ($P > 0.05$; Table 1 and Fig. 4a); however, the learned changes in VOR gain that they induced were different (Fig. 4c). Moreover, the changes in VOR gain were correlated with the simple spike responses during training (Fig. 4b,c); for these three stimuli, the biggest decreases in VOR gain occurred when the Purkinje cell simple spike responses were most similar to those during the standard ×0T/×0BG training stimulus.

Learning with various combinations of instructive signals

The observation of motor learning in the absence of instructive signals in the climbing fibers does not in any way exclude a contribution of climbing fiber–triggered plasticity mechanisms to VOR learning. We used an additional training stimulus, ×1T/×0BG, to isolate the climbing fiber contribution by eliminating the putative instructive signals in the Purkinje cell simple spikes. This stimulus drove the climbing fibers to respond in a manner similar to their response during ×0T/×0BG training, which induces a decrease in VOR gain (Fig. 4a,c). In contrast, the simple spike responses of the Purkinje cells were eliminated in monkey L and reversed in monkey E (Fig. 4b), so they were more similar to the response elicited by the ×2T/×2BG stimulus, which induces an increase in VOR gain. At the behavioral level, training with the ×1T/×0BG stimulus induced a decrease in VOR gain in both monkeys (Fig. 4c), as one might predict from the climbing fiber response.

Together, our results indicate that motor learning can occur when information about the required direction of learning is carried by both the climbing fibers and Purkinje cell simple spikes or when only one of these two instructive signals is available. To evaluate how instructive signals carried by the simple spikes and climbing fibers may interact to control the induction of learning, we recorded climbing fiber and Purkinje cell simple spike responses to many novel training stimuli (see Online Methods) and measured the effectiveness of each training stimulus at inducing VOR learning. These stimuli elicited different combinations of instructive signals in the climbing fibers and Purkinje cell simple spikes (Fig. 5a), which allowed us to analyze their individual contribution to the induction of learning.

For each pair of training stimuli, we calculated the difference in climbing fiber response (Δclimbing fiber), the difference in Purkinje cell simple spike response (Δsimple spike) and the difference in learning (Δlearning). If the climbing fibers provide instructive signals guiding learning, then the difference in learning induced by any pair of training stimuli should be related to the difference in the climbing fiber signals that they elicit during

training. Indeed, Δclimbing fiber was linearly correlated with Δlearning (Fig. 5b). We then divided all the stimulus pairs into three groups on the basis of the Δsimple spike value associated with each pair. For a given Δclimbing fiber, Δlearning systematically varied with Δsimple spike. Moreover, Δlearning was linearly correlated with Δsimple spike, particularly when the data were grouped according to Δclimbing fiber (Fig. 5c). This influence of both Δsimple spike and Δclimbing fiber on Δlearning is consistent with an independent contribution of both the climbing fibers and simple spikes to learning.

The contributions of the two putative neural instructive signals to learning were estimated by using the average correlation coefficients obtained from the pair-wise analyses (Fig. 5b,c) to predict the behavioral changes induced by each training stimulus (see Online Methods). When only the signals in the climbing fibers were considered, the predicted learning was well correlated with the observed learning; however, the amount of learning was underestimated by 30–40% (correlation coefficient = 0.68 in monkey L and 0.63 in monkey E; Fig. 5d). In contrast, when the signal in the simple spikes was used along with the signal in the climbing fibers, the predicted learning was very close to equal to the observed learning (correlation coefficient = 0.98 in monkey L and 1.02 in monkey E; Fig. 5d). Thus, a linear combination of climbing fiber and Purkinje cell simple spike instructive signals better accounted for motor learning in the VOR than climbing fiber signals alone.

DISCUSSION

VOR circuit physiology and the Marr-Albus-Ito model

Models of motor learning in the VOR are constrained by a great deal of information about how activity at each site in the circuit should affect the gain of the VOR. For the appropriate changes in Purkinje cell output to be accomplished by cerebellar LTD, as suggested by the influential Marr-Albus-Ito model of cerebellum-dependent learning[1–3], there should be selective depression of those parallel fibers that fire during ipsiversive head movement to increase the VOR gain, whereas a decrease in VOR gain would require selective depression of the parallel fibers that fire during contraversive head movement (Fig. 1b). Because climbing fiber activation can trigger LTD in parallel fibers active simultaneously[6], the responses present during the ×2T/×2BG and ×0T/×0BG training stimuli would be expected to trigger LTD in the appropriate vestibular parallel fibers to account for the observed changes in VOR gain (Figs. 1 and 2). In contrast, there is no reason to believe that the ×0T/×2BG training stimulus could induce selective LTD or LTP of the appropriate parallel fibers to produce the observed decrease in VOR gain. During ×0T/×2BG training, the climbing fibers fired with the same probability during ipsiversive and contraversive head movement (Fig. 2), making it equally likely that parallel fibers active during contraversive or ipsiversive head movements would undergo LTD. LTD of both groups of parallel fibers may decrease the average firing rate of the Purkinje cells, but should not cause the change in firing-rate modulation during head movements required to decrease the VOR gain. Moreover, if climbing fiber activity at the spontaneous rate is not effective at inducing LTD *in vivo*, then there may be no LTD at the parallel fiber–to–Purkinje cell synapses during ×0T/×2BG training.

Our experiments measured the neural instructive signals that were available to guide learning at the beginning of training, when the gain of the VOR was at baseline. Each training stimulus was presented for just 1–2 min, so that the responses of a single neuron to many stimuli could be compared. It is possible that climbing fiber responses to the training stimuli could emerge as learning progresses, even if no response was present at the beginning of training. However, most of the behavioral changes occurred early in the training session (Supplementary Fig. 4) and therefore cannot depend on any late-developing climbing fiber responses. Thus, even if climbing fibers did make a small contribution to

learning late in ×0T/×2BG training, it would have to be secondary to a climbing fiber–independent plasticity mechanism that drove the initial changes in the circuit.

Do other climbing fibers provide instructive signals?

During standard ×2T/×2BG and ×0T/×0BG training stimuli, the population of climbing fibers that we recorded carried robust signals, which were previously hypothesized to guide VOR learning. Therefore, our sample is drawn from the same population that has been previously implicated in VOR learning. However, these very same climbing fibers carried no instructive signals during our novel training stimuli (×0T/×2BG, ×0.5T/×1BG and ×1.5T/×0.5BG; Figs. 2 and 4).

It is unlikely that there is another population of unrecorded climbing fibers that carry signals during the training stimuli that elicited no responses in the recorded climbing fibers. In the floccular complex, we tested all of the Purkinje cells that we isolated with any task-related activity and found no significant response of their climbing fiber inputs during the ×0T/×2BG training stimulus ($P > 0.05$, one-sample t test; Supplementary Fig. 5). It is unlikely that climbing fibers in other parts of the cerebellum carry instructive signals to guide motor learning in the VOR. There is compelling evidence from several previous studies that VOR gain learning requires the floccular complex and not other regions of the cerebellum[30,31].

It is also unlikely that a subpopulation of the climbing fibers that we recorded can account for the decreases in VOR gain induced by the ×0T/×2BG stimulus. A subset of the individual climbing fibers (23 of 45) had small responses in the correct, ‘gain decrease’ direction during the ×0T/×2BG stimulus to account for the observed decrease in VOR gain; however, only 2 out of those 23 responses were significant ($P < 0.05$; Fig. 2d). Moreover, 22 of 45 climbing fibers had small responses in the incorrect, ‘gain increase’ direction, three of which were significant ($P < 0.05$). Thus, any effects of the climbing fibers with small gain decrease responses should be cancelled by the effects of the equal number of climbing fibers with small gain increase responses, unless the Purkinje cells receiving the gain decrease climbing fibers have unique properties that endow them with privileged control over the behavior, and there was no evidence for this. The neurons receiving small gain increase versus gain decrease instructive signals during ×0T/×2BG training were indistinguishable in all other respects (Supplementary Table 1 and Supplementary Fig. 6). Finally, the responses of the population of climbing fibers during ×0T/×2BG training were normally distributed, consistent with the responses being drawn from a single, uniform population rather than two distinct populations ($P = 0.26$, D’Agostino-Pearson test). Thus, variation around the mean climbing fiber response of zero during the ×0T/×2BG stimulus appears to be biological noise, rather than any kind of signal that could guide the induction of the observed behavioral changes.

Other instructive signals for cerebellar learning

When the instructive signals were eliminated from the climbing fibers, learning was correlated with the Purkinje cell simple spike responses present during training, suggesting that the signals carried by the simple spikes may contribute to the induction of learning. Purkinje cell simple spikes have previously been proposed as an instructive signal for motor learning[23]. Purkinje cells are well positioned to control the induction of plasticity in their main target, the vestibular nuclei or deep cerebellar nuclei, and there is evidence for changes in the vestibular nuclei/deep cerebellar nuclei during learning[7,10,11,41,42].

We cannot rule out the possibility that other neural instructive signals, in addition to those carried by climbing fibers and Purkinje cell simple spikes, could contribute to the induction of learning. In particular, the mossy fiber pathways upstream of the Purkinje cell simple

spikes must, together, carry the same information as the simple spikes. However, it is not the simple spike activity in isolation, but the timing of simple spike activity relative to head movement that correlated with the behavioral changes. Therefore, the observed learning cannot be readily explained by a nonassociative plasticity mechanism that depends only on signals carried by the simple spikes or upstream pathways (for example, LTP or LTD that depends only on parallel fiber activity)[43],[44]. In contrast, in the vestibular nucleus, there is a convergence of simple spike and vestibular signals (Fig. 1a), making it plausible that the correlation of activity in these two inputs could induce synaptic changes in the vestibular nucleus that decrease or increase VOR gain. Plasticity at sites outside the cerebellar cortex has generally been viewed as secondary to LTD at the parallel fiber–to–Purkinje cell synapses[7],[10],[14] or dependent on cerebellar LTD for its appropriate expression[45]. However, our results suggest that instructive signals carried by Purkinje cell simple spikes may induce learning in the absence of any climbing fiber–triggered plasticity.

Multiple instructive signals for cerebellar learning

Our results suggest that neither instructive signals carried by the climbing fibers nor instructive signals carried by the Purkinje cell simple spikes are necessary for motor learning. Instead, each of these neural instructive signals may operate in parallel, with each being capable of inducing learning in the other's absence, thus imparting the cerebellum with distinct, independent ways to control the induction of motor learning. For the set of training stimuli used in this study, the contribution of the climbing fibers to the induction of learning appeared to be greater, on average, than the contribution of the Purkinje cell simple spikes. The climbing fiber responses during training accounted for 60–70% of the observed learning, whereas the simple spike responses accounted for another 30–40% (Fig. 5d). However, the training stimuli that we used elicited climbing fiber responses that covered much of their physiological range (±1 spikes per s), but relatively modest simple spike responses (about ± 20 spikes per s) compared with their physiological range of at least ±40–50 spikes per s. Therefore, the climbing fiber contribution to learning measured using the set of stimuli in this study may be near maximal, whereas training stimuli that drive bigger simple spike responses may be able to recruit a bigger simple spike–triggered component of learning.

When instructive signals are carried by both the climbing fibers and Purkinje cell simple spikes during training, their effects appear to sum linearly, suggesting that climbing fiber and simple spike instructive signals operate independently and in parallel during the induction of learning. The independent operation of multiple instructive signals offers at least three advantages. First, it provides more than one way to achieve a similar behavioral outcome. The learning induced by ×0T/×2BG training and ×1T/×0BG training was similar at the behavioral level; in each case, there was a decrease in VOR gain. However, different neural instructive signals are available during these two training stimuli. Therefore, the underlying memory traces may be quite different, potentially involving distinct locations in the circuit. The use of different plasticity mechanisms may influence behavioral properties of the memory, such as its resistance to forgetting and the extent to which it generalizes to conditions different from those occurring during training. Second, the use of multiple instructive signals could allow learning to occur under a broader range of conditions because each neural instructive signal encodes different aspects of the learning environment, with the climbing fibers being more sensitive to motion of the visual background and the Purkinje cells being more sensitive to the visually driven eye movements made during training. Under natural viewing conditions, discrepant motion of the background visual stimuli versus the visual target and eye movement is common, because of motion parallax. Third, when multiple instructive signals are recruited, more learning is induced. Thus, to optimize motor

learning in clinical or other settings, one should design the training environment to recruit each of the available instructive signals.

METHODS

General procedures

Experiments were conducted on two male rhesus monkeys trained to perform a visual fixation task to obtain liquid reinforcement. Previously described surgical procedures were used to implant orthopedic plates for restraining the head[42,46], a coil of wire in one eye for measuring eye position[47] and a stereotaxically localized recording cylinder. During experiments, each monkey sat in a specially designed primate chair to which his implanted head holder was secured. Vestibular stimuli were delivered using a servo-controlled turntable (Ideal Aerosmith) that rotated the monkey, the primate chair and a set of magnetic coils (CNC Engineering) together about an earth-vertical axis. Visual motion stimuli were provided by a visual target subtending 0.5° of visual angle, which the monkey was rewarded for tracking, and a 20° × 30° visual background consisting of a high-contrast black and white checkerboard pattern. The visual stimuli were reflected off mirror galvanometers onto the back of a tangent screen 114 cm in front of the eyes. All surgical and behavioral procedures conformed to guidelines established by the US Department of Health and Human Services (US National Institutes of Health) Guide for the Care and Use of Laboratory Animals as approved by Stanford University.

Behavioral experiments

Motor learning in the VOR was induced by presenting combined visual-vestibular stimuli for 1 h (monkey E) or 2 h (monkey L). VOR performance was tested before and after training and at 15-min (monkey E) or 30-min intervals (monkey L) during training by delivering the vestibular stimulus in total darkness. The vestibular stimulus used to measure the VOR and induce learning had a sinusoidal velocity profile (0.5 Hz, peak velocity ± 10° per s, or in a few cases, where noted, ± 20° per s).

The visual-vestibular training stimuli are described by the eye velocity gain (relative to head movement) required to stabilize the image of the target (T) and the background (BG) on the retina. If the target moved exactly with the head, the training stimulus is described as ×0T, as the VOR gain required to stabilize the image of the target on the retina is zero. If the visual target moved at the same speed as the head, but 180° out of phase with the head, then the VOR gain required to stabilize the target on the retina was 2 (eye speed equal to twice head speed), and the stimulus was described as ×2T. During ×0.5T and ×1.5T training stimuli, the target moved in phase or 180° out of phase with the head, respectively, at one-half the head speed. During training stimuli with ×1T, the visual target was earth-stationary.

With the exception of two experiments conducted in monkey L (×0T only and ×2T only), the visual stimulus included a visual background that either moved together with the target or independently. During the ×0T/×0BG, ×2T/×2BG, ×0.5T/×0.5BG and ×1.5T/×1.5BG stimuli, the visual background moved exactly with the target. During ×0T/×2BG, ×0.5T/×1.5BG, ×1.5T/×0.5BG and ×2T/×0BG stimuli, the visual background moved at the same speed as the target, but was 180° out of phase with target motion. During the ×0T/×1BG, ×0.5T/×1BG, ×1.5T/×1BG and ×2T/×1BG stimuli, the visual background was earth-stationary. During the ×0T/×0.5BG and ×2T/×1.5BG stimuli, the background moved at one-half the speed of the target and was in-phase with target motion. During the ×0.5T/×0BG and ×1.5T/×2BG stimuli, the background moved at twice the speed and was in phase with target motion. For the ×1T/×0BG and ×1T/×2BG stimuli, the background moved at the same speed as the head and was in phase or 180° out of phase with the head motion, respectively.

Experiments were separated by at least 24 h to allow the gain of the VOR to readapt to its normal value before the next experiment. In each monkey, there were a minimum of three replications of the behavioral experiments for each training stimulus.

Electrophysiology

Tungsten electrodes (FHC, Microprobe) were used to make extracellular recordings from Purkinje cells in the floccular complex of the cerebellum, comprising the cerebellar flocculus and ventral paraflocculus. After a Purkinje cell was isolated, its sensitivity to eye velocity and head velocity were first measured by recording its responses during smooth pursuit eye movements evoked by horizontal motion of the visual target with a sinusoidal velocity profile at a frequency of 0.5 Hz and a peak velocity of 20° per s or greater and as the monkey cancelled his VOR by tracking a visual target that moved exactly with sinusoidal head rotation about an earth-vertical axis at 0.5 Hz and at a peak velocity of 20° per s or greater. Purkinje cells were classified as HGVPs if the simple spike firing rate was modulated by at least ± 0.3 spikes per s per degree per s and there was a phase difference of less than 45° between peak firing rate and peak ipsiversive eye velocity during horizontal smooth pursuit eye movements, and if the simple spike firing rate was modulated by at least ± 0.3 spikes per s per deg per s and the phase difference between peak firing rate and peak ipsiversive head velocity was less than 45° during cancellation of the VOR[34,36].

We compared the instructive signals carried by the same neuron during several different training stimuli used to induce motor learning in the VOR. Each training stimulus was presented for 60–90 s. Recordings were made when the gain of the VOR was at baseline and the training stimuli were not presented long enough to induce measurable changes in VOR performance as measured in the dark.

Data analysis

Voltages related to the position and velocity of eye, head and visual stimulus were recorded during the experiments at 500 Hz per channel. Eye velocity records were edited to remove the rapid deflections caused by saccades. The data were then analyzed by aligning stimulus cycles on head or target velocity and averaging. Most averages contained ten or more cycles and analyses were limited to cycles for which gaze position was within 15 deg of straight-ahead gaze. Average eye and head velocity traces were subjected to a sines fit. The gain of the VOR was calculated as the ratio of peak eye velocity to peak head velocity derived from the fitted sinusoidal functions.

The simple-spike activity of Purkinje cells was detected with a hardware window discriminator and the times of the resulting pulses were recorded to the nearest 10 μs. In addition, unit activity was sampled at 50 kHz, and complex spikes were discriminated using off-line spike sorting with time and amplitude windows or template matching algorithms (Spike2, Cambridge Electronic Design). In addition, the occurrence of each complex spike was confirmed by visual inspection of the raw traces by the investigators.

Data analysis was performed in Matlab (Mathworks) and Excel (Microsoft). The simple spike data were analyzed after the experiment by aligning the records on head velocity or visual stimulus position. The amplitude of firing-rate modulation and phase of the simple spike responses relative to peak contraversive head velocity were estimated as the amplitude and phase of the fundamental components provided by Fourier analysis of the averages.

Because of firing rate cutoff, the climbing fiber responses were not always well described by a sinusoid. Therefore, to quantify climbing fiber responses during training stimuli, complex spike data were analyzed using a vector analysis. Stimulus cycles were aligned on head velocity and averaged. The stimulus cycle was divided into 1,000 equal bins. Each time bin

was represented as a vector, with the magnitude of the vector being equal to the average firing rate in that bin and the phase being determined by the phase of the bin relative to peak ipsiversive head velocity. The phase and amplitude of the climbing fiber response were calculated as the phase and one-half the amplitude of the vector sum.

The component of the climbing fiber response or simple spike response aligned with peak head velocity was calculated by multiplying the amplitude of the response with the cosine of the phase and these values were used for statistical analysis. The significance of each neural response was determined by performing the vector analysis on each cycle of head movement during a given training stimulus. Significance was tested using a one-sample *t* test.

To test for temporally restricted climbing fiber responses, we divided the stimulus cycle into ten 200-ms epochs and calculated the average probability of a complex spike for each epoch. For spontaneous and shuffled control conditions, climbing fiber responses were divided into 2,000-ms trials aligned to the onset of recording and the same analysis was performed.

The baseline probability of climbing fiber firing was estimated from all recordings of spontaneous activity in climbing fibers. Spike trains of spontaneous activity were divided into 200-ms bins and the probability of complex spike firing was calculated from a random selection, without replacement, of 35 200-ms bins (the typical number of bins used to calculate the firing probability during a training stimulus). This measure was repeated to derive the mean and s.d. used as the baseline probability.

The pair-wise analysis in Figure 5 was based on the neural and behavioral responses to 16 training stimuli for monkey L and 17 training stimuli for monkey E. For each training stimulus, the average response in the climbing fibers and Purkinje cell simple spike populations was used. For learning, the median value of the behavioral replications was used. For each pair of training stimuli, Δsimple spike, Δclimbing fiber and Δlearning were calculated.

To assess the contribution of climbing fiber signals to learning (Fig. 5b), we subtracted the neural responses and learning induced by the two training stimuli in each pair in the order that yielded a positive Δclimbing fiber value, where positive Δclimbing fiber was defined as a bigger increase (or smaller decrease) in firing during ipsiversive head movements. The training stimulus pairs were then grouped into three bins according to their Δsimple spike values: stimulus pairs with Δsimple spike above 33.3% of the maximum absolute Δsimple spike value in the set of stimulus pairs, stimulus pairs with Δsimple spike between −33.3% and 33.3% of the maximum, and stimulus pairs with Δsimple spike below −33.3% of the maximum. A linear regression was performed on Δlearning and Δclimbing fiber for the stimulus pairs in each bin. The correlation coefficients obtained from the three bins were averaged and this value (C_{CF}) was used to estimate the climbing fiber contribution to learning.

To assess the contribution of simple spike signals to learning (Fig. 5c), we subtracted the neural responses and learning induced by the training stimuli in each pair in the order that yielded a positive Δsimple spike value, where positive Δsimple spike was defined as a bigger increase or smaller decrease in firing during contraversive head movements. The training stimulus pairs were then grouped into three bins according to their Δclimbing fiber values: stimulus pairs with Δclimbing fiber above 33.3% of the maximum absolute Δclimbing fiber value, stimulus pairs with Δclimbing fiber between −33.3% and 33.3% and stimulus pairs with Δclimbing fiber below −33.3% of the maximum. A linear regression was performed on Δlearning and Δsimple spike for the stimulus pairs in each bin. The correlation coefficients

obtained from the three bins were averaged and this value (C_{SS}) was used to estimate the Purkinje cell simple spike contribution to learning.

The coefficients, C_{CF} and C_{SS}, derived from the pairwise analysis were used to predict the amount of learning induced by each training stimulus. To predict the amount of learning on the basis of only the climbing fiber instructive signals present during each training stimulus (Fig. 5d), we used the following equation:

$$L_{\mathrm{CF}}(\mathrm{stimulus}) = C_{\mathrm{CF}} \times CF_{\mathrm{measured}}(\mathrm{stimulus})$$

where L_{CF}(stimulus) is the predicted learning for the given training stimulus and $CF_{measured}$(stimulus) is the measured climbing fiber response during that stimulus.

To predict the amount of learning based on both Purkinje cell simple spike and climbing fiber responses (Fig. 5d), we calculated the amount of learning predicted from each signal and then summed:

$$L_{\mathrm{total}}(\mathrm{stimulus}) = C_{\mathrm{CF}} \times CF_{\mathrm{measured}}(\mathrm{stimulus}) + C_{\mathrm{ss}} \times SS_{\mathrm{measured}}(\mathrm{stimulus})$$

where $SS_{measured}$(stimulus) is the measured simple spike response during the training stimulus. The regression analysis was constrained to pass through the origin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Albus J. A theory of cerebellar function. Math Biosci. 1971; 10:25–61.
2. Marr D. A theory of cerebellar cortex. J Physiol (Lond). 1969; 202:437–470. [PubMed: 5784296]
3. Ito M. Neural design of the cerebellar motor control system. Brain Res. 1972; 40:81–84. [PubMed: 4338265]
4. Simpson JI, Alley KE. Visual climbing fiber input to rabbit vestibulo-cerebellum: a source of direction-specific information. Brain Res. 1974; 82:302–308. [PubMed: 4441896]
5. Gilbert PF, Thach WT. Purkinje cell activity during motor learning. Brain Res. 1977; 128:309–328. [PubMed: 194656]
6. Ito M, Kano M. Long-lasting depression of parallel fiber-Purkinje cell transmission induced by conjunctive stimulation of parallel fibers and climbing fibers in the cerebellar cortex. Neurosci Lett. 1982; 33:253–258. [PubMed: 6298664]
7. Shutoh F, et al. Memory trace of motor learning shifts transsynaptically from cerebellar cortex to nuclei for consolidation. Neuroscience. 2006; 139:767–777. [PubMed: 16458438]
8. Boyden ES, Katoh A, Raymond JL. Cerebellum-dependent learning: the role of multiple plasticity mechanisms. Annu Rev Neurosci. 2004; 27:581–609. [PubMed: 15217344]
9. Boyden ES, et al. Selective engagement of plasticity mechanisms for motor memory storage. Neuron. 2006; 51:823–834. [PubMed: 16982426]

10. De Zeeuw CI, Yeo CH. Time and tide in cerebellar memory formation. Curr Opin Neurobiol. 2005; 15:667–674. [PubMed: 16271462]
11. Lisberger SG, Sejnowski TJ. Motor learning in a recurrent network model based on the vestibulo-ocular reflex. Nature. 1992; 360:159–161. [PubMed: 1436091]
12. Jörntell H, Ekerot CF. Reciprocal bidirectional plasticity of parallel fiber receptive fields in cerebellar Purkinje cells and their afferent interneurons. Neuron. 2002; 34:797–806. [PubMed: 12062025]
13. Lisberger SG. Neural basis for motor learning in the vestibuloocular reflex of primates. III. Computational and behavioral analysis of the sites of learning. J Neurophysiol. 1994; 72:974–998. [PubMed: 7983549]
14. Medina JF, et al. Mechanisms of cerebellar learning suggested by eyelid conditioning. Curr Opin Neurobiol. 2000; 10:717–724. [PubMed: 11240280]
15. Hirata Y, Highstein SM. Acute adaptation of the vestibuloocular reflex: signal processing by floccular and ventral parafloccular Purkinje cells. J Neurophysiol. 2001; 85:2267–2288. [PubMed: 11353040]
16. Jirenhed DA, Bengtsson F, Hesslow G. Acquisition, extinction and reacquisition of a cerebellar cortical memory trace. J Neurosci. 2007; 27:2493–2502. [PubMed: 17344387]
17. Coesmans M, et al. Bidirectional parallel fiber plasticity in the cerebellum under climbing fiber control. Neuron. 2004; 44:691–700. [PubMed: 15541316]
18. Lev-Ram V, et al. Reversing cerebellar long-term depression. Proc Natl Acad Sci USA. 2003; 100:15989–15993. [PubMed: 14671315]
19. Rancillac A, Crepel F. Synapses between parallel fibres and stellate cells express long-term changes in synaptic efficacy in rat cerebellum. J Physiol (Lond). 2004; 554:707–720. [PubMed: 14617674]
20. Shinoda Y, et al. The entire trajectory of single climbing and mossy fibers in the cerebellar nuclei and cortex. Prog Brain Res. 2000; 124:173–186. [PubMed: 10943124]
21. Evans GJ. Synaptic signaling in cerebellar plasticity. Biol Cell. 2007; 99:363–378. [PubMed: 17567263]
22. Mittmann W, Hausser M. Linking synaptic plasticity and spike output at excitatory and inhibitory synapses onto cerebellar Purkinje cells. J Neurosci. 2007; 27:5559–5570. [PubMed: 17522301]
23. Miles FA, Lisberger SG. Plasticity in the vestibulo-ocular reflex: a new hypothesis. Annu Rev Neurosci. 1981; 4:273–299. [PubMed: 6784658]
24. Haddad GM, Demer JL, Robinson DA. The effect of lesions of the dorsal cap of the inferior olive on the vestibulo-ocular and optokinetic systems of the cat. Brain Res. 1980; 185:265–275. [PubMed: 6965604]
25. Mintz M, et al. Unilateral inferior olive NMDA lesion leads to unilateral deficit in acquisition and retention of eyelid classical conditioning. Behav Neural Biol. 1994; 61:218–224. [PubMed: 8067977]
26. Zbarska S, Bloedel JR, Bracha V. Cerebellar dysfunction explains the extinction-like abolition of conditioned eyeblinks after NBQX injections in the inferior olive. J Neurosci. 2008; 28:10–20. [PubMed: 18171918]
27. Colin F, Manil J, Desclin JC. The olivocerebellar system. I. Delayed and slow inhibitory effects: an overlooked salient feature of cerebellar climbing fibers. Brain Res. 1980; 187:3–27. [PubMed: 7357475]
28. Rambold H, et al. Partial ablations of the flocculus and ventral paraflocculus in monkeys cause linked deficits in smooth pursuit eye movements and adaptive modification of the VOR. J Neurophysiol. 2002; 87:912–924. [PubMed: 11826056]
29. Robinson DA. Adaptive gain control of vestibuloocular reflex by the cerebellum. J Neurophysiol. 1976; 39:954–969. [PubMed: 1086347]
30. Torte MP, et al. Anatomical segregation of different adaptative processes within the vestibulocerebellum of the cat. Exp Brain Res. 1994; 99:441–454. [PubMed: 7957724]
31. Ito M, Jastreboff PJ, Miyashita Y. Specific effects of unilateral lesions in the flocculus upon eye movements in albino rabbits. Exp Brain Res. 1982; 45:233–242. [PubMed: 7056329]

32. Cohen H, et al. Habituation and adaptation of the vestibuloocular reflex: a model of differential control by the vestibulocerebellum. Exp Brain Res. 1992; 90:526–538. [PubMed: 1426111]
33. Miles FA, et al. Long-term adaptive changes in primate vestibuloocular reflex. III. Electrophysiological observations in flocculus of normal monkeys. J Neurophysiol. 1980; 43:1437–1476. [PubMed: 6768853]
34. Lisberger SG, Fuchs AF. Role of primate flocculus during rapid behavioral modification of vestibuloocular reflex. I. Purkinje cell activity during visually guided horizontal smooth-pursuit eye movements and passive head rotation. J Neurophysiol. 1978; 41:733–763. [PubMed: 96225]
35. Eccles JC, Llinas R, Sasaki K. The excitatory synaptic action of climbing fibres on the purinje cells of the cerebellum. J Physiol (Lond). 1966; 182:268–296. [PubMed: 5944665]
36. Raymond JL, Lisberger SG. Neural learning rules for the vestibulo-ocular reflex. J Neurosci. 1998; 18:9112–9129. [PubMed: 9787014]
37. Ito M, Nisimaru N, Yamamoto M. Specific patterns of neuronal connexions involved in the control of the rabbit's vestibulo-ocular reflexes by the cerebellar flocculus. J Physiol (Lond). 1977; 265:833–854. [PubMed: 300801]
38. Maekawa K, Takeda T. Origin of descending afferents to the rostral part of dorsal cap of inferior olive which transfers contralateral optic activities to the flocculus. A horseradish peroxidase study. Brain Res. 1979; 172:393–405. [PubMed: 476490]
39. Ilg UJ, Hoffmann KP. Responses of neurons of the nucleus of the optic tract and the dorsal terminal nucleus of the accessory optic tract in the awake monkey. Eur J Neurosci. 1996; 8:92–105. [PubMed: 8713453]
40. Mustari MJ, Fuchs AF. Discharge patterns of neurons in the pretectal nucleus of the optic tract (NOT) in the behaving primate. J Neurophysiol. 1990; 64:77–90. [PubMed: 2388076]
41. Ohyama T, et al. Learning-induced plasticity in deep cerebellar nucleus. J Neurosci. 2006; 26:12656–12663. [PubMed: 17151268]
42. Lisberger SG, Pavelko TA, Broussard DM. Neural basis for motor learning in the vestibuloocular reflex of primates. I. Changes in the responses of brain stem neurons. J Neurophysiol. 1994; 72:928–953. [PubMed: 7983547]
43. Han VZ, et al. Synaptic plasticity and calcium signaling in Purkinje cells of the central cerebellar lobes of mormyrid fish. J Neurosci. 2007; 27:13499–13512. [PubMed: 18057208]
44. Hartell NA. Strong activation of parallel fibers produces localized calcium transients and a form of LTD that spreads to distant synapses. Neuron. 1996; 16:601–610. [PubMed: 8785057]
45. Medina JF, Garcia KS, Mauk MD. A mechanism for savings in the cerebellum. J Neurosci. 2001; 21:4081–4089. [PubMed: 11356896]
46. Raymond JL, Lisberger SG. Behavioral analysis of signals that guide learned changes in the amplitude and dynamics of the vestibulo-ocular reflex. J Neurosci. 1996; 16:7791–7802. [PubMed: 8922435]
47. Robinson DA. A method of measuring eye movement using a scleral search coil in a magnetic field. IEEE Trans Biomed Eng. 1963; 10:137–145. [PubMed: 14121113]

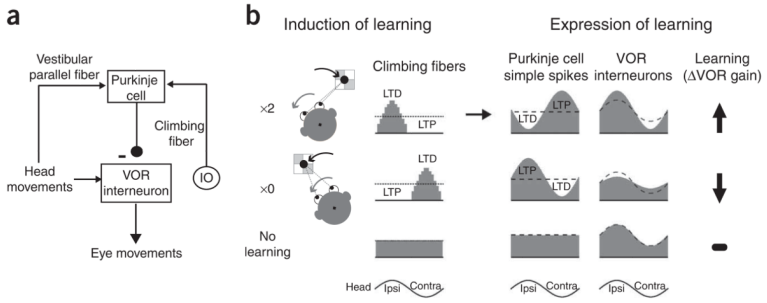


Figure 1.

VOR circuit and Marr-Albus-Ito hypothesis for VOR learning. (**a**) VOR circuit. The climbing fiber input to the Purkinje cells originates from the inferior olive (IO), carries visual signals and is thought to control the induction of plasticity at the vestibular parallel fiber–Purkinje cell synapses. (**b**) Marr-Albus-Ito hypothesis. To induce VOR learning, we paired head movements with a visual stimulus that moves exactly opposite the head (×2) or with the head (×0), which drives climbing fiber responses with peak firing during ipsiversive (×2) or contraversive (×0) head movement, respectively. During training (induction of learning), increases in climbing fiber activity above baseline (dotted line) should induce LTD in the vestibular parallel fibers that are simultaneously active. During subsequent testing with head movements in total darkness (expression of learning), the LTD induced by ×2 training should alter Purkinje cell simple spike output during head movements so that this inhibition is more out-of-phase with the activity of the VOR interneurons, thereby increasing the response of the interneurons and the gain of the VOR (histograms, post-training responses; dashed traces, pre-training). In contrast, the LTD induced by ×0 training should cause Purkinje cells to fire more in-phase with VOR interneurons, thereby decreasing the VOR gain. The decrease in climbing fiber activity below baseline during the induction of learning may also induce LTP of parallel fibers firing during the corresponding phase of head movement, which would complement the effects of LTD on interneuron response amplitude.

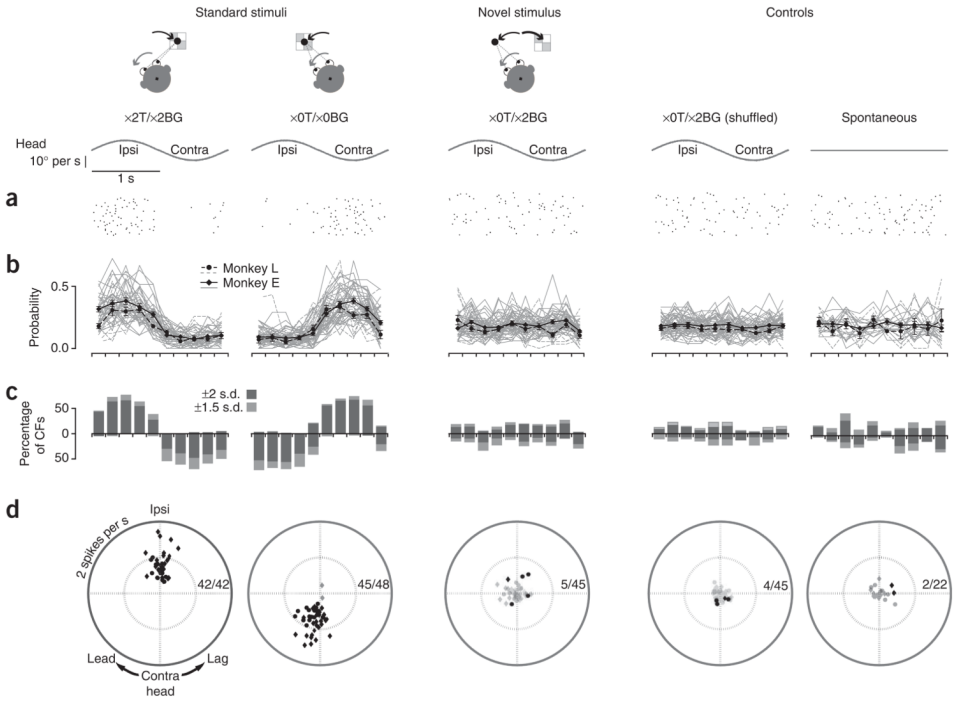


Figure 2.

Climbing fiber responses to standard and novel training stimuli. (**a**) Rasters of activity in a single climbing fiber during ~35 cycles of the standard visual-vestibular training stimuli (left), with coherent motion of a visual target (T) and background (BG) during the head movement; or a novel training stimulus (middle), with opposite motion of T and BG (×0T/×2BG). Right, spike trains generated by randomly shuffling interspike intervals measured during the ×0T/×2BG stimulus and spontaneous activity in the absence of head or image motion. (**b**) Probability of climbing fiber (CF) firing during 200-ms epochs of the stimuli in individual climbing fibers (gray lines) and the population of climbing fibers (black lines) recorded in monkeys L (circles) and E (diamonds). Error bars indicate s.e.m. (**c**) Percent of climbing fibers whose probability of firing was 1.5 (light gray) or 2 (dark gray) s.d. above or below spontaneous for each 200-ms epoch of the stimulus cycle. (**d**) Amplitude and phase of climbing fiber firing rate modulation. Distance from the origin represents the amplitude of a climbing fiber response and phase represents the timing of peak firing relative to head velocity. Responses significantly different from zero ($P < 0.05$) are represented by black symbols and the number of climbing fibers with significant responses out of the total recorded is noted on each plot. Climbing fiber responses with peak firing during ipsiversive head velocity, which are typically associated with VOR gain increases, are plotted above the horizontal axis. Clockwise rotation represents increased phase lead.

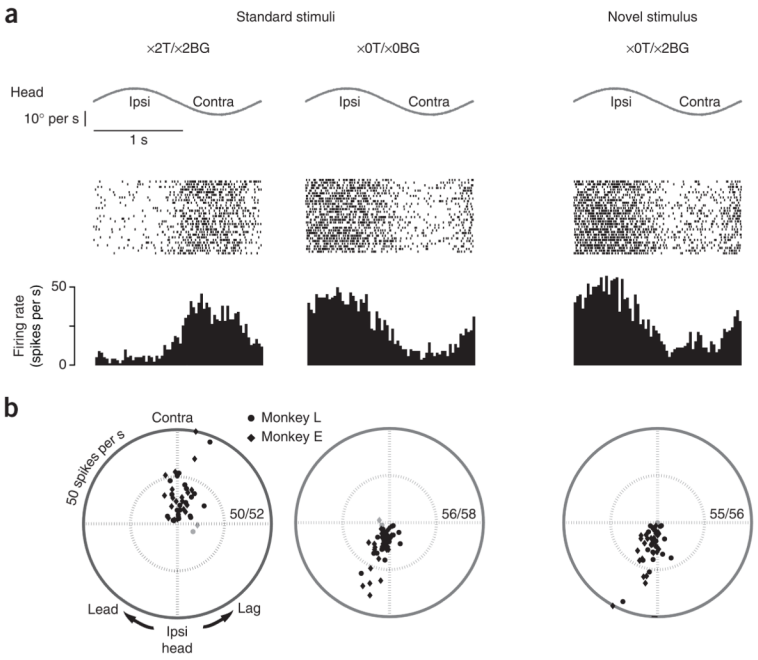


Figure 3.

Purkinje cell simple spike responses during training. (**a**) Individual Purkinje cell. Rasters and peristimulus time histograms of simple spike activity, aligned on the onset of ipsiversive head movement. (**b**) Purkinje cell population. Polar plots summarizing the amplitude and phase of simple spike firing rate modulation in each Purkinje cell. Note that the axis is rotated, compared with Figure 2d, so that simple spike responses with peak activity during contraversive head velocity plot above the horizontal axis, as such responses are typically associated with increases in VOR gain. Black symbols represent responses that were significantly different from zero ($P < 0.05$).

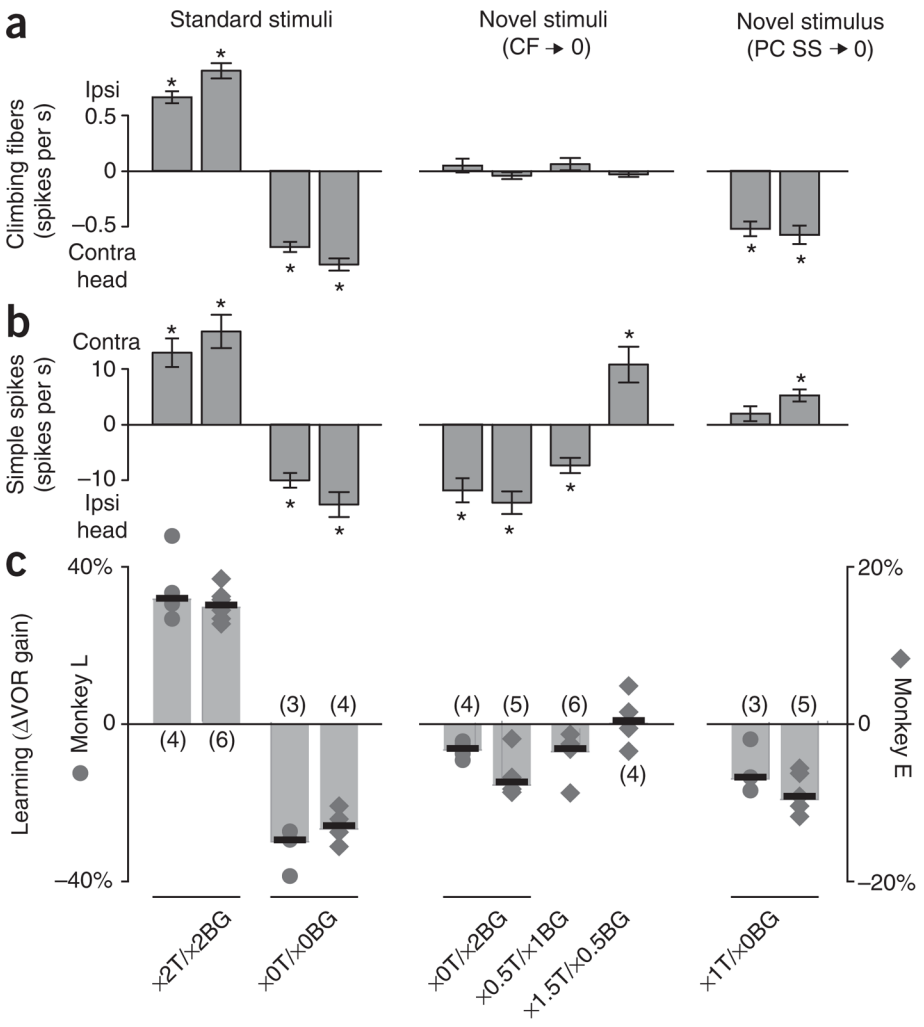
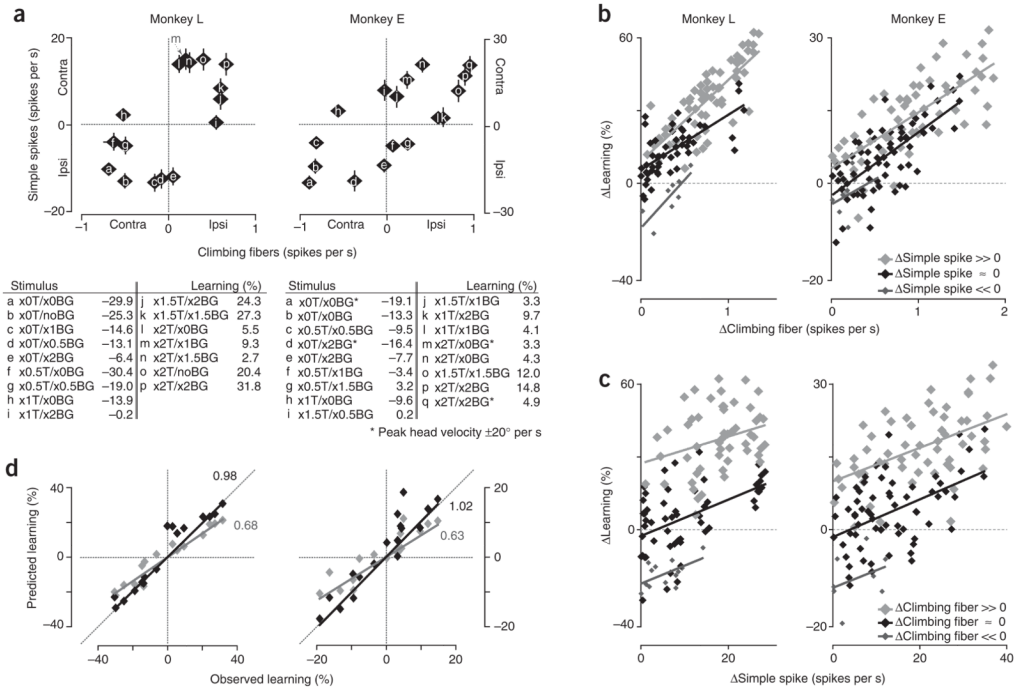


Figure 4.

In the absence of instructive signals in the climbing fibers, learning was correlated with simple spike responses during training. (**a**) Climbing fiber responses. Bars represent the component of the climbing fiber response aligned with peak head velocity during standard training stimuli (×2T/×2BG, ×0T/×0BG), novel training stimuli that elicited no significant climbing fiber response (×0T/×2BG, ×0.5T/×1BG, ×1.5T/×0.5BG) and a novel training stimulus that elicited no significant Purkinje cell response (×1T/×0BG). * $P < 0.05$, one sample *t* test. (See Supplementary Fig. 7 for the responses of individual neurons to the ×0.5T/×1BG, ×1.5T/×0.5BG and ×1T/×0BG training stimuli.) Positive and negative values correspond to increased activity during ipsiversive and contraversive head movement, respectively. Error bars signify s.e.m. See Online Methods for detailed description of stimuli. (**b**) Purkinje cell simple spikes. Positive and negative values correspond to increased activity during contraversive and ipsiversive head movement, respectively (the axis is flipped relative to **a**, so that responses typically associated with gain increases are positive). (**c**) Learning. Each point represents the percent change in VOR gain in a single replication of a behavioral training session in one monkey, with a black bar representing the median. VOR learning was measured after 1 h (monkey E) or 2 h (monkey L) of training. The number of replications is listed in parentheses. Note the different scales for the two monkeys.



Stimulus		Learning (%)	Stimulus		Learning (%)
a x0T/x0BG	−29.9	j x1.5T/x2BG 24.3	a x0T/x0BG*	−19.1	j x1.5T/x1BG 3.3
b x0T/noBG	−25.3	k x1.5T/x1.5BG 27.3	b x0T/x0BG	−13.3	k x1T/x2BG 9.7
c x0T/x1BG	−14.6	l x2T/x0BG 5.5	c x0.5T/x0.5BG	−9.5	l x1T/x1BG 4.1
d x0T/x0.5BG	−13.1	m x2T/x1BG 9.3	d x0T/x2BG*	−16.4	m x2T/x0BG* 3.3
e x0T/x2BG	−6.4	n x2T/x1.5BG 2.7	e x0T/x2BG	−7.7	n x2T/x0BG 4.3
f x0.5T/x0BG	−30.4	o x2T/noBG 20.4	f x0.5T/x1BG	−3.4	o x1.5T/x1.5BG 12.0
g x0.5T/x0.5BG	−19.0	p x2T/x2BG 31.8	g x0.5T/x1.5BG	3.2	p x2T/x2BG 14.8
h x1T/x0BG	−13.9		h x1T/x0BG	−9.6	q x2T/x2BG* 4.9
i x1T/x2BG	−0.2		i x1.5T/x0.5BG	0.2	

* Peak head velocity ±20° per s

Figure 5.

Purkinje cell simple spike and climbing fiber signals together predict learned behavioral changes. (**a**) Different combinations of instructive signals. Each point represents the average climbing fiber response (abscissa) and simple spike response (ordinate) to one training stimulus. The letter on each symbol and the corresponding tables identify the training stimuli and the median percent change in VOR gain induced by each stimulus. Error bars represent s.e.m. (**b**) Climbing fiber responses were correlated with learning. Each data point represents the difference in climbing fiber responses (abscissa) and the difference in learning (ordinate) induced by one pair of training stimuli. The stimulus pairs are grouped into three bins, according to the difference in their simple spike responses (gray shades). (**c**) Purkinje cell simple spike responses were correlated with learning. Each data point represents the difference in simple spike responses and difference in learning for one pair of training stimuli. The stimulus pairs are grouped into three bins, according to the difference in their climbing fiber responses (gray shades). (**d**) Learning could be predicted from the climbing fiber and Purkinje cell simple spike responses during training. The changes in VOR gain predicted from the instructive signals carried by the climbing fibers alone (gray) or from a linear combination of climbing fiber and Purkinje cell simple spike instructive signals (black) are plotted against the observed changes in VOR gain induced by each training stimulus. The correlation coefficients for each fit are indicated.

Table 1

Statistical evaluation of climbing fiber and Purkinje cell simple spike responses to the training stimuli

Monkey L

Climbing fiber instructive signal

	ρ (spikes per s)	θ (°)	*P* value	*n*
×0T/×0BG	0.81	147.8	<0.001	15
×2T/×2BG	0.66	1.8	<0.001	15
×0T/×2BG	0.05	5.3	0.39	15
×2T/×0BG	0.21	54.0	0.02	14
×1T/×0BG	0.59	152.1	<0.001	12

Paired *t* test		*P* value	*N*
×0T/×0BG	×0T/×2BG	<0.001	15
×0T/×0BG	×1T/×0BG	0.01	12
×0T/×0BG	×2T/×2BG	<0.001	15
×2T/×2BG	×2T/×0BG	<0.001	14
×0T/×2BG	×2T/×0BG	0.50	14

Purkinje cell simple spike instructive signal

	ρ (spikes per s)	θ (°)	*P* value	*n*
×0T/×0BG	10.4	−15.0	<0.001	24
×2T/×2BG	14.5	163.2	<0.001	23
×0T/×2BG	12.3	−13.1	<0.001	24
×2T/×0BG	14.7	161.5	<0.001	21
×1T/×0BG	2.5	152.8	0.25	19

Monkey E

Climbing fiber instructive signal

	ρ (spikes per s)	θ (°)	*P* value	*n*
×0T/×0BG	0.84	171.7	<0.001	33
×2T/×2BG	0.91	−3.9	<0.001	27
×0T/×2BG	0.08	115.3	0.84	30
×2T/×0BG	0.24	−18.6	0.002	26
×1T/×0BG	0.59	163.9	<0.001	25
×0.5T/×1BG	0.07	15.8	0.24	14
×1.5T/×0.5BG	0.07	−111.1	0.31	15

Paired *t* test		*P* value	*n*
×0T/×0BG	×0T/×2BG	<0.001	30
×0T/×0BG	×1T/×0BG	<0.001	25
×0T/×0BG	×2T/×2BG	<0.001	27
×2T/×2BG	×2T/×0BG	<0.001	26
×0T/×2BG	×2T/×0BG	<0.001	24
×0T/×2BG	×0.5T/×1BG	0.18	13
×0T/×2BG	×1.5T/×0.5BG	0.95	14

Purkinje cell simple spike instructive signal

	ρ (spikes per s)	θ (°)	*P* value	*n*
×0T/×0BG	15.0	−16.7	<0.001	34
×2T/×2BG	16.8	176.9	<0.001	29
×0T/×2BG	14.7	−17.1	<0.001	32
×2T/×0BG	15.6	174.2	<0.001	28
×1T/×0BG	4.8	−173.2	<0.001	29
×0.5T/×1BG	8.1	−24.2	<0.001	16
×1.5T/×0.5BG	11.9	178.9	0.003	17

Monkey L				**Monkey E**			
Paired *t* test		*P* value	*n*	Paired *t* test		*P* value	*n*
×0T/×0BG	×0T/×2BG	0.19	24	×0T/×0BG	×0T/×2BG	0.58	32
×2T/×2BG	×2T/×0BG	0.51	21	×2T/×2BG	×2T/×0BG	0.06	28
×0T/×0BG	×1T/×0BG	<0.001	19	×0T/×0BG	×1T/×0BG	<0.001	25
×0T/×0BG	×2T/×2BG	<0.001	22	×0T/×0BG	×2T/×2BG	<0.001	27
				×0T/×2BG	×2T/×0BG	<0.001	25
				×0T/×2BG	×0.5T/×1BG	<0.001	14
				×0T/×2BG	×1.5T/×0.5BG	<0.001	15

Population average of the climbing fiber and Purkinje cell simple spike responses elicited by each training stimulus in monkey L (left) and E (right). The population response was calculated as the vector average of the responses in all individual neurons. θ indicates the phase of the neural responses relative to peak ipsiversive head velocity, with positive values reflecting phase lag. A one-sample *t* test was used to evaluate whether the population response was significantly different from zero during each training stimulus. Paired *t* tests were used to compare the neural responses to different training stimuli. ρ indicates the amplitude of firing rate modulation.