Mechanisms of cerebellar learning suggested by eyelid conditioning

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Classical eyelid conditioning has been used to great advantage in demonstrating that the cerebellum helps to improve movements through experience, and in identifying the underlying mechanisms. Results from recent studies support the hypotheses that learning occurs in both the cerebellar nucleus and cortex, and that these sites make different contributions. Specifically, results indicate that the cerebellar cortex is responsible for temporally specific learning. A combination of experimental and computational studies has been important for arriving at these conclusions, which seem to be applicable to the broad range of movements to which the cerebellum contributes.

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Abbreviations

CS	conditioned stimulus
GABA	γ-aminobutyric acid
gr→Pkj	synapses between granule cells and Purkinje cells
LTD	long-term depression
LTP	long-term potentiation
mf→nuc	synapses between mossy fibers and cerebellar nucleus cells
NMDA	*N*-methyl-D-aspartate
US	unconditioned stimulus
VOR	vestibulo-ocular reflex

Introduction

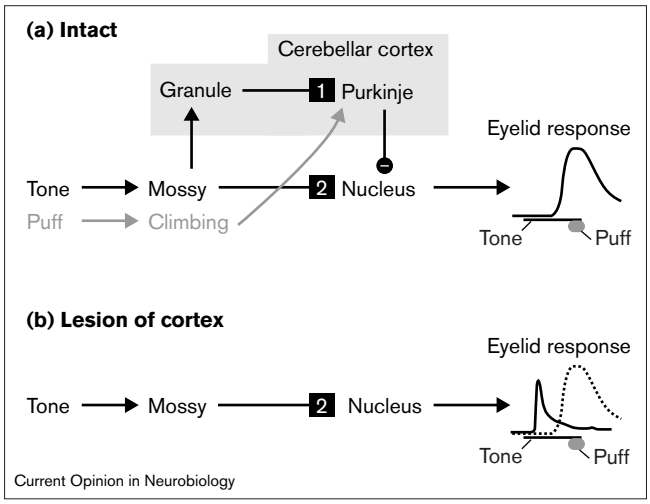
One of the most notable features of the cerebellum is the consistency or regularity of synaptic organization throughout its different regions [1,2]. Thus, although these different regions clearly contribute to different movements, and some perhaps even to non-motor processes [3–6], the computation that the cerebellum contributes in each case is likely to be the same [1]. For this reason, the cerebellum lends itself well to analyses examining a variety of movements, each of which has its own investigative strengths. Examples include well-practiced single and multi-joint movements in monkeys [7], adaptation of eye movement systems (vestibulo-ocular reflex [VOR] [8,9], saccades [8,10], smooth pursuit [11,12]), and classical or Pavlovian conditioning of eyelid responses [13,14]. A relatively consistent picture of how the cerebellum functions seems to be emerging from these various systems. Here we outline how the inherent advantages of eyelid conditioning have been used to help characterize the role of the cerebellum in motor learning.

Eyelid conditioning involves paired presentations of a neutral conditioned stimulus (CS) such as a tone, and a reinforcing unconditioned stimulus (US) such as a puff of air in the eye. (To avoid jargon, we will use tone and puff instead of the more general CS and US, respectively, in describing the stimuli used in eyelid conditioning. However, the reader should not assume that the results discussed are necessarily specific to the use of these two stimuli.) Whereas initially the tone does not elicit an eyelid response, after a few hundred tone+puff training trials the eyelid will close in response to the tone. A variety of techniques that include lesion [15–19,20••], recording [15,21], stimulation [22], reversible inactivation [23,24], brain-imaging [25,26] and computer simulations [27••,28–30] indicate that the cerebellum is essential for the acquisition and expression of these conditioned eyelid responses. Two advantages of eyelid conditioning, experimenter control over stimuli and the relatively direct mapping of stimuli onto cerebellar afferents (Figure 1), have been instrumental in distinguishing the relative contributions of the cerebellar cortex and cerebellar nuclei, and in characterizing the temporal specificity of cerebellar-mediated learning.

Eyelid conditioning reveals cerebellar input/output transformations

The utility of eyelid conditioning as a means of studying the cerebellum stems from the relatively direct mapping of stimuli to cerebellar inputs and of cerebellar outputs to behavior [13,14]. Previous work has shown that during eyelid conditioning the conditioned stimuli, such as tones, are conveyed to the cerebellum via mossy fiber afferents [22,31], and the reinforcing stimuli, such as puffs in the eye, are conveyed via climbing fibers [22,32] (Figure 1a). Moreover, output from the cerebellum via one of the cerebellar deep nuclei (the anterior interpositus nucleus) drives the expression of conditioned eyelid responses [15]. Thus, the behavioral properties of eyelid conditioning reflect the input/output transformations of the cerebellum. For example, the ability of paired tone+puff presentations to promote conditioned eyelid responses reveals two basic features of cerebellar input/output transformations: first, paired activation of mossy fiber and climbing fiber inputs eventually leads to increased cerebellar output in response to the same mossy fiber input; and second, the particular set of climbing fiber inputs activated by the puff determines the precise cerebellar nucleus cells and muscles that will come to be activated after learning. During eyelid conditioning, activation of eyelid-related climbing fibers by the puff will result in increased activity in the anterior interpositus nucleus, which contains the subset of nucleus cells responsible for closing the eyelid. Analysis of VOR adaptation suggests the generality of this input/output transformation capability: head acceleration (mossy fibers) repeatedly paired with image motion on the retina (climbing fibers) influences cerebellar output neurons that adjust the gain of the VOR and reduce the image motion [14].

Figure 1



A schematic representation of the basic circuitry of the cerebellum and its relationship to eyelid conditioning. **(a)** The tone and puff stimuli in eyelid conditioning are conveyed to the cerebellum by mossy fiber and climbing fiber afferents, respectively. Similarly, output from the cerebellar interpositus nucleus begins the motor pathway that is necessary and sufficient for the expression of conditioned responses. This mapping of stimuli onto cerebellar inputs and cerebellar output onto responses makes the behavioral properties of eyelid conditioning a good model of the input/output properties of the cerebellum. Evidence also suggests that two sites of plasticity are involved in eyelid conditioning. These excitatory, modifiable synapses are shown as squares and labeled 1 (granule to Purkinje synapses, *gr→Pkj*) and 2 (mossy fiber to cerebellar nucleus synapses, *mf→nuc*). For the remaining synapses, arrows indicate excitatory synapses and circles inhibitory. **(b)** Evidence indicates that lesion of the cerebellar cortex unmasks short-latency responses that may be mediated by some form of plasticity in the interpositus nucleus. These results also suggest that learning in the cerebellar cortex is temporally specific.

The temporal properties of eyelid conditioning have been particularly useful in revealing important features of cerebellar processing. For learning to occur, the tone must precede puff onset by at least 100 ms, and the ability to learn gradually worsens as the interval increases beyond 500 ms [33,34]. The time interval between the onsets of the tone and puff also affects the timing of conditioned eyelid responses. Response onsets are delayed and rise-times gauged such that maximum eyelid closure always occurs at the time when the puff is expected [35,36] (Figure 1a). As this learned timing occurs even when direct electrical stimulation of mossy fibers and climbing fibers substitutes for tone and puff respectively [22,31,32], the capacity for temporally specific learning appears to be an intrinsic property of cerebellar processing, and one that provides clues about the general computation accomplished by the cerebellum. In support, recent evidence indicates that adaptation of the VOR displays similar temporal specificity [14].

These findings indicate that the cerebellum solves a temporal variant of the so-called 'credit assignment problem': that is, what should the cerebellum change when a particular mossy fiber input (tone) consistently predicts the need to move a particular part of the body as signaled by the climbing fiber input (puff)? The solution cannot simply entail learning a new cerebellar output in response to the mossy fiber input, because that may not result in movement at the appropriate time (i.e. protecting the eye by closing the eyelid right before the puff is delivered). Consider the events that occur when you stub your toe while walking and a particular set of climbing fibers is activated signaling a motor error and the need to modify the stride. There are distinct mossy fiber inputs occurring at different times during the stride that convey to the cerebellum information about the current position and angle of individual joints. If the cerebellum changed movement components temporally tied to all of the mossy fiber inputs activated during the stride, the resulting changes might affect portions of the stride that were appropriate and did not need to be fixed. Instead, as suggested by eyelid conditioning, the ability of the cerebellum to change its output with proper delays with respect to mossy fiber inputs so that the errant component of the movement is specifically fixed is central to what the cerebellum accomplishes. How does the cerebellum accomplish this temporally specific learning? Recent lesion, stimulation and modeling studies suggest a central role of the cerebellar cortex.

Role of the cerebellar cortex in temporally specific motor learning

Debates over the role of the cerebellar cortex in motor learning have been fueled by a collection of controversial results regarding the effects of cerebellar cortex lesions on eyelid conditioning. Although early studies showed that lesions of the cerebellar cortex had no effect on previously acquired responses [15], later experiments showed that responses were either transiently or permanently abolished by lesions of the HVI region of cerebellar cortex [16,17]. Subsequent studies showed that learning of new responses was impaired, but not prevented, by either large cerebellar cortex lesions [37] or those aimed at the HVI region [38]. Adding more fuel to the controversy, Perrett and others later showed that cerebellar cortex lesions that included a different region, the anterior lobe, spared previously learned conditioned responses but disrupted their learned timing [19]. Post-lesion responses were found to display a short and relatively fixed latency to onset that was independent of their pre-lesion timing (Figure 1b). This timing effect not only suggested a role of the cerebellar cortex in temporally specific learning but, as discussed below, it also provided a means to resolve debates about the controversial role of the cerebellar cortex in acquiring and extinguishing responses [18,20••].

Recent lesion and reversible inactivation studies

The main undercurrent throughout these debates has been the identification of the region of cerebellar cortex relevant to eyelid conditioning. This issue is complicated by difficulties inherent in identifying the functional extent of lesions or ensuring that they do not involve unintended damage to other essential structures [17]. Recent studies

employing reversible disconnection of the cerebellar cortex have provided a way to obviate these limitations [24]. Krupa *et al*. [23] first showed that inactivation of the interpositus nucleus with the GABA agonist muscimol abolishes conditioned eyelid responses. Subsequently, Garcia and Mauk [24] replicated these findings, ensuring that that the tip of each cannula was positioned in the eyelid region of the interpositus nucleus. Then, in the same animals, they showed that infusion of the GABA antagonist picrotoxin through the same cannula, which blocks the inhibitory synapses between the Purkinje and nucleus cells, disrupted the timing of conditioned responses. Because this manipulation is the equivalent of removing all cerebellar cortical input to the critical eyelid region of the interpositus nucleus, the results provide further support that the cerebellar cortex is necessary for response timing and also suggest criteria for identifying lesions that include regions of the cerebellar cortex relevant to eyelid conditioning. Lesions that do not affect the timing of previously learned responses are proposed to have missed a crucial region of cerebellar cortex, whereas lesions that abolish responses completely are likely to have included unintended damage to the interpositus nucleus or to related pathways that are necessary for response expression. Studies using these lesion criteria have shown that the cerebellar cortex is necessary for extinction of already learned responses [18] and for acquisition of new responses [20••].

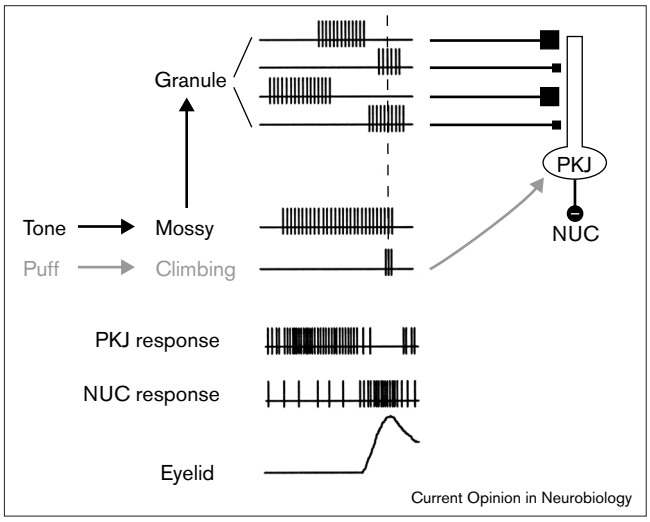
Recent stimulation studies

Although lesions can demonstrate that the cerebellar cortex is necessary for temporally specific learning, alone they cannot distinguish temporal coding mechanisms within the cerebellar cortex from temporal coding that is conveyed to the cerebellum. Indeed, there are two competing ideas about how and where temporally specific motor learning is achieved. One idea states that cells in the pontine nuclei could pre-process input and provide time-varying activation of mossy fibers which could then be used to generate a well-timed response [29]. The alternative view proposes that the intrinsic circuitry of the cerebellar cortex can produce properly timed responses even without time-varying mossy fiber inputs [27••,28,30].

Recent studies by Hesslow's group [39•] seem to support this second hypothesis [40••]. After standard training, very brief direct stimulation of mossy fibers could substitute as the 'tone' and produce well-timed conditioned responses. The stimulation-elicited responses were unaffected by pharmacological blockade behind the stimulation site, which provided an important control to ensure that the cerebellum was the only target of the stimulation. These data show that the cerebellum does not require time-varying mossy fiber activity in order to generate properly timed responses.

As the Purkinje cells in the cerebellar cortex are known to inhibit the nucleus cells responsible for generating the conditioned response [2,41], it appears that response timing is mediated by learning a well-timed pause in Purkinje cell activity in response to the tone (Figure 2). This hypothesis is consistent with Purkinje cell recordings from identified eyelid regions of cerebellar cortex [42] and with the observation that direct stimulation of the cerebellar cortex prevents the expression of responses [43] (presumably by overriding the learned pause in Purkinje cell activity).

Figure 2



Proposed cerebellar cortex mechanisms involved in temporally specific learning. When the tone activates certain mossy fibers, interactions in the cerebellar cortex give rise to activation of different granule cells at different times during the stimulus. Those synapses that are active when the puff (US) arrives and activates a climbing fiber input undergo LTD, as schematized by the smaller squares. In contrast, synapses that are active in the absence of the climbing fiber input (during the early parts of the tone) undergo LTP. The induction of these changes during learning allows the Purkinje cell (PKJ) to pause in activity at just the right time, producing a properly timed conditioned response by disinhibiting the nucleus cell (NUC).

Computer simulation studies

As with all brain systems, the cerebellum receives inputs and generates outputs according to its internal rules for information processing. In trying to understand how the brain works, computer simulations can be particularly useful because they inherently address function at this basic level. In the case of the cerebellum, the development of detailed computer simulations has benefited greatly from the wealth of information about cerebellar circuitry and physiology [2] and about how this circuitry is engaged by simple forms of motor learning such as adaptation of the VOR and eyelid conditioning [14]. These biologically constrained simulations also incorporate climbing-fiber-controlled plasticity at granule to Purkinje (*gr→Pkj*) synapses in the cerebellar cortex [27••,28–30]. This is supported by evidence regarding long-term depression (LTD) and long-term potentiation (LTP) at these synapses [44–48]. LTD occurs when *gr→Pkj* synapses are active in the presence of a climbing fiber input, whereas activating the synapses without a climbing fiber input produces LTP.

A large-scale computer simulation of eyelid conditioning has recently suggested new ideas about how the cerebellum may accomplish temporally specific learning [27••]. The activity of Purkinje cells in simulations that have been trained using the standard eyelid conditioning protocol showed a learned bi-phasic modulation in response to the tone. As shown in Figure 2, the simulated Purkinje cells showed an initial increase in activity just after the onset of the tone (produced by LTP at a different set of *gr→Pkj* synapses), followed by a pause around the time of the puff (produced by LTD of *gr→Pkj* synapses). Thus, Purkinje cells learned not only to pause in their responding at the time of the puff, but also learned to actively suppress responding at earlier times when the tone was present but a response was not required.

The hypothesis that Purkinje cells contribute to timing in part by learning to suppress responding at times when a response is not needed led to an unusual prediction about the pattern of responding that would be expected after partial lesions of the cerebellar cortex [27••]. This pattern of responding was confirmed in a group of rabbits that were subjected to small, electrolytic lesions of cerebellar cortex after having received extensive training with standard eyelid conditioning trials [27••]. Immediately after the lesion, the timing of conditioned responses showed a maladaptive short-latency component in addition to the timed component. This observation is consistent with a partial disruption of the learned increases in Purkinje cell activity that in the intact rabbits help suppress responding early during the presentation of the tone (when a response is not required). With further training, however, the short-latency component gradually decreased, consistent with a partially spared learning capacity of the remaining Purkinje cells to suppress the early maladaptive component.

Plasticity in the cerebellar cortex

One of the most fundamental challenges in neuroscience is to link behavioral properties of learning with cellular properties of plasticity. The well-characterized form of climbing-fiber-dependent plasticity at *gr→Pkj* synapses offers great potential in this regard. However, recent cerebellar simulations of eyelid conditioning have revealed that the properties of plasticity in the cerebellar cortex, as they are currently understood, may not be capable of explaining the cellular changes associated with cerebellar-dependent motor learning.

One potential shortcoming relates to the need for LTP and LTD mechanisms at *gr→Pkj* synapses to be mutually reversing. As might be expected intuitively, the computer simulations require this property; otherwise both LTP and LTD soon saturate and no further learning is possible. Although evidence to date suggests that LTD and LTP at *gr→Pkj* synapses are not mutually reversing because the expression of the former seems to be presynaptic [47,48] whereas that of the latter is clearly postsynaptic [46], these studies cannot rule out the possibility of additional LTP/LTD mechanisms that reverse each other. In fact, Bell and colleagues have recently provided evidence for bidirectional and reversing plasticity at these synapses in weakly electric fish [49,50•].

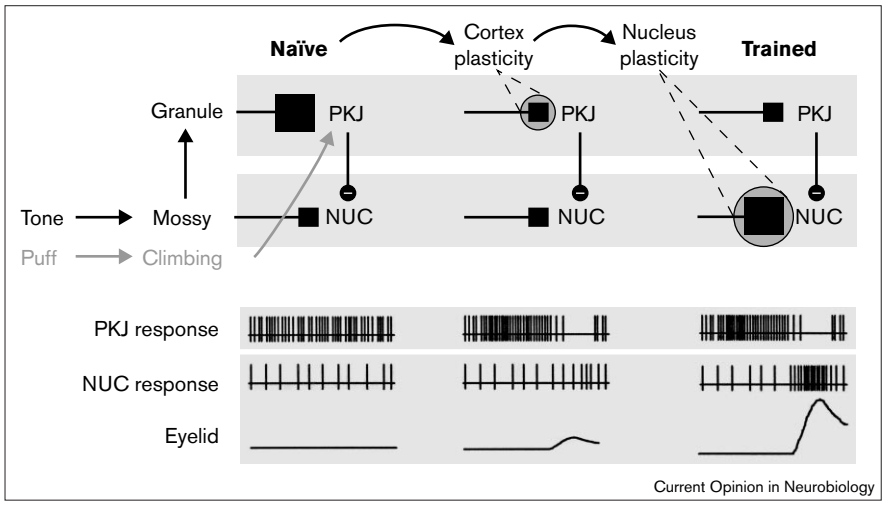
The timing of granule and climbing fiber inputs required for the induction of LTD at *gr→Pkj* synapses is often cited as damaging for theories that attempt to link this form of plasticity to cerebellar motor learning [51,52]. Early studies showed that almost simultaneous activation of granule cell and climbing fiber inputs is effective for inducing LTD [53–55]. When this property is incorporated, simulations learn appropriately timed responses, but they display one important flaw. The simulations learn even when the tone (granule cells) and puff (climbing fiber) are presented simultaneously, whereas rabbits require the tone to precede the puff by at least 100 ms in order to support eyelid conditioning [33,34]. This raises an important question: does this apparent mismatch between the properties of LTD and eyelid conditioning mean the former does not contribute to the latter?

Recent results from Raymond and Lisberger may provide new insights to this question [56]. These authors used Purkinje cell recordings to examine the patterns of granule cell and climbing fiber inputs during adaptation of the VOR. Their fascinating results suggest that climbing fiber inputs may induce learning in granule cell inputs that were active approximately 100 ms earlier. In preliminary results from simulations that incorporate this rule for LTD, we find that the simulations learn properly timed responses with appropriate sensitivity to the interval of time between the tone and the puff. It remains unclear whether this implies that the signals for the induction of LTD at *gr→Pkj* synapses are slightly different *in vitro* and *in vivo*, or whether a new form of climbing-fiber-controlled plasticity exists *in vivo* that has not yet been characterized *in vitro*.

Plasticity in the cerebellar nucleus

Accumulating evidence from eyelid conditioning and adaptation of the VOR indicates that plasticity in the cerebellar cortex is not sufficient to account for all of the learning that takes place during cerebellar-dependent motor learning [14,57]. For example, the observation that full lesions of the cerebellar cortex do not completely abolish conditioned responses has been interpreted as evidence for learning in the cerebellar nucleus [19,24]. Thus, learning-induced changes at mossy fiber to nucleus (*mf→nuc*) synapses, or perhaps changes in nucleus cell excitability, are presumed to produce the short-latency responses that remain after a cerebellar cortex lesion [19,24] (Figure 1b). This hypothesis is consistent with a variety of lesion, stimulation, recording, and reversible inactivation studies that indirectly point to the cerebellar nucleus as a potential site of plasticity [14,15,19,23,24,57,58]. At the moment, however, the involvement of other pre-cerebellar sites in generating the short-latency response cannot be completely excluded and the role played by plasticity in the nucleus during motor

Figure 3



Sequence of events believed to occur during acquisition of a well-timed conditioned eyelid response. In the naïve animal (left column) the *gr→Pkj* synapses activated by the tone are relatively strong (as indicated by the large square) and the *mf→nuc* synapses activated by the tone are relatively weak (as indicated by the small square). When the tone is presented, the Purkinje cells (PKJ) maintain their high ongoing activity, inhibiting nucleus cells (NUC) and precluding a response. Paired presentations of tone+puff lead to LTD at the tone-activated *gr→Pkj* synapses (cortex plasticity, middle column). For this reason, Purkinje cell activity comes to a pause during the tone (for timing, see Figure 2). However, at this time the conditioned responses may be small or absent as a result of lack of plasticity in the cerebellar nucleus. We have suggested that the learned pause in Purkinje cell activity could serve as a signal to induce plasticity (LTP at *mf→nuc* synapses or perhaps increased nucleus cell excitability, or both) in the nucleus. However, the precise nature of the signals controlling the induction of plasticity in the nucleus is still under investigation. After the induction of plasticity in the nucleus (nucleus plasticity, right column), robust conditioned responses are possible.

learning remains an important and unresolved question. We end with a brief review of recent findings that support the hypothesis of plasticity in the cerebellar nucleus.

Physiological evidence

Despite the indirect evidence for its proposed role during motor learning, physiological analyses of plasticity in the cerebellar nucleus have been sparse. In addition to the solitary report of plasticity at the *mf→nuc* synapses published a number of years ago [59], there have been more recent discoveries of plasticity at the Purkinje to nucleus synapses [60] and of activity-dependent changes in excitability within the nucleus cells [61••]. In this latter study, it was demonstrated that neurons of the cerebellar deep nuclei show persistent increases in excitability following Ca^{2+} entry, either by activation of NMDA receptors, by direct depolarization, or possibly by a burst–pause stimulus delivered to the Purkinje cells. To our knowledge, these forms of plasticity have not yet been incorporated into a simulation of the cerebellum. In coming years, it will be interesting to examine the implications of these forms of plasticity for theories and models of cerebellar-dependent learning.

Signals that guide learning in the cerebellar nuclei: open questions

In addition to being used as a tool to identify sites of plasticity, eyelid conditioning can also provide insights into the patterns of neural activity that are necessary for the induction of this plasticity *in vivo*. During eyelid conditioning, the induction of plasticity in the cerebellar cortex is normally presumed to follow the rules implied by electrophysiological studies of plasticity at *gr→Pkj* synapses [46–48]. Thus, there is general agreement that during acquisition, activation of the climbing fiber pathway by the US leads to the induction of LTD at *gr→Pkj* synapses that are activated by the tone. This decrease in synaptic strength would ultimately result in a decrease in Purkinje cell activity during the tone, which could contribute to the generation of the conditioned response by disinhibiting the nucleus cells [27••,41] (Figures 2 and 3). Although it is known that major inputs to the cerebellar nuclei arise from the Purkinje cell axons as well as from collaterals of both mossy fibers and climbing fibers [2], much less is known about which combinations of these inputs are required to induce plasticity in the cerebellar nucleus of awake behaving animals.

One recent hypothesis suggests that pauses in the ongoing inhibitory input from Purkinje cells are well-suited to signal the induction of LTP in the cerebellar nucleus [62•], possibly by releasing nucleus cells from hyperpolarization and activating a T-type calcium conductance [60,61••,63]. According to this proposition, learning takes place in the cerebellar cortex first, and then the resulting changes in Purkinje cell activity drive learning in the cerebellar nucleus (Figure 3). Consistent with this hypothesis that plasticity in the cerebellar nucleus is under the control of Purkinje cell inputs, lesions of the anterior lobe of cerebellar cortex have recently been shown to prevent the acquisition [20••] and extinction [18] of conditioned eyelid responses. Also consistent with this hypothesis, Purkinje cell activity from the floccular complex has been shown to contain information that is appropriate for guiding adaptation of the VOR at low frequencies [14].

A second hypothesis suggests that the cerebellar cortex plays only a minor role and is not required for learning in the cerebellar nucleus. In support of this proposition,

mutant mice lacking Purkinje cells have been shown to be impaired during eyelid conditioning but still to be capable of acquiring conditioned eyelid responses, whereas the same mutants with lesions of the cerebellar nucleus cannot learn at all [64,65••]. Given the potential for compensatory mechanisms in both animals with permanent lesions and mutants, addressing the controversial role played by the cerebellar cortex in the induction of plasticity in the cerebellar nucleus will probably require the use of reversible lesions or inducible mutations.

Conclusions

Although there are a number of important questions that remain unanswered, analysis of eyelid conditioning has reached a critical mass that permits quite concrete hypotheses regarding the events that occur during cerebellar-dependent motor learning (Figure 3). These studies suggest that cerebellar learning engages plasticity in both the cerebellar cortex and cerebellar nucleus [14,57]. Bidirectional plasticity at *gr→Pkj* synapses in the cerebellar cortex is well suited to contribute to temporally specific learning [27••,66] by generating appropriately timed increases and decreases in Purkinje cell activity [42], which ultimately modulate cerebellar nucleus output [2,41]. The role played by plasticity in the cerebellar nucleus, as well as the conditions required for its induction, are less well understood. However, as evidence suggests that plasticity in the cerebellar nucleus is necessary for response expression [23], understanding more about this plasticity seems essential.

Thus, while debates continue regarding the precise nature of cerebellar function (motor, cognitive, timing, etc.) [1,3–7,67], we believe that the cerebellum is best understood in terms of how it accomplishes its input/output transformations. By understanding the computation performed during simple forms of motor learning such as eyelid conditioning, we may be better able to appreciate how other brain systems tap into the cerebellum in order to influence various motor and cognitive functions.

Acknowledgements

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