Neuron 51, 823–834, September 21, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.neuron.2006.08.026

Selective Engagement of Plasticity Mechanisms for Motor Memory Storage

Edward S. Boyden,[1,2] Akira Katoh,[1] Jason L. Pyle,[2] Talal A. Chatila,[3] Richard W. Tsien,[2] and Jennifer L. Raymond[1,*]
[1] Department of Neurobiology
[2] Department of Molecular and Cellular Physiology
Stanford University
Stanford, California 94305
[3] Department of Pediatrics
The David Geffen School of Medicine
University of California at Los Angeles
Los Angeles, California 10833

Summary

The number and diversity of plasticity mechanisms in the brain raises a central question: does a neural circuit store all memories by stereotyped application of the available plasticity mechanisms, or can subsets of these mechanisms be selectively engaged for specific memories? The uniform architecture of the cerebellum has inspired the idea that plasticity mechanisms like cerebellar long-term depression (LTD) contribute universally to memory storage. To test this idea, we investigated a set of closely related, cerebellum-dependent motor memories. In mutant mice lacking Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV), the maintenance of cerebellar LTD is abolished. Although memory for an increase in the gain of the vestibulo-ocular reflex (VOR) induced with high-frequency stimuli was impaired in these mice, memories for decreases in VOR gain and increases in gain induced with low-frequency stimuli were intact. Thus, a particular plasticity mechanism need not support all cerebellum-dependent memories, but can be engaged selectively according to the parameters of training.

Introduction

Much neuroscience research aims to uncover the fundamental algorithm for information storage in each brain structure specialized for memory storage—e.g., the algorithm used by the hippocampus for declarative memories, or by the cerebellum for motor memories. In vitro studies have revealed a profusion of plasticity mechanisms throughout such structures (reviewed in Hansel et al., 2001; Martin et al., 2000); over a dozen have been described within the cerebellar circuit alone (e.g., Armano et al., 2000; Caria et al., 2001; Coesmans et al., 2004; Hansel and Linden, 2000; Ito et al., 1982; Kano et al., 1992; Lev-Ram et al., 2003; Nelson et al., 2003; Rancillac and Crepel, 2004; Salin et al., 1996; Smith and Otis, 2003). How are all these plasticity mechanisms used for memory storage? A common assumption is that brain structures like the cerebellum store memories via stereotyped usage of their plasticity mechanisms (Albus, 1971; Marr, 1969). The search for a universal mechanism (or set of mechanisms) for memory storage has been particularly intense in the case of the cerebellum due to its simple, almost crystalline anatomy. However, another possibility is that different experiences or paradigms can engage distinct, combinatorial subsets of the plasticity mechanisms available in the cerebellar circuitry. Combinatorial usage of plasticity mechanisms could allow for more flexibility in memory encoding than is possible with a stereotyped usage of a set of mechanisms.

Testing these opposing hypotheses calls for an experimental system that not only stores memories in a form accessible to behavioral and electrophysiological analysis, but also allows molecular manipulation of candidate plasticity mechanisms. The vestibulo-ocular reflex (VOR) is well-suited for this purpose. During head movement, the VOR stabilizes images on the retina by producing eye movements in the direction opposite to the head, thus preventing blurred vision. Cerebellum-dependent motor learning keeps the VOR calibrated by adaptively altering the amplitude (gain) of the reflex (Ito et al., 1974; Koekkoek et al., 1997; Nagao, 1983; Robinson, 1976). The formation and storage of motor memories can be assessed in the laboratory using combinations of visual and vestibular stimuli that increase or decrease VOR gain. The VOR has been a key testing ground for the leading model of cerebellum-dependent memory, which goes back more than three decades to the classical work of Ito (1972, 1982). This model invokes a universal, indeed unitary, plasticity mechanism, long-term depression of parallel fiber-Purkinje cell synapses ("cerebellar LTD") as playing the central role in motor learning.

We conducted a rigorous test of the idea of a universally applicable memory mechanism or set of mechanisms for motor memory storage in the cerebellum by measuring retention of multiple memories in a mutant mouse in which long-term plasticity was targeted. In particular, we used knockout mice lacking Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) (Ho et al., 2000). These mice offer an advantage in that they undergo successful induction of cerebellar LTD but cannot enduringly maintain it (Ahn et al., 1999; Ho et al., 2000). One can therefore expect the mutant mice to display normal acquisition of learning, importantly confirming that all processes necessary for the acquisition of learning and expression of the learned response are intact; then, the memories that depend on LTD will be forgotten, whereas LTD-independent memories will be retained. Using these mice, we found that neural plasticity mechanisms such as cerebellar LTD can be selectively engaged according to the specific parameters of training, even within a narrow class of learning tasks. This provides critical evidence against the prevailing hypothesis that plasticity mechanisms within a brain structure are used in a universal, stereotyped fashion for memory storage.

*Correspondence: jenr@stanford.edu

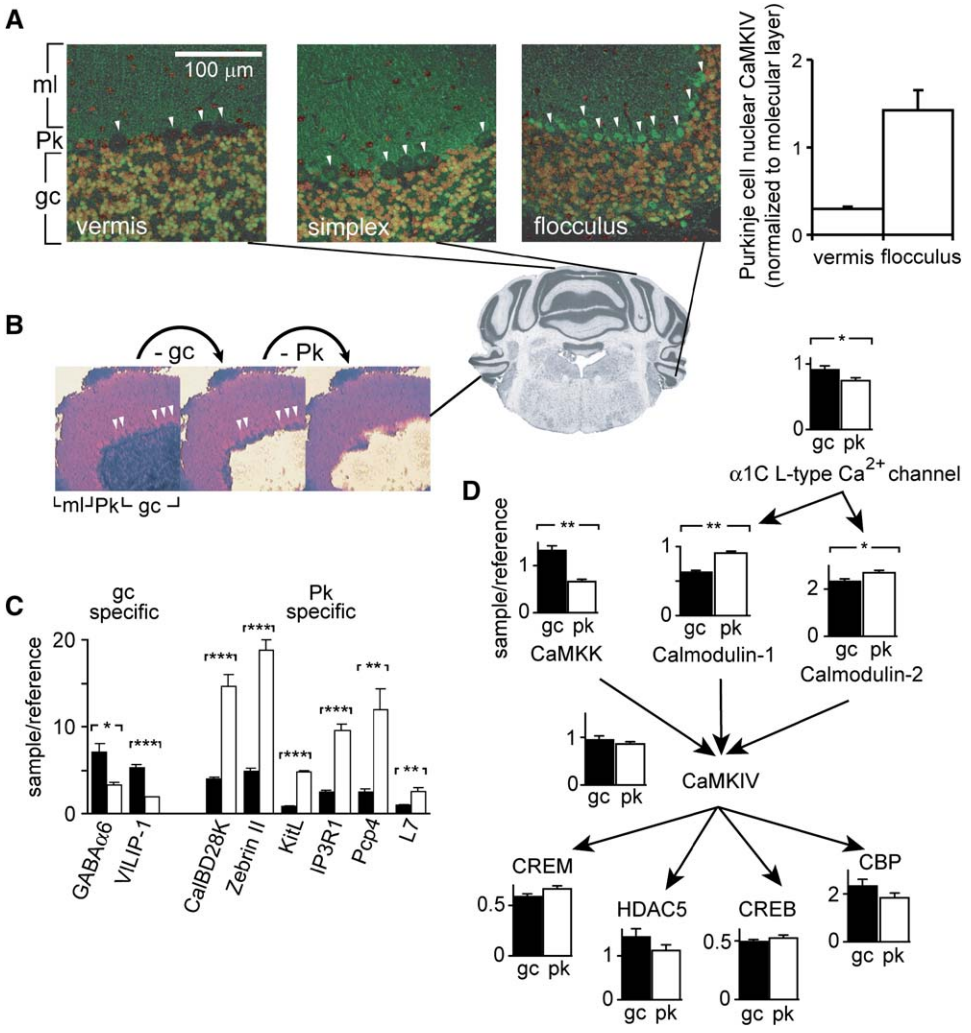

Figure 1. Distribution of the CaMKIV Signaling Pathway in the VOR Circuit

(A) Immunostaining for CaMKIV (green) and nuclei (red) in vermis, lateral cerebellum, and flocculus. gc, granule cell layer; Pk, Purkinje cell layer; ml, molecular layer. Purkinje cells are indicated by arrowheads. (Bottom) Coronal section of mouse brain, reprinted from Plate 79 of Paxinos and Franklin, 2000, with permission from Elsevier. (Right) Quantitation of Pk cell nuclear CaMKIV, relative to the molecular layer (20–24 cells, 3 wild-type mice).

(B) Laser capture microdissection of gc and Pk cells. (Left) Original 14 μm thick section of the flocculus stained with hematoxylin/eosin; (middle) after granule cell capture; (right) after Purkinje cell capture. Five of the Purkinje cells are indicated by arrowheads.

(C) Microarray analysis of granule cell-specific (left) and Purkinje cell-specific (right) genes, in granule cell (black bars) and Purkinje cell (white bars) samples. Bars indicate mean ± SEM of gene expression levels (across all array spots for a gene) (n = 18 gc samples, n = 23 Pk samples). Asterisks indicate significant difference between gc and Pk samples (*$p < 0.05$; **$p < 10^{-4}$; ***$p < 10^{-8}$, unpaired t test). References: GABAα6 (Luddens et al., 1990); Vilip-1 (Bernstein et al., 1999); CalbD28K, calbindin-D28K (Jande et al., 1981); Zebrin II (Brochu et al., 1990); kitL, kit ligand (Morii et al., 1992); IP_3R_1 (Nakanishi et al., 1991); Pcp4 (Mugnaini et al., 1987); L7 (Oberdick et al., 1988).

(D) Schematic of CaMKIV signaling pathway, with gene expression levels for granule cells (black bars) and Purkinje cells (white bars) plotted for each molecule. Asterisks are as in Figure 1C. CaMKK, calmodulin-dependent protein kinase kinase; CREM, cAMP-responsive element modulator; HDAC5, histone deacetylase 5; CBP, CREB binding protein.

Data points indicate mean ± SEM.

Results

CaMKIV Is Expressed in Purkinje Cells in the VOR Circuit

We first clarified the anatomical distribution of the expression of molecules in the CaMKIV cascade. Although cultured Purkinje cells require CaMKIV for the persistence of LTD (Ahn et al., 1999; Ho et al., 2000), previous anatomical studies have reported that adult Purkinje cells contain little CaMKIV (Jensen et al., 1991; Sakagami et al., 1992). Our own immunostaining confirmed that this was the case in adult cerebellar vermis (Figure 1A), the region of focus in the earlier immunocytochemical studies. Importantly, however, we found prominent immunoreactivity to CaMKIV in adult Purkinje cells in cerebellar regions essential for motor learning in the VOR (flocculus, Figure 1A). We quantified the CaMKIV fluorescence in the Purkinje cell nucleus, normalizing this value to the mean fluorescence of the molecular layer to facilitate between-animal comparisons. CaMKIV

levels, thus measured, were 4.8-fold higher in floccular Purkinje cells than in vermal Purkinje cells ($p < 10^{-4}$, unpaired t test; n = 24 flocculus Purkinje cells, 20 vermis Purkinje cells, from three wild-type mice; Figure 1A, right). To pursue this further, we used cDNA microarrays to probe for transcripts for CaMKIV and related signaling molecules. mRNAs were isolated, using laser capture microdissection, from neurons in the cerebellar region known to contribute to the horizontal VOR (Balaban et al., 2000; Ruigrok and Voogd, 2000). We obtained samples of the Purkinje cell (Pk) and granule cell (gc) layers of the cerebellar flocculus (Figure 1B). This procedure preserved the transcriptional identity of adjacently located cell types, as reflected by enrichment of well-known granule cell- and Purkinje cell-specific mRNAs in their respective samples (Figure 1C). Transcripts for CaMKIV were as abundant in the floccular Purkinje cell population as in the granule cell population, which is known to express CaMKIV at high levels (Figure 1D). This was also true for other members of the CaMKIV signaling pathway (Anderson et al., 2004; Bito et al., 1996; Impey et al., 2002; Kimura et al., 2002; McKinsey et al., 2000; Means et al., 1997), including elements upstream of CaMKIV such as L-type calcium channels, calmodulin-dependent protein kinase kinase (CaMKK), and calmodulin, as well as downstream elements such as CREB, cAMP-responsive element modulator (CREM), histone deacetylase 5 (HDAC5), and CREB binding protein (CBP) (see Figure 1 legend). Thus, the molecular components required for CaMKIV-dependent signaling and plasticity were well-represented in adult Purkinje cells in cerebellar regions that support motor learning in the VOR. This result supports the idea that CaMKIV plays a similar role in adult plasticity in certain regions of the cerebellum, as it does in the cultured Purkinje cell preparation that has provided a method for studying the molecular mechanisms of late phase cerebellar LTD (Ho et al., 2000).

Selective Memory Impairment for an Increase, but Not a Decrease, in VOR Gain

We induced motor learning in the VOR by pairing head rotation with rotation of a striped optokinetic drum. Moving the drum in the opposite direction from the head (gain-up stimulus; Figure 2A) caused the VOR gain to increase adaptively, whereas moving the drum in the same direction as the head (gain-down stimulus; Figure 2B) caused the VOR gain to decrease. After 30 min of training, the change in VOR gain was measured, and mice were then placed in darkness for 24 hr to prevent further adaptive changes. In mice lacking CaMKIV, learned increases and decreases in VOR gain were acquired no differently than in wild-type mice (Figures 2C and 2D; $p > 0.80$ for increases, $p > 0.85$ for decreases, unpaired t test). This fulfills the expectation that these mutant mice would have normal learning, and indicates that any memory impairment is not due to disruption of the neural processing underlying the acquisition of learning or the ability to perform the learned response. In contrast to the normal acquisition of motor learning, memory for the increase in VOR gain was impaired in the CaMKIV knockout mice. When the VOR was remeasured 24 hr later, the learned increase in VOR gain had declined significantly in the knockout

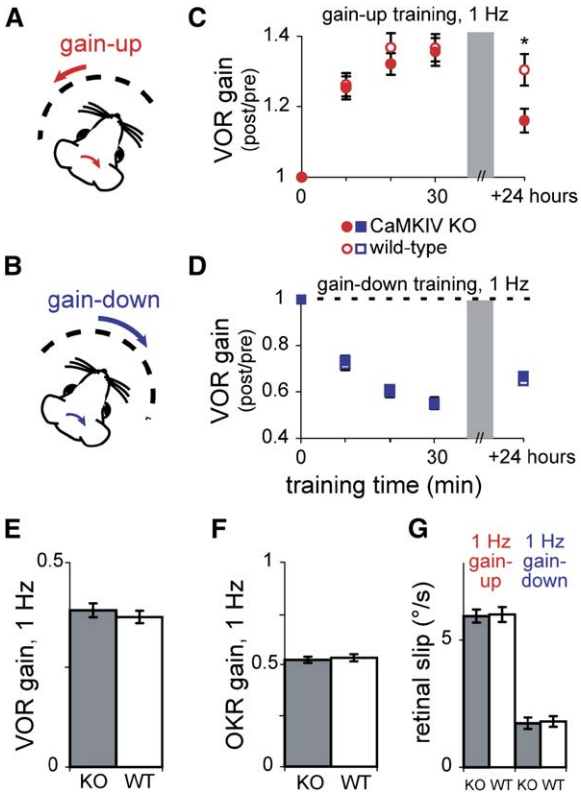

Figure 2. Learning and Memory of Changes in VOR Gain in CaMKIV Knockout Mice

(A) Gain-up stimulus. Optokinetic drum (dashed circle) and head move in opposite directions, with peak drum speed equal to 50% of peak head speed.

(B) Gain-down stimulus. The optokinetic drum moves with the same direction and speed as the head.

(C) Change in VOR gain induced by 30 min of gain-up training, in CaMKIV knockout (filled symbols; n = 23) and wild-type (open symbols; n = 23) mice. VOR gain is normalized to initial VOR gain. The data at time point "+24 hours" indicate the VOR gain measured after a 24 hr memory retention period in darkness (indicated by shaded bar). (*) indicates a significant difference between wild-type and knockout mice at a given time point ($p < 0.05$, unpaired t test). Error bars were omitted when smaller than the experimental symbols.

(D) Change in VOR gain induced by 30 min of gain-down training, and memory retention 24 hr later ("+24 hours"), in knockout (n = 20) and wild-type (n = 20) mice.

(E) Baseline VOR gain for CaMKIV knockout mice (filled gray bars; n = 30) and wild-type mice (open bars; n = 30).

(F) Baseline gain of the optokinetic reflex (OKR).

(G) Peak retinal image slip velocity during tracking of gain-up and gain-down stimuli.

Data points indicate mean ± SEM.

mice relative to wild-type ("+24 hours" in Figure 2C; $p < 0.01$). In striking contrast, memory for a learned decrease in VOR gain was intact in the knockout ("+24 hours" in Figure 2D; $p > 0.95$), as was memory for the change in VOR timing associated with the decrease in gain (see Figure S1 in the Supplemental Data). The specific forgetting of the effects of gain-up training but not gain-down training indicates a differential use of cerebellar LTD or some other CaMKIV-dependent mechanism for memories induced by two closely related, cerebellum-dependent learning paradigms.

We performed control experiments to check whether the observed behavioral deficits were specifically

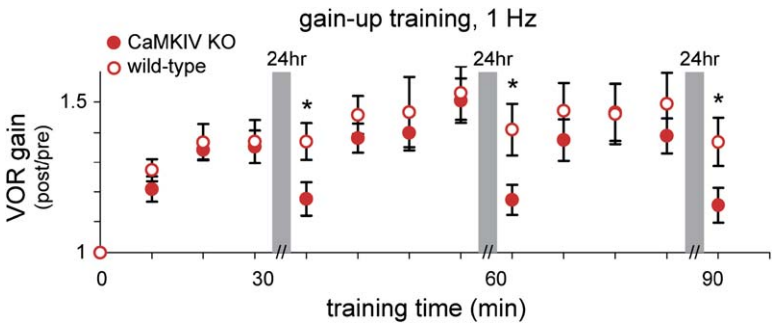

Figure 3. Changes in VOR Gain Induced with Repeated Gain-Up Training Sessions

Increase in VOR gain induced by three 30 min gain-up training sessions in CaMKIV knockout (filled symbols; n = 13) and wild-type (open symbols; n = 13) mice. The shaded bars indicate 24 hr memory retention periods in darkness. The abscissa indicates the cumulative time of gain-up training. (*) indicates a significant difference between wild-type and knockout mice at a given time point ($p < 0.05$, unpaired t test). Data points indicate mean ± SEM.

related to learning or merely resulted from sensory or motor deficits that impaired task performance. In a detailed examination of baseline parameters critical for learning, no difference was found between CaMKIV knockout and wild-type mice in baseline VOR gain (Figure 2E; $p > 0.45$), suggesting normal vestibular function. Furthermore, knockout and wild-type mice were not significantly different in the gain of the optokinetic reflex (OKR) (Figure 2F; $p > 0.60$), suggesting normal visual function. In addition, tracking of the visual stimulus during training, and therefore any error signals provided by the movement of the visual stimulus relative to the eye (retinal slip), was normal for CaMKIV knockout mice for both learning tasks (Figure 2G; $p > 0.85$ for gain-up, $p > 0.55$ for gain-down). The normal magnitudes of VOR, OKR, and tracking of the visual stimulus during training indicate normal oculomotor function as well. Along with the magnitude of each of these measures, their time dynamics were also similar in knockout and wild-type mice (see phase measurements in the Supplemental Data). Thus, the normal sensorimotor performance of CaMKIV knockout mice suggests that the observed difference in retention of increases and decreases in VOR gain reflects a true memory impairment. In this respect, the measurements of baseline VOR, OKR, and retinal slip buttress the central observation that CaMKIV knockout mice acquired increases and decreases in VOR gain no differently than wild-type mice (Figures 2C and 2D). From multiple perspectives, knockout mice were not deficient in the signal processing required for the acquisition or expression of learning. This leads to the conclusion that the clear differences in the retention of the motor memories must arise from differences in the plasticity mechanisms used for storing the motor memories.

Spaced Training Does Not Rescue Memory for an Increase in VOR Gain

The memory for an increase in VOR gain, although clearly impaired, was not completely abolished in the CaMKIV knockout when remeasured after 24 hr (Figure 2C; $p < 0.0001$, paired t test comparing retained VOR to pretraining VOR gain), indicating a CaMKIV-independent component of the memory for an increase in gain. We examined whether this component could compensate for the loss of the CaMKIV-dependent component of an increase in gain with the help of additional training sessions. In CREB mutant mice, repeated training has been shown to restore spatial memory retention to normal levels, despite impairments in memory after limited training or training with brief intertrial intervals (Kogan et al., 1997). In addition, a recent study suggests that cerebellum-dependent adaptation of the OKR is less dependent on plasticity in the cerebellar cortex and more dependent on changes in the vestibular nucleus after multiple days of training as compared with a single training session (Shutoh et al., 2006). Accordingly, we exposed mice to additional training sessions with the gain-up stimulus. After three gain-up training sessions separated by 24 hr intervals, memory for an increase in gain was still impaired in the CaMKIV knockout mice compared to wild-type mice (Figure 3; $p < 0.05$ after both the second and third retention periods, unpaired t test). The knockout mice repeatedly reacquired an increased VOR gain during successive training periods, only to lose the increase over the next 24 hr retention interval. Despite the significant forgetting after each 24 hr interval, the gains observed at the 24 hr retention time points were still significantly higher than baseline VOR gain ($p < 0.05$ for all genotypes/time points, paired t test). This spared component suggests that a CaMKIV-independent mechanism contributes to the increase in gain. However, the fact that the spared component of motor memory was still significantly smaller in the knockout mouse than in the wild-type mouse, even after multiple days of training, suggests that CaMKIV makes an essential contribution to the persistent memory for an increased VOR gain.

Reducing Training Stimulus Frequency Rescues Memory for an Increase in VOR Gain

Although multiple training sessions with the same stimulus could not rescue the impairment of increases in VOR gain, we found that increases in gain could be made CaMKIV-independent by reducing the rotational frequency of the training stimulus used to induce learning (Figure 4). This experiment was prompted by previous behavioral and electrophysiological observations in monkeys suggesting that the mechanism of VOR memory storage may vary with training stimulus frequency (Raymond and Lisberger, 1996, 1998). In addition to the 1 Hz sinusoidal head and image rotation used in Figures 1–3, we tested both higher (2 Hz) and lower (0.5 Hz, 0.66 Hz) frequencies. As observed at 1 Hz, CaMKIV knockout mice displayed normal acquisition of motor learning after 30 min of gain-up training at the other stimulus frequencies, which was not significantly different from their wild-type counterparts (Figure 4A; $p > 0.25$ for 0.5 Hz, $p > 0.65$ for 0.66 Hz, $p > 0.05$ for 2 Hz). However, a clear frequency-dependence

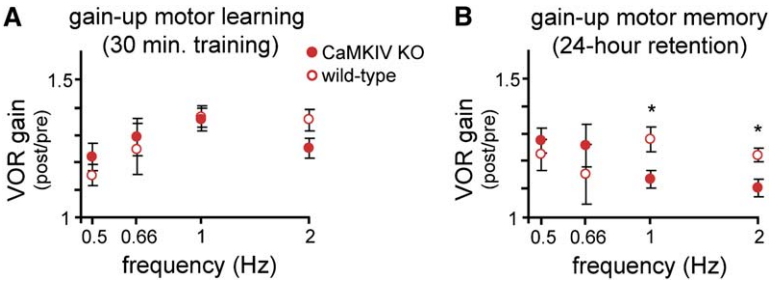

Figure 4. CaMKIV Dependence of the Memory for an Increase in VOR Gain Varies with Rotational Stimulus Frequency

(A) Changes in VOR gain induced by 30 min of gain-up training with 0.5, 0.66, 1, or 2 Hz rotational stimuli in CaMKIV knockout (filled symbols; n = 20 for 0.5 Hz, n = 20 for 0.66 Hz, n = 23 for 1 Hz, n = 25 for 2 Hz) and wild-type (open symbols; n = 23 for 0.5 Hz, n = 17 for 0.66 Hz, n = 23 for 1 Hz, n = 23 for 2 Hz) mice.

(B) Motor memory retention 24 hr after gain-up training with 0.5, 0.66, 1, or 2 Hz stimuli. (*) indicates a significant difference between wild-type and knockout mice at a given time point ($p < 0.05/4 = 0.0125$, Bonferroni-corrected t test).

Data points indicate mean ± SEM.

of memory emerged when the learned increases in VOR gain were remeasured 24 hr later (Figure 4B). With 2 Hz stimuli, similar to the 1 Hz case described above, there was partial sparing of the memory for the increase in VOR gain ($p < 0.0001$; paired t test comparing retained VOR to pretraining VOR gain); however, VOR gain declined significantly in knockout mice relative to wild-type mice (Figure 4B; $p < 0.0125$). In contrast, for the lower training frequencies (0.5 Hz, 0.66 Hz), the learned increase in VOR gain was intact in the knockout 24 hr after training (Figure 4B; $p > 0.45$ knockout versus wild-type for 0.5 Hz, $p > 0.65$ for 0.66 Hz). We also found no difference between wild-type and knockout mice for gain-down learning or memory at any of these four stimulus frequencies (Figure S2). The selective retention of increased VOR gain at 0.5 Hz and 0.66 Hz indicates that the reliance on CaMKIV is not universal even for all forms of gain-up training, but is restricted according to the training stimulus frequency.

None of the sensory or motor performance parameters were significantly different between knockout and wild-type mice, including baseline VOR gain (Figure 5A; $p > 0.15$ for 0.5 Hz, $p > 0.60$ for 0.66 Hz, $p > 0.35$ for 2 Hz), OKR gain (Figure 5B; $p > 0.85$ for 0.5 Hz, no data for 0.66 Hz, $p > 0.80$ for 2 Hz), or retinal slip (Figure 5C, $p > 0.50$ for 0.5 Hz, $p > 0.50$ for 0.66 Hz, $p > 0.85$ for 2 Hz) during gain-up training. Accordingly, the frequency-dependence of the effect of CaMKIV deletion cannot be attributed to a difference in baseline sensory or motor performance or available error signals.

We noted, however, that in both wild-type and knockout mice, VOR gains were, on average, lower immediately after the end of 0.5-Hz gain-up training than 24 hr later (Figures 4A and 4B). This suggested that 0.5 Hz rotation could be inducing VOR habituation, as had been reported to result from low-frequency rotation in earlier work (Dow and Anastasio, 1996; Jager and Henn, 1981), including a study in mice (Stahl, 2004). VOR habituation is thought to depend on the vermis (Torte et al., 1994), where CaMKIV expression is low in the Purkinje cells but high in the granule cells. If this habituation were different between CaMKIV knockout and wild-type mice, such a difference could potentially mask or masquerade as a difference in the adaptive changes induced by paired visual and vestibular stimuli. Accordingly, we tested whether 30 min of head rotation in the absence of a visual stimulus had any transient and/or prolonged effect on VOR gain. We found that 30 min of head rotation in the dark at 0.5 Hz did indeed induce habituation of the VOR, but this habituation did not differ between wild-type and knockout mice ($p > 0.95$, Figure 6A). In addition, there was no long-term retention of the habituation; 24 hr later; both wild-type and knockout mice had recovered to their baseline VOR gain ($p > 0.30$ for both, paired t test comparing retained VOR to pretraining VOR gain), and there was no difference

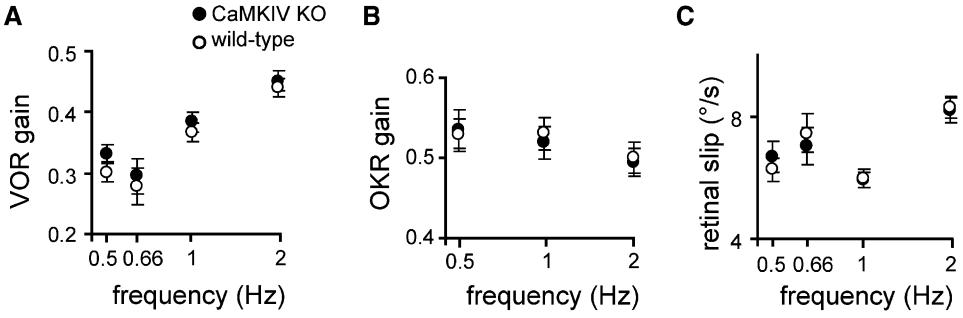

Figure 5. Sensory and Motor Performance Controls

(A) Baseline VOR gain for 0.5, 0.66, 1, or 2 Hz rotation in CaMKIV knockout (filled symbols; n = 21 for 0.5 Hz, n = 20 for 0.66 Hz, n = 23 for 1 Hz, n = 25 for 2 Hz) and wild-type (open symbols; n = 23 for 0.5 Hz, n = 18 for 0.66 Hz, n = 23 for 1 Hz, n = 23 for 2 Hz) mice.

(B) Baseline OKR gain.

(C) Peak retinal image slip velocity during tracking of gain-up stimulus.

Data points indicate mean ± SEM.

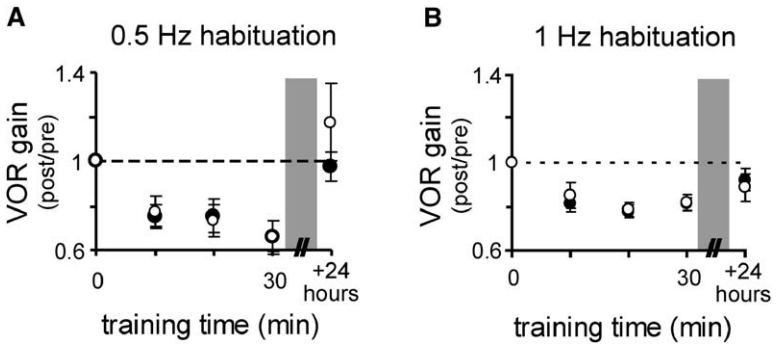

Figure 6. Induction and Retention of VOR Habituation in CaMKIV Knockout and Wild-Type Mice

(A) Changes in VOR gain induced by 30 min of 0.5 Hz head rotation in the dark, for CaMKIV knockout (filled symbols; n = 14) and wild-type (open symbols; n = 12) mice.

(B) Changes in VOR gain induced by 30 min of 1 Hz head rotation in the dark, for CaMKIV knockout (closed symbols; n = 8) and wild-type (open symbols; n = 8) mice.

Data points indicate mean ± SEM.

between wild-type and knockout mice ($p > 0.25$, unpaired t test). Thus, no genotypic difference in habituation at 0.5 Hz could mask a difference between knockout and wild-type mice in adaptive learning. A smaller degree of habituation was observed with 30 min of 1 Hz rotation in the dark (Figure 6B), consistent with earlier reports that VOR habituation is reduced at higher frequencies (Dow and Anastasio, 1996; Jager and Henn, 1981). 24 hr later, this habituation recovered to baseline levels ($p > 0.10$ for wild-type, $p > 0.20$ for knockout, paired t test). Once again, neither the habituation observed at 30 min nor its retention differed between wild-type and knockout mice ($p > 0.85$ for habituation measured at 30 min, $p > 0.65$ for the habituation measured 24 hr later, unpaired t test). Therefore, habituation of the VOR is CaMKIV-independent, and hence cannot explain our finding of a selective impairment of the memory for adaptive VOR gain increases, but not gain decreases, at high training frequencies.

Discussion

We have performed a rigorous test of the idea that the cerebellar circuit stores all motor memories via stereotyped usage of its plasticity mechanisms (Albus, 1971; Marr, 1969). We found that the plasticity-mediating kinase CaMKIV was selectively engaged for memories of increases, but not decreases, in VOR gain. Furthermore, alteration of the training stimulus frequency could completely alter the CaMKIV dependence of motor memory storage for an increase in VOR gain. Our results therefore challenge the assumption that a universal plasticity mechanism (or set of mechanisms), applied in a stereotyped fashion to appropriate synapses, mediates the storage of all cerebellum-dependent memories. Below, we discuss two key conclusions that emerge from our data: (1) task-selective usage of plasticity mechanisms may be an important, general property of memory storage systems, and (2) for the case of the cerebellum, parallel fiber-Purkinje cell LTD may play a specific, circumscribed role in memory encoding.

Task-Selective Mechanisms of Memory Storage

The classical model of Ito attributed both an increase and a decrease in VOR gain to a single synaptic plasticity mechanism by suggesting that cerebellar LTD operates independently on parallel fibers that are active at different times during the VOR (Ito, 1982). Specifically, this model suggested that LTD of parallel fibers firing during ipsiversive head turns would mediate an increase in VOR gain, whereas LTD of parallel fibers firing during contraversive head turns would mediate a decrease in VOR gain. However, behavioral differences between increases and decreases in the gain of the VOR (Boyden and Raymond, 2003; Cohen et al., 2004; Kuki et al., 2004; Miles and Eighmy, 1980) suggested that these motor memories might be stored with different plasticity mechanisms (Boyden and Raymond, 2003). This prediction was borne out here by our finding of a key molecular difference between the way increases and decreases in the gain of the VOR are stored—namely, that they are affected differentially by CaMKIV deletion. The dissociation between the effects of CaMKIV disruption on increases and decreases in VOR gain, and between the effects on increases in VOR gain induced at different training frequencies, leads to a clear conclusion: increases in gain induced with high-frequency training must engage different cellular/molecular plasticity mechanisms than those recruited by decreases in gain or increases in gain induced with low-frequency training. It is important to note that this central conclusion about task-selective usage of plasticity mechanisms in the VOR circuit holds even if CaMKIV deletion causes additional neural changes beyond disruption of cerebellar LTD. This finding requires the revision of old models that attributed a universal role in motor learning and memory to specific cellular/molecular plasticity mechanisms such as cerebellar LTD: plasticity mechanisms within the VOR circuit are selectively recruited during certain training paradigms and not others.

Is task-selective plasticity a general feature of memory storage systems? There is tantalizing evidence for such specific memory mechanisms in the hippocampus, based on mutant mouse studies (Bach et al., 1995; Peters et al., 2003). Deletion of CaMKKβ, a kinase upstream of CaMKIV, affected spatial long-term memory but not contextual fear memory, despite the hippocampal dependence of both learning paradigms (Peters et al., 2003). However, this finding appears to conflict with other work (Abel et al., 1997; Wong et al., 1999), including a study showing that CaMKIV impairment in the forebrain affects contextual fear conditioning (Kang et al., 2001). In such complex behaviors, it is possible that multiple aspects of memory processing, including the storage, consolidation, and even retrieval of memories could depend on details of the training. More studies are needed to resolve these questions and to put the behavioral findings in the context of circuit function.

Cerebellar LTD as a Task-Selective Plasticity Mechanism

Although the general limitations of molecular interventional approaches preclude incontrovertible conclusions regarding the specific role of cerebellar LTD in VOR learning, our results are nevertheless highly suggestive. Earlier studies of motor learning in mice lacking LTD induction obtained inconsistent results: some studies reported more dramatic impairments than others, with the majority reporting greater impairment than we detected in the CaMKIV knockout (Aiba et al., 1994; De Zeeuw et al., 1998; Feil et al., 2003; Ito, 1982; Koekkoek et al., 2003; Li et al., 1995; Nagao and Ito, 1991; Shibuki et al., 1996; Van Alphen and De Zeeuw, 2002; Welsh et al., 2005). It is possible that the experiments that found greater behavioral impairments disrupted not only LTD itself, but also the patterns of spiking activity important for the induction of plasticity at other sites in the circuit (Hansel and Linden, 2000; Maffei et al., 2003; Smith and Otis, 2003; Wall, 2003). Here we minimized such complications by focusing on a mutant with normal induction but impaired persistence of LTD. The behavioral results in these mice paralleled the LTD physiology: removing CaMKIV selectively disrupted memory while sparing the acquisition of learning across all the motor tasks we examined, even those that engaged CaMKIV for memory storage. This allowed us to distinguish between memories that were preserved and those that were forgotten, in the absence of confounding factors that disrupt the acquisition or expression of learning, making it more likely that the behavioral deficits observed can be attributed to the disruption of long-lasting cerebellar LTD, rather than other, nonspecific effects of the mutation.

Our results provide partial support for the classical hypothesis that cerebellar LTD mediates motor memory (Albus, 1971; Ito, 1982; Marr, 1969), since the CaMKIV knockout mice, which lack long-lasting cerebellar LTD, forget increases in VOR gain relative to wild-type mice. However, in these same mice, other motor memories are completely spared, which suggests a circumscribed role of LTD in motor memory storage as opposed to the universal role assumed in most accounts of cerebellar function.

A New Model: Combinatorial Selection of Plasticity Mechanisms for Cerebellar Memory Storage

Prior behavioral experiments and in vivo electrophysiological recording studies laid the groundwork for our current analysis of cerebellar memory storage. The role we have delineated for cerebellar LTD allows us to synthesize prior and current results into a unifying model of cerebellar operation for multiple changes in the VOR (Figure 7).

Previous measurements of the vestibular sensitivity of Purkinje cells in the VOR circuit suggested that the vestibular parallel fiber input to Purkinje cells is greatest during ipsiversive head movement (Lisberger and Fuchs, 1978; Lisberger et al., 1994; Miles et al., 1980; Pastor et al., 1997; Raymond and Lisberger, 1997; Stone and Lisberger, 1990). This observation raised the possibility that LTD of the prevailing, ipsiversive-responding (vestibular type I) parallel fibers could make a bigger contribution to increases in VOR gain than LTD of the weaker input from contraversive-responding (vestibular type II) parallel fibers could make to decreases in VOR gain. This prediction was confirmed by our finding of no significant impairment of decreases in VOR gain in the LTD-deficient mice. How is the decrease in VOR gain implemented? Behavioral studies of the reversal properties of increases and decreases in VOR gain (Boyden and Raymond, 2003; Cohen et al., 2004; Kuki et al., 2004; Miles and Eighmy, 1980) led us to formulate a model in which LTD of ipsiversive-responding vestibular parallel fibers supported increases in VOR gain, whereas LTP of the same set of synapses supported decreases in VOR gain (Boyden and Raymond, 2003). The plausibility of this model was bolstered by the discovery that both LTD and LTP exist at parallel fiber-Purkinje cell synapses, and that they have different reversal

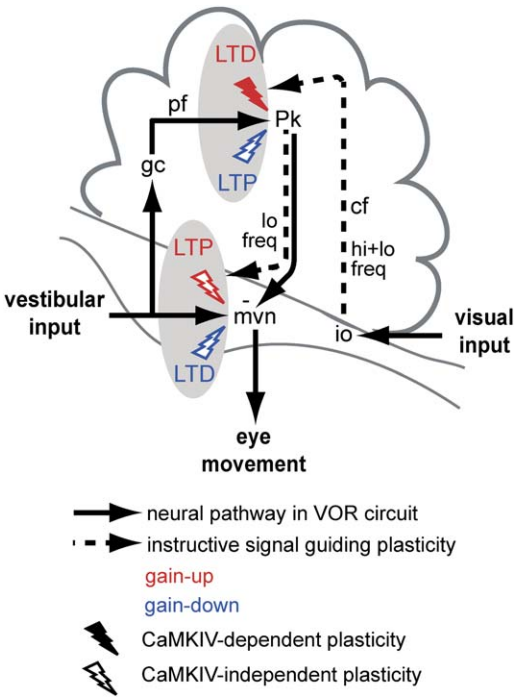

Figure 7. Selective Engagement of Plasticity Mechanisms for Cerebellar Memory Storage

A model that integrates previous and current behavioral and physiological data. Solid lines indicate the brainstem (vestibular input → medial vestibular nucleus (mvn) → eye movement) and cerebellar (vestibular input → granule cells (gc)/parallel fibers (pf) → Purkinje cells (Pk) → mvn) pathways for the VOR. Candidate neural instructive signals (dashed lines), which convey information about the required direction of learning, include the Purkinje cells and the climbing fibers (cf) from the inferior olive (io) (Ito, 1982; Miles and Lisberger, 1981). These two signals could regulate plasticity at different sites (gray ovals) in the VOR circuit. Purkinje cells carry information about the required direction of learning for low training stimulus frequencies only, whereas climbing fibers carry instructive signals during both high- and low-frequency training (Raymond and Lisberger, 1998). Thus, during low-frequency training, Purkinje cells could induce CaMKIV-independent plasticity (open lightning bolts) in the mvn, whereas during high-frequency training, learning would rely on climbing fiber-triggered plasticity in the cerebellum. CaMKIV-dependent (filled lightning bolts) pf-Pk LTD (Ahn et al., 1999; Ho et al., 2000) could increase VOR gain (red), and CaMKIV-independent (open lightning bolts) pf-Pk LTP (Lev-Ram et al., 2003; Salin et al., 1996) could decrease VOR gain (blue).

properties (Coesmans et al., 2004; Lev-Ram et al., 2003). Our study of the CaMKIV knockout is consistent with the inverse-mechanism model, since the disruption of LTD selectively disrupted increases, but not decreases, in VOR gain. One potential caveat is that the CaMKIV independence of parallel fiber LTP, assumed in the current model, has not been tested; further experiments may require revision of the model. Note also that increases in gain induced with high-frequency stimuli were partly spared in the CaMKIV knockout, suggesting that CaMKIV-dependent mechanisms such as cerebellar LTD are only a subset of the changes supporting these memories.

Our finding that high-frequency-induced increases in gain were impaired in the CaMKIV knockout, whereas low-frequency-induced changes were spared, provides support for a second idea raised by earlier studies: memories produced by high- and low-frequency training use plasticity mechanisms at different sites in the VOR circuit. Behavioral differences in the generalization of changes induced with high- versus low-frequency training stimuli hinted that different plasticity mechanisms were engaged at different training frequencies (Kimpo et al., 2005; Raymond and Lisberger, 1996). Subsequent electrophysiological recordings of candidate neural instructive signals provided additional evidence that the neural instructive signals controlling the induction of plasticity in vivo might vary with the rotational frequency of the training stimulus (Raymond and Lisberger, 1998). The induction of cerebellar LTD is controlled by climbing fibers, whereas plasticity in the vestibular nuclei may be controlled by the output from the Purkinje cells (Miles and Lisberger, 1981). At high frequencies, the climbing fibers, but not Purkinje cells, carry information about the required direction of learning, implicating climbing fiber-triggered cerebellar LTD in learning under such conditions. At low frequencies, however, both climbing fibers and Purkinje cells convey instructive signals, raising the possibility that learning could be mediated preferentially by Purkinje cell-triggered plasticity in the vestibular nuclei, and could be less-dependent on cerebellar LTD. Indeed, we observed completely intact VOR memory at low frequencies, lending support to the idea that plasticity could be preferentially engaged in the vestibular nuclei during low-frequency VOR training (Figure 7).

Our results from the CaMKIV mutant mouse provide a molecular basis for understanding several prior behavioral and physiological results in the context of a cohesive model of cerebellar operation. The task-selective use of plasticity mechanisms may allow for multiple strategies of information encoding, each appropriate for the specific demands of a particular training condition or instance of memory storage.

Experimental Procedures

Behavioral experiments were performed on 93 CaMKIV knockout mice (backcrossed >12 generations to C57BL/6 mice from Charles River Labs [Wilmington, MA] and bred in the Stanford Research Animal Facility) and 88 wild-type mice on the C57BL/6 background (from Charles River Labs), 8 to 16 weeks old. All procedures were approved by the Stanford Administrative Panel for Laboratory Animal Care (APLAC). All experiments were done blind to mouse genotype. Protocols for mouse headpost and eye coil surgery, experimental equipment, and behavioral protocols were similar to those described previously (Boyden and Raymond, 2003).

Surgical Protocols

Mice were anesthetized and three screws were implanted in the skull. An 80 turn copper scleral search coil was implanted under the conjunctiva of the eye, and held in place with n-butyl cyanoacrylate glue (Vetbond). Eye coils allow stable and repeatable precision in the measurement of mouse eye movements, over timescales from milliseconds to days (Boyden and Raymond, 2003), and coil methods are therefore particularly reliable for measuring learning- and memory-related changes in the VOR (Coesmans et al., 2003; De Zeeuw et al., 1998; Kimpo et al., 2005; Kistler et al., 2002; Koekkoek et al., 1997; van Alphen et al., 2001). The twisted wire leads were threaded through the top of the eye, emerging from under the scalp near bregma, and soldered to a 2 pin connector. This connector and a plastic headpost (placed approximately over lambda) were cemented with dental acrylic to the screws.

Equipment

During each behavioral experiment, the head of the mouse was immobilized by placing it in a custom-made restrainer to which its headpost was fixed. Vestibular stimuli were delivered to the mouse by rotating this restrainer, which was mounted on a computer-controlled turntable (Carco IGTS, Pittsburgh, PA). Optokinetic stimuli were applied by rotating a hemispherical drum (30 cm in diameter, white with black vertical stripes subtending 7.5° visual angle), mounted on a motor with shaft encoder. The drum was backlit by two 60 watt bulbs placed approximately six inches outside the drum. A silvered acrylic mirror was placed under the mouse to provide nearly full-field visual motion. Magnetic field coils (CNC Engineering, Seattle, WA) fixed to the turntable provided the signals for measuring eye position using the mouse's scleral search coil.

Behavioral Protocols

On the sixth day after surgery, each mouse was acclimatized to head restraint for two 15 min sessions. During the first of these sessions, the mouse's scleral search coil was calibrated by rotating the magnetic field coils sinusoidally (±10°/s peak velocity) around the mouse, which was held stationary in darkness. During the second 15 min acclimatization session, the VOR gain, OKR gain, and eye movement responses to the gain-up and gain-down stimuli were measured.

The VOR gain was measured by delivering 0.5, 0.66, 1, or 2 Hz ±10°/s peak velocity sinusoidal turntable rotations in the dark. Measurements were taken in 30 s blocks. Any cycle containing a saccade or motion artifact was deleted from the analysis. Head and eye velocity traces were aligned on the zero crossings of head velocity, and then averaged. Fourier analysis was then used to extract amplitude and phase from the averaged traces (Boyden and Raymond, 2003). The VOR gain was calculated to be the ratio of the eye velocity amplitude to the head velocity amplitude, and the VOR phase was calculated to be the eye velocity phase minus the head velocity phase, minus 180°. A perfectly compensatory VOR would thus have a phase of zero. We adopt the sign convention that negative phase indicates peak eye velocity preceding peak head velocity, and positive phase indicates peak eye velocity following peak head velocity (note that this is opposite to the sign convention used in Boyden and Raymond, 2003). The OKR gain was measured by delivering 0.5, 1, or 2 Hz ±10°/s peak sinusoidal rotation of an illuminated striped optokinetic drum, and calculated as the ratio of averaged eye velocity amplitude to averaged drum velocity amplitude.

We induced motor learning in the VOR by pairing head rotation with rotation of the optokinetic drum. The gain-up stimulus consisted of ±10°/s peak velocity, sinusoidal turntable rotation paired with oppositely directed ±5°/s, sinusoidal drum rotation (with both speeds measured relative to the external world; Figure 2A). This configuration is sometimes referred to as an x1.5 stimulus. For the gain-down stimulus, the illuminated drum was held stationary relative to the mouse, while ±10°/s sinusoidal turntable rotation was delivered (Figure 2B). This configuration is sometimes referred to as an x0 stimulus. The frequency of the sinusoidal rotation for gain-up and gain-down stimuli was 0.5, 0.66, 1, or 2 Hz. Habituation experiments

involved ±10°/s sinusoidal turntable rotation in the dark at either 0.5 or 1 Hz. For each training session, mice were trained in three 10 min periods. After each 10 min period, the VOR was measured during two 30 s blocks of turntable rotation in the dark. In between the two blocks, a bright light lasting approximately 1/6 s was flashed to maintain animal alertness, followed by an 8 s pause before beginning the second block of eye movement measurement (Boyden and Raymond, 2003). The two blocks were averaged in a weighted fashion so that each cycle would make equal contributions to the final value of the VOR. In addition, measurements were made of the eye movements in the presence of the gain-up or gain-down stimuli at the beginning of the first 10 min training period. Retinal image slip was calculated by extracting the amplitude and phase from the averaged difference between optokinetic drum velocity and eye movement velocity. To test memory retention, mice were transferred to a completely dark chamber for 24 hr periods following training. The transfer itself was also done in darkness; the investigator wore night vision goggles.

Some mice were run on more than one experiment, in which case mice were allowed to rest in their home cage with normal light-dark cycles until VOR gain had recovered to normal (at least 2 days after training, which allowed the gain to return to its naive value; Boyden and Raymond, 2003; Kimpo et al., 2005). For these mice, experiment order was randomized. No mouse was run on the same experiment more than once. All VOR data acquired during learning were normalized by dividing by the mouse's baseline VOR, measured at the beginning of the experiment. Phase changes during learning were measured with respect to each mouse's initial phase. Raw values of baseline VOR gain and phase are shown in Figures 2 and 5 and Figure S1.

We excluded from the analysis any experiments in which VOR gains were unreliable because too few sinusoidal cycles of eye movement remained for averaging after exclusion of cycles containing saccades or motion artifacts at either of two critical time points—the beginning of the experiment (which provides the baseline VOR to which all subsequent measurements were normalized) or the end of the experiment (which provides the critical 24 hr memory retention time point). The threshold value for exclusion was that less than 5 cycles were collected for a 0.5 or 0.66 Hz experiment, or that less than 10 cycles were collected for a 1 or 2 Hz experiment. This criterion resulted in the exclusion of 5 experiments from analysis out of a total of 288 experiments conducted—one 2 Hz gain-up wild-type, one 0.5 Hz gain-up knockout, one 0.66 Hz gain-up wild-type, one 0.66 Hz gain-down knockout, and one 0.5 Hz dark rotation wild-type.

Immunostaining

Adult (8- to 16-week-old) wild-type C57BL/6 mice were perfused with 0.5% heparin in room temperature PBS, followed by cold 4% paraformaldehyde, at a rate of 10 mL/min for 30 min. The cerebellum was removed and postfixed overnight, then cut into 50 μm coronal sections in PBS with a Vibratome. Slices were permeabilized with 0.25% Triton and 100 mM glycine, blocked in donkey serum, then stained with goat-anti-CaMKIV (Santa Cruz Biotechnology) and To-Pro-3. Slices were mounted with Vectashield, and different regions of the cerebellum were imaged on a Zeiss LSM510 confocal with a 40× objective (optical section 1 μm). Maximum intensity projections 10 μm thick were created for the sample images in Figure 1. Quantitation of CaMKIV in Purkinje cell nuclei was performed by identifying Purkinje cell nuclei in single optical sections by their characteristic appearance with To-Pro-3 staining and normalizing the signal to CaMKIV staining in the molecular layer of that same optical section. No CaMKIV staining was observed in CaMKIV knockout mice. Statistics were done with Excel.

Microarray Procedures

Thirty-five adult (8- to 16-week-old) C57BL/6 mice (which had undergone previous behavioral protocols) were anesthetized with isoflurane, and the brains were removed quickly, embedded in OCT, and frozen in a dry ice-ethanol mixture. The interval between decapitation and complete freezing was <3 min. The brain was warmed up to −20°C and cut into 10–14 μm sections with a cryostat. Serial coronal sections were placed on slides, dipped in 100% ethanol, and stained with hematoxylin and eosin, followed by both dehydration with increasing concentrations of ethanol and finally xylene.

We focused on two cell types, the Purkinje and granule cells of the dorsal flocculus. We used an Arcturus PixCell II laser capture microdissection scope to capture cells from the appropriate areas, obtaining ten samples per cell type per mouse. We estimate that each Pk sample contained about 50–100 Purkinje cells, and that each gc sample contained a few thousand granule cells. Granule cell samples were captured before Purkinje cell samples to reduce contamination of the smaller Purkinje cell samples by granule cell material. Samples were removed with lysis buffer (Qiagen) and then kept at −80°C. For each mouse, all the samples of a given cell type were pooled; samples from different mice were not pooled.

Total RNA was isolated using RNEasy kits (Qiagen) and amplified in two rounds of in vitro transcription (IVT) (Ambion). IVT-amplified samples were hybridized to microarrays if the end product after the second amplification round was of concentration $\geq$0.2 μg/μl, and at least ten times the negative control signal (measured after two amplification rounds of a null sample). Due to the small starting sample sizes, samples often were rejected due to insufficient quantity for hybridization: thus, 18/35 granule cell samples and 23/35 Purkinje cell samples survived this quality control process and were subsequently run on arrays (41 total).

Mouse cDNA microarrays ("MM arrays") were obtained from the Stanford Functional Genomics facility (http://www.microarray.org/). We used a "type II" experiment design, where all samples were hybridized against a common reference sample. The reference sample comprised mRNA extracted from neonatal and adult brain and liver, twice amplified. We used standard protocols for cDNA labeling, array hybridization, washing, scanning, and data analysis (http://cmgm.stanford.edu/pbrown/protocols/index.html). Briefly, 6 μg of amplified RNA was reverse transcribed with random hexamers and Superscript II (Invitrogen), labeled with Cy5-dUTP. The amplified brain-liver RNA was reverse transcribed, labeled with Cy3-dUTP. The labeled sample and reference were hybridized for 17 hr on the arrays, then washed and scanned on a GenePix scanner. Spots were analyzed semiautomatically with GenePix 3.0 (Axon Instruments). We used standard criteria for spot quality: spots with red-green regression correlation <0.6 or signal/background ratio <2.5 were not used. We extracted the difference between the mean signal and the mean background (defined as the region outside the spot, with a radius 3× the radius of the spot) for both the Cy5 and Cy3 channels, and computed the resultant Cy5/Cy3 ratio. Each array was normalized by setting the mean of the Cy5/Cy3 ratios, over all spots on the array, to be 1. Statistical analysis was carried out by applying an unpaired, two-tailed t test without Bonferroni correction (Excel) to genes of interest, an appropriate test since only preselected genes were analyzed (Piedras-Renteria et al., 2004). If more than one spot was present on the array for a given gene, data were pooled across spots. All raw and processed data is published on the Stanford Microarray Database (http://genome-www.stanford.edu/microarray).

Statistics

All statistics were performed using Excel (t tests, paired or unpaired) and Statview (ANOVA). The t test was used to compare CaMKIV versus wild-type data, using a criterion for significance of $p < 0.05$. Whenever all four training frequencies were considered (e.g., Figures 4 and 6, and Figure S2), we utilized a Bonferroni-corrected threshold p value for declaring significance ($p < 0.05/4$ test frequencies = 0.0125). None of our conclusions about which genotypic differences in memory retention are significant and which are not significant are dependent on whether the p values were Bonferroni-corrected. The results from the t tests are also consistent with the values obtained from a two-factor ANOVA (see the Supplemental Data), which confirmed our conclusion that the CaMKIV dependence of gain-up memory was frequency selective.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/51/6/823/DC1/.

Acknowledgments

Thanks to E. Knudsen, M. Schnitzer, D. Feldman, D. Wetmore, and members of the Raymond, Tsien, and Knudsen labs for helpful

discussions. Thanks also to P. Louderback, S. Dack, S. Teitge, and M. Neogi for technical help. This research was supported by NIH R01 DC04154, a McKnight Scholar Award, a Klingenstein Award in Neuroscience, and an EJLB Foundation Award to J.L.R.; R01s from the NIMH and NINDS for R.W.T.; a MSTP fellowship to J.L.P.; a fellowship from the Human Frontier Science Program for A.K.; and a Fannie & John Hertz Fellowship, NIH F31 DC07006, a Dan David Prize Scholarship, and a Helen Hay Whitney Fellowship to E.S.B. We would like to thank M. Fero and the staff of the Stanford Functional Genomics Facility for supplying us with the microarrays.

Received: April 4, 2005
Revised: January 3, 2006
Accepted: August 22, 2006
Published: September 20, 2006

References

Abel, T., Nguyen, P.V., Barad, M., Deuel, T.A., Kandel, E.R., and Bourtchouladze, R. (1997). Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. Cell *88*, 615–626.

Ahn, S., Ginty, D.D., and Linden, D.J. (1999). A late phase of cerebellar long-term depression requires activation of CaMKIV and CREB. Neuron *23*, 559–568.

Aiba, A., Kano, M., Chen, C., Stanton, M.E., Fox, G.D., Herrup, K., Zwingman, T.A., and Tonegawa, S. (1994). Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. Cell *79*, 377–388.

Albus, J. (1971). A theory of cerebellar function. Math. Biosci. *10*, 25–61.

Anderson, K.A., Noeldner, P.K., Reece, K., Wadzinski, B.E., and Means, A.R. (2004). Regulation and function of the calcium/calmodulin-dependent protein kinase IV/protein serine/threonine phosphatase 2A signaling complex. J. Biol. Chem. *279*, 31708–31716. Published online May 13, 2004. 10.1074/jbc.M404523200.

Armano, S., Rossi, P., Taglietti, V., and D'Angelo, E. (2000). Long-term potentiation of intrinsic excitability at the mossy fiber-granule cell synapse of rat cerebellum. J. Neurosci. *20*, 5208–5216.

Bach, M.E., Hawkins, R.D., Osman, M., Kandel, E.R., and Mayford, M. (1995). Impairment of spatial but not contextual memory in CaMKII mutant mice with a selective loss of hippocampal LTP in the range of the theta frequency. Cell *81*, 905–915.

Balaban, C.D., Schuerger, R.J., and Porter, J.D. (2000). Zonal organization of flocculo-vestibular connections in rats. Neuroscience *99*, 669–682.

Bernstein, H.G., Baumann, B., Danos, P., Diekmann, S., Bogerts, B., Gundelfinger, E.D., and Braunewell, K.H. (1999). Regional and cellular distribution of neural visinin-like protein immunoreactivities (VILIP-1 and VILIP-3) in human brain. J. Neurocytol. *28*, 655–662.

Bito, H., Deisseroth, K., and Tsien, R.W. (1996). CREB phosphorylation and dephosphorylation: a Ca(2+)- and stimulus duration-dependent switch for hippocampal gene expression. Cell *87*, 1203–1214.

Boyden, E.S., and Raymond, J.L. (2003). Active reversal of motor memories reveals rules governing memory encoding. Neuron *39*, 1031–1042.

Brochu, G., Maler, L., and Hawkes, R. (1990). Zebrin II: a polypeptide antigen expressed selectively by Purkinje cells reveals compartments in rat and fish cerebellum. J. Comp. Neurol. *291*, 538–552.

Caria, M.A., Melis, F., Solinas, A., Tavera, C., and Mameli, O. (2001). Frequency-dependent LTP/LTD in guinea pig Deiters' nucleus. Neuroreport *12*, 2353–2358.

Coesmans, M., Smitt, P.A., Linden, D.J., Shigemoto, R., Hirano, T., Yamakawa, Y., van Alphen, A.M., Luo, C., van der Geest, J.N., Kros, J.M., et al. (2003). Mechanisms underlying cerebellar motor deficits due to mGluR1-autoantibodies. Ann. Neurol. *53*, 325–336.

Coesmans, M., Weber, J.T., De Zeeuw, C.I., and Hansel, C. (2004). Bidirectional parallel fiber plasticity in the cerebellum under climbing fiber control. Neuron *44*, 691–700.

Cohen, M.R., Meissner, G.W., Schafer, R.J., and Raymond, J.L. (2004). Reversal of motor learning in the vestibulo-ocular reflex in the absence of visual input. Learn. Mem. *11*, 559–565.

De Zeeuw, C.I., Hansel, C., Bian, F., Koekkoek, S.K., van Alphen, A.M., Linden, D.J., and Oberdick, J. (1998). Expression of a protein kinase C inhibitor in Purkinje cells blocks cerebellar LTD and adaptation of the vestibulo-ocular reflex. Neuron *20*, 495–508.

Dow, E.R., and Anastasio, T.J. (1996). Violation of superposition by the vestibulo-ocular reflex of the goldfish. Neuroreport *7*, 1305–1309.

Feil, R., Hartmann, J., Luo, C., Wolfsgruber, W., Schilling, K., Feil, S., Barski, J.J., Meyer, M., Konnerth, A., De Zeeuw, C.I., and Hofmann, F. (2003). Impairment of LTD and cerebellar learning by Purkinje cell-specific ablation of cGMP-dependent protein kinase I. J. Cell Biol. *163*, 295–302.

Hansel, C., and Linden, D.J. (2000). Long-term depression of the cerebellar climbing fiber–Purkinje neuron synapse. Neuron *26*, 473–482.

Hansel, C., Linden, D.J., and D'Angelo, E. (2001). Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. Nat. Neurosci. *4*, 467–475.

Ho, N., Liauw, J.A., Blaeser, F., Wei, F., Hanissian, S., Muglia, L.M., Wozniak, D.F., Nardi, A., Arvin, K.L., Holtzman, D.M., et al. (2000). Impaired synaptic plasticity and cAMP response element-binding protein activation in Ca2+/calmodulin-dependent protein kinase type IV/Gr-deficient mice. J. Neurosci. *20*, 6459–6472.

Impey, S., Fong, A.L., Wang, Y., Cardinaux, J.R., Fass, D.M., Obrietan, K., Wayman, G.A., Storm, D.R., Soderling, T.R., and Goodman, R.H. (2002). Phosphorylation of CBP mediates transcriptional activation by neural activity and CaM kinase IV. Neuron *34*, 235–244.

Ito, M. (1972). Neural design of the cerebellar motor control system. Brain. Res. *40*, 81–84.

Ito, M. (1982). Cerebellar control of the vestibulo-ocular reflex–around the flocculus hypothesis. Annu. Rev. Neurosci. *5*, 275–296.

Ito, M., Shiida, T., Yagi, N., and Yamamoto, M. (1974). The cerebellar modification of rabbit's horizontal vestibulo-ocular reflex induced by sustained head rotation combined with visual stimulation. Proc. Jpn. Acad. *50*, 85–89.

Ito, M., Sakurai, M., and Tongroach, P. (1982). Climbing fibre induced depression of both mossy fibre responsiveness and glutamate sensitivity of cerebellar Purkinje cells. J. Physiol. *324*, 113–134.

Jager, J., and Henn, V. (1981). Habituation of the vestibulo-ocular reflex (VOR) in the monkey during sinusoidal rotation in the dark. Exp. Brain Res. *41*, 108–114.

Jande, S.S., Maler, L., and Lawson, D.E. (1981). Immunohistochemical mapping of vitamin D-dependent calcium-binding protein in brain. Nature *294*, 765–767.

Jensen, K.F., Ohmstede, C.A., Fisher, R.S., Olin, J.K., and Sahyoun, N. (1991). Acquisition and loss of a neuronal Ca2+/calmodulin-dependent protein kinase during neuronal differentiation. Proc. Natl. Acad. Sci. USA *88*, 4050–4053.

Kang, H., Sun, L.D., Atkins, C.M., Soderling, T.R., Wilson, M.A., and Tonegawa, S. (2001). An important role of neural activity-dependent CaMKIV signaling in the consolidation of long-term memory. Cell *106*, 771–783.

Kano, M., Rexhausen, U., Dreessen, J., and Konnerth, A. (1992). Synaptic excitation produces a long-lasting rebound potentiation of inhibitory synaptic signals in cerebellar Purkinje cells. Nature *356*, 601–604.

Kimpo, R.R., Boyden, E.S., Katoh, A., Ke, M.C., and Raymond, J.L. (2005). Distinct patterns of stimulus generalization of increases and decreases in VOR gain. J. Neurophysiol. *94*, 3092–3100.

Kimura, Y., Corcoran, E.E., Eto, K., Gengyo-Ando, K., Muramatsu, M.A., Kobayashi, R., Freedman, J.H., Mitani, S., Hagiwara, M., Means, A.R., and Tokumitsu, H. (2002). A CaMK cascade activates CRE-mediated transcription in neurons of Caenorhabditis elegans. EMBO Rep. *3*, 962–966.

Kistler, W.M., De Jeu, M.T., Elgersma, Y., Van Der Giessen, R.S., Hensbroek, R., Luo, C., Koekkoek, S.K., Hoogenraad, C.C., Hamers, F.P., Gueldenagel, M., et al. (2002). Analysis of Cx36 knockout does not support tenet that olivary gap junctions are required for complex

spike synchronization and normal motor performance. Ann. N. Y. Acad. Sci. *978*, 391–404.

Koekkoek, S.K., v Alphen, A.M., vd Burg, J., Grosveld, F., Galjart, N., and De Zeeuw, C.I. (1997). Gain adaptation and phase dynamics of compensatory eye movements in mice. Genes Funct. *1*, 175–190.

Koekkoek, S.K., Hulscher, H.C., Dortland, B.R., Hensbroek, R.A., Elgersma, Y., Ruigrok, T.J., and De Zeeuw, C.I. (2003). Cerebellar LTD and learning-dependent timing of conditioned eyelid responses. Science *301*, 1736–1739.

Kogan, J.H., Frankland, P.W., Blendy, J.A., Coblentz, J., Marowitz, Z., Schutz, G., and Silva, A.J. (1997). Spaced training induces normal long-term memory in CREB mutant mice. Curr. Biol. *7*, 1–11.

Kuki, Y., Hirata, Y., Blazquez, P.M., Heiney, S.A., and Highstein, S.M. (2004). Memory retention of vestibuloocular reflex motor learning in squirrel monkeys. Neuroreport *15*, 1007–1011.

Lev-Ram, V., Mehta, S.B., Kleinfeld, D., and Tsien, R.Y. (2003). Reversing cerebellar long-term depression. Proc. Natl. Acad. Sci. USA *100*, 15989–15993.

Li, J., Smith, S.S., and McElligott, J.G. (1995). Cerebellar nitric oxide is necessary for vestibulo-ocular reflex adaptation, a sensorimotor model of learning. J. Neurophysiol. *74*, 489–494.

Lisberger, S.G., and Fuchs, A.F. (1978). 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. *41*, 733–763.

Lisberger, S.G., Pavelko, T.A., Bronte-Stewart, H.M., and Stone, L.S. (1994). Neural basis for motor learning in the vestibuloocular reflex of primates. II. Changes in the responses of horizontal gaze velocity Purkinje cells in the cerebellar flocculus and ventral paraflocculus. J. Neurophysiol. *72*, 954–973.

Luddens, H., Pritchett, D.B., Kohler, M., Killisch, I., Keinanen, K., Monyer, H., Sprengel, R., and Seeburg, P.H. (1990). Cerebellar GABAA receptor selective for a behavioural alcohol antagonist. Nature *346*, 648–651.

Maffei, A., Prestori, F., Shibuki, K., Rossi, P., Taglietti, V., and D'Angelo, E. (2003). NO enhances presynaptic currents during cerebellar mossy fiber-granule cell LTP. J. Neurophysiol. *90*, 2478–2483.

Marr, D. (1969). A theory of cerebellar cortex. J. Physiol. *202*, 437–470.

Martin, S.J., Grimwood, P.D., and Morris, R.G. (2000). Synaptic plasticity and memory: an evaluation of the hypothesis. Annu. Rev. Neurosci. *23*, 649–711.

McKinsey, T.A., Zhang, C.L., Lu, J., and Olson, E.N. (2000). Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. Nature *408*, 106–111.

Means, A.R., Ribar, T.J., Kane, C.D., Hook, S.S., and Anderson, K.A. (1997). Regulation and properties of the rat Ca2+/calmodulin-dependent protein kinase IV gene and its protein products. Recent Prog. Horm. Res. *52*, 389–406.

Miles, F.A., and Eighmy, B.B. (1980). Long-term adaptive changes in primate vestibuloocular reflex. I. Behavioral observations. J. Neurophysiol. *43*, 1406–1425.

Miles, F.A., and Lisberger, S.G. (1981). Plasticity in the vestibulo-ocular reflex: a new hypothesis. Annu. Rev. Neurosci. *4*, 273–299.

Miles, F.A., Fuller, J.H., Braitman, D.J., and Dow, B.M. (1980). Long-term adaptive changes in primate vestibuloocular reflex. III. Electrophysiological observations in flocculus of normal monkeys. J. Neurophysiol. *43*, 1437–1476.

Morii, E., Hirota, S., Kim, H.M., Mikoshiba, K., Nishimune, Y., Kitamura, Y., and Nomura, S. (1992). Spatial expression of genes encoding c-kit receptors and their ligands in mouse cerebellum as revealed by in situ hybridization. Brain Res. Dev. Brain Res. *65*, 123–126.

Mugnaini, E., Berrebi, A.S., Dahl, A.L., and Morgan, J.I. (1987). The polypeptide PEP-19 is a marker for Purkinje neurons in cerebellar cortex and cartwheel neurons in the dorsal cochlear nucleus. Arch. Ital. Biol. *126*, 41–67.

Nagao, S. (1983). Effects of vestibulocerebellar lesions upon dynamic characteristics and adaptation of vestibulo-ocular and optokinetic responses in pigmented rabbits. Exp. Brain Res. *53*, 36–46.

Nagao, S., and Ito, M. (1991). Subdural application of hemoglobin to the cerebellum blocks vestibuloocular reflex adaptation. Neuroreport *2*, 193–196.

Nakanishi, S., Maeda, N., and Mikoshiba, K. (1991). Immunohistochemical localization of an inositol 1,4,5-trisphosphate receptor, P400, in neural tissue: studies in developing and adult mouse brain. J. Neurosci. *11*, 2075–2086.

Nelson, A.B., Krispel, C.M., Sekirnjak, C., and du Lac, S. (2003). Long-lasting increases in intrinsic excitability triggered by inhibition. Neuron *40*, 609–620.

Oberdick, J., Levinthal, F., and Levinthal, C. (1988). A Purkinje cell differentiation marker shows a partial DNA sequence homology to the cellular sis/PDGF2 gene. Neuron *1*, 367–376.

Pastor, A.M., De la Cruz, R.R., and Baker, R. (1997). Characterization of Purkinje cells in the goldfish cerebellum during eye movement and adaptive modification of the vestibulo-ocular reflex. Prog. Brain Res. *114*, 359–381.

Paxinos, G., and Franklin, K.B.J. (2000). The Mouse Brain in Stereotaxic Coordinates (Deluxe Edition), Second Edition (New York: Academic Press).

Peters, M., Mizuno, K., Ris, L., Angelo, M., Godaux, E., and Giese, K.P. (2003). Loss of Ca2+/calmodulin kinase kinase beta affects the formation of some, but not all, types of hippocampus-dependent long-term memory. J. Neurosci. *23*, 9752–9760.

Piedras-Renteria, E.S., Pyle, J.L., Diehn, M., Glickfeld, L.L., Harata, N.C., Cao, Y., Kavalali, E.T., Brown, P.O., and Tsien, R.W. (2004). Presynaptic homeostasis at CNS nerve terminals compensates for lack of a key Ca2+ entry pathway. Proc. Natl. Acad. Sci. USA *101*, 3609–3614.

Rancillac, A., and Crepel, F. (2004). Synapses between parallel fibres and stellate cells express long-term changes in synaptic efficacy in rat cerebellum. J. Physiol. *554*, 707–720.

Raymond, J.L., and Lisberger, S.G. (1996). Behavioral analysis of signals that guide learned changes in the amplitude and dynamics of the vestibulo-ocular reflex. J. Neurosci. *16*, 7791–7802.

Raymond, J.L., and Lisberger, S.G. (1997). Multiple subclasses of Purkinje cells in the primate floccular complex provide similar signals to guide learning in the vestibulo-ocular reflex. Learn. Mem. *3*, 503–518.

Raymond, J.L., and Lisberger, S.G. (1998). Neural learning rules for the vestibulo-ocular reflex. J. Neurosci. *18*, 9112–9129.

Robinson, D.A. (1976). Adaptive gain control of vestibuloocular reflex by the cerebellum. J. Neurophysiol. *39*, 954–969.

Ruigrok, T.J., and Voogd, J. (2000). Organization of projections from the inferior olive to the cerebellar nuclei in the rat. J. Comp. Neurol. *426*, 209–228.

Sakagami, H., Watanabe, M., and Kondo, H. (1992). Gene expression of Ca2+/calmodulin-dependent protein kinase of the cerebellar granule cell type or type IV in the mature and developing rat brain. Brain Res. Mol. Brain Res. *16*, 20–28.

Salin, P.A., Malenka, R.C., and Nicoll, R.A. (1996). Cyclic AMP mediates a presynaptic form of LTP at cerebellar parallel fiber synapses. Neuron *16*, 797–803.

Shibuki, K., Gomi, H., Chen, L., Bao, S., Kim, J.J., Wakatsuki, H., Fujisaki, T., Fujimoto, K., Katoh, A., Ikeda, T., et al. (1996). Deficient cerebellar long-term depression, impaired eyeblink conditioning, and normal motor coordination in GFAP mutant mice. Neuron *16*, 587–599.

Shutoh, F., Ohki, M., Kitazawa, H., Itohara, S., and Nagao, S. (2006). Memory trace of motor learning shifts transsynaptically from cerebellar cortex to nuclei for consolidation. Neuroscience *139*, 767–777.

Smith, S.L., and Otis, T.S. (2003). Persistent changes in spontaneous firing of Purkinje neurons triggered by the nitric oxide signaling cascade. J. Neurosci. *23*, 367–372.

Stahl, J.S. (2004). Eye movements of the murine P/Q calcium channel mutant rocker, and the impact of aging. J. Neurophysiol. *91*, 2066–2078.

Stone, L.S., and Lisberger, S.G. (1990). Visual responses of Purkinje cells in the cerebellar flocculus during smooth-pursuit eye movements in monkeys. I. Simple spikes. J. Neurophysiol. *63*, 1241–1261.

Torte, M.P., Courjon, J.H., Flandrin, J.M., Magnin, M., and Magenes, G. (1994). Anatomical segregation of different adaptative processes within the vestibulocerebellum of the cat. Exp. Brain Res. *99*, 441–454.

Van Alphen, A.M., and De Zeeuw, C.I. (2002). Cerebellar LTD facilitates but is not essential for long-term adaptation of the vestibulo-ocular reflex. Eur. J. Neurosci. *16*, 486–490.

van Alphen, A.M., Stahl, J.S., and De Zeeuw, C.I. (2001). The dynamic characteristics of the mouse horizontal vestibulo-ocular and optokinetic response. Brain Res. *890*, 296–305.

Wall, M.J. (2003). Endogenous nitric oxide modulates GABAergic transmission to granule cells in adult rat cerebellum. Eur. J. Neurosci. *18*, 869–878.

Welsh, J.P., Yamaguchi, H., Zeng, X.H., Kojo, M., Nakada, Y., Takagi, A., Sugimori, M., and Llinas, R.R. (2005). Normal motor learning during pharmacological prevention of Purkinje cell long-term depression. Proc. Natl. Acad. Sci. USA *102*, 17166–17171.

Wong, S.T., Athos, J., Figueroa, X.A., Pineda, V.V., Schaefer, M.L., Chavkin, C.C., Muglia, L.J., and Storm, D.R. (1999). Calcium-stimulated adenylyl cyclase activity is critical for hippocampus-dependent long-term memory and late phase LTP. Neuron *23*, 787–798.