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Does Cerebellar LTD Mediate Motor Learning? Toward a Resolution without a Smoking Gun

Minireview

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"Every time I read a paper that says the cerebellum mediates motor learning, it makes me want to vomit."

—A cerebellar researcher.

In a humorous way, this comment typifies the intensity and the futility of the ongoing debate regarding the putative role of cerebellar synaptic plasticity in motor learning. This debate intensified in 1969 when David Marr proposed a theory suggesting how the cerebellar cortex could contribute to motor learning. A key component of this theory states that the climbing fiber input to a Purkinje cell induces plasticity at coactive granule cell synapses (gr→Pkj) onto the same Purkinje cell (Figure 2). Thirteen years later, Ito et al. (see Ito, 1989) provided the first support for this proposition by showing that coactivation of granule cell and climbing fiber inputs produces a long-term depression (LTD) of gr→Pkj synaptic strength. However, as illustrated by the quote, the role of LTD in cerebellar-mediated motor learning remains a hotly debated, and even emotional, issue.

Anyone with a passing interest in the cerebellum or motor learning has probably witnessed a stormy session at a neuroscience meeting and could easily find dozens of reviews arguing passionately for or against a role for LTD in motor learning. Many colleagues have complained that this controversy, while important and spirited, has become repetitious. We agree. As such, another review that simply extols the virtues of one side seems useless. Instead, our goal here is to consider a path that leads to light at the end of the tunnel and to a resolution of this debate. We will propose a series of criteria that it should be possible to satisfy if LTD is causally related to cerebellar-mediated motor learning.

Why is it necessary to specify a set of criteria rather than a single, smoking-gun observation that could resolve this issue? The answer largely derives from the sparse, distributed nature of stimulus representations in the vertebrate nervous system. Each Purkinje cell, for example, receives excitatory input from upwards of 200,000 granule cells. Since any stimulus is likely to activate only a small percentage of these inputs, learning would involve the induction of LTD in a small proportion of gr→Pkj synapses. Since the small percentage of synapses that change are probably not spatially segregated, detecting plasticity with standard stimulate-and-record techniques would be extraordinarily difficult. Worse still, any tendency for other synapses onto the same cell to increase in strength during learning would make this task virtually impossible. Thus, smoking gun experiments will probably require new technologies such as in vivo imaging of (many) individual synapses or the ability to tag recently modified synapses with a marker.

Until then, the best approaches will require a series of indirect observations that together support involvement of plasticity in a particular form of learning. Because they are indirect, satisfying any of the criteria would not establish a causal link between cerebellar LTD and motor learning. However, clearly contradicting any one criterion, with a caveat or two, should make it possible to reject LTD as a candidate mechanism for the target form of learning. To the extent that these criteria are comprehensive, satisfying each of them would constitute, given the absence of a smoking gun technology, the strongest evidence possible for a causal link between LTD and motor learning.

Necessary Starting Conditions

Evaluating the functional significance of synaptic plasticity and applying the criteria we propose requires a form of learning whose behavioral properties and relationships to the target brain system are well characterized. Analysis of many mutant mice, for example, has emphasized the effects of the mutation on tasks such as the rotorod. While it is clear that cerebellar damage impairs rotorod performance, the relationship is not commutative; rotorod deficits do not necessarily imply cerebellar dysfunction. In contrast, analyses of the role of LTD in motor learning benefit greatly from the way in which relatively simple forms of motor learning such as adaptation of the vestibulo-ocular reflex (VOR) and Pavlovian eyelid conditioning satisfy this necessity (Raymond et al., 1996). For brevity, the present discussion will be expressed in terms of cerebellar involvement in eyelid conditioning.

Establishing cerebellar involvement in eyelid conditioning has been facilitated by the relative simplicity and tractability of Pavlovian conditioning. In eyelid conditioning, paired presentation of a conditioned stimulus, such as a tone, with a reinforcing stimulus, such as an air puff in the eye, promotes the acquisition of a well-timed conditioned eyelid response (Figure 1). In a trained animal, presentation of the tone elicits a response in which the onset is delayed and rise time gauged so that eyelid closure peaks near the time at which the reinforcing stimulus usually arrives. Thus, the animal not only learns to close its eye, but also learns the appropriate response timing. Given this simplicity, it has been relatively easy to demonstrate that cerebellar output drives the expression of the conditioned responses and that lesions of the cerebellar nuclei abolish previously learned responses and prevent all subsequent learning (Thompson, 1986; Mauk and Donegan, 1997). These observations make it possible to ask: does the induction of LTD at gr→Pkj synapses contribute to the ability of the tone to elicit the conditioned responses through well-timed activation of the appropriate cerebellar outputs?

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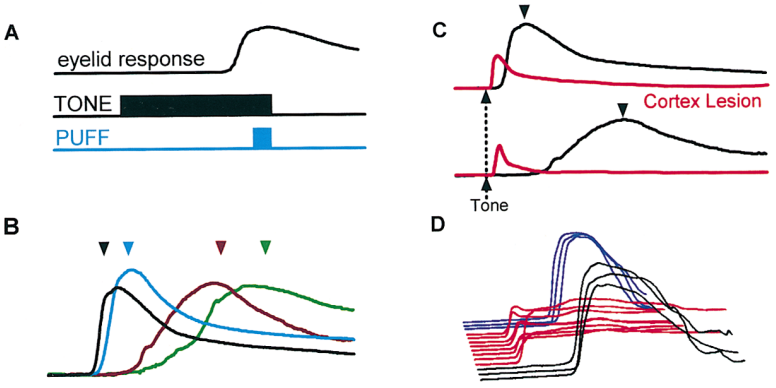

Figure 1. Behavioral Properties of Eyelid Conditioning

(A) In eyelid conditioning, the paired presentation of a tone and an air puff directed at the eye results in the acquisition of a learned eyelid closure in response to the tone.

(B) The timing of the conditioned responses is also learned; responses are delayed to peak near puff onset (color coded arrows).

(C) Removal of the cerebellar cortex abolishes response timing; following a lesion, responses are smaller and occur at a fixed, short latency independent of pre-lesion timing.

(D) Reversible pharmacological block of cerebellar cortex output similarly affects the timing of conditioned responses; normal responses are black, responses after pharmacological block are red, and normally timed responses seen the next day are blue.

Criteria

If LTD at gr→Pkj synapses contributes to eyelid conditioning, it should be possible to satisfy the following criteria.

(1) Necessity of convergence. Since eyelid conditioning requires the copresentation of the tone and puff, gr→Pkj synapses must be at a site of convergence between pathways activated by these stimuli.

(2) Sufficiency of induction. Eyelid conditioning procedures should elicit patterns of granule cell and climbing fiber activity that are sufficient to induce LTD.

(3) Capacity for expression. Changes in Purkinje cell activity that would result from the induction of LTD at gr→Pkj synapses should have the capacity to contribute to the expression of conditioned eyelid responses.

(4) Necessity for learning. Blocking LTD should prevent learning, and removing or reversing LTD should abolish the expression of that learning.

Without the capabilities for a definitive, smoking-gun experiment, debates about the involvement of LTD in motor learning can benefit from the focus provided by the above criteria. With the remainder of this review we will consider, not in this order, the extent to which these criteria have been addressed in the context of eyelid conditioning.

Testing Capacity for Expression

Capacity for expression is an expectation about the relationship between LTD at gr→Pkj synapses and the anatomy downstream from the Purkinje cells. The ability to address this criterion is provided by experiments showing that cerebellar output drives the expression of conditioned responses (Figure 2). Briefly, the necessity and sufficiency of cerebellar nucleus output in eliciting the conditioned responses is supported by studies showing that (1) micro-stimulation of the interpositus nucleus can elicit robust eyelid responses, even in untrained animals; (2) lesions of the interpositus nucleus abolish conditioned eyelid responses; and (3) interpositus neurons show increased activity during the expression of responses.

It seems reasonably clear that the induction of LTD at gr→Pkj synapses has the capacity to contribute to the expression of conditioned eyelid responses. Since activity in the appropriate interpositus cells is necessary and sufficient to elicit responses, and since Purkinje cells inhibit nucleus cells, activity in Purkinje cells should suppress the expression of responses, and decreases in activity would therefore have the capacity to help elicit conditioned responses. Indeed, because Purkinje cells display high rates of ongoing activity, and because stimulation of the cerebellar cortex suppresses conditioned responses (Hesslow, 1994), learned decreases in activity during the tone seem necessary for response expression. In support, recording studies have revealed decreases in Purkinje activity during the expression of conditioned eyelid responses. Increases in Purkinje cell activity during eyelid responses have also been seen. However, the capacity for expression criterion should only apply to those Purkinje cells whose climbing fiber input is activated by the puff; many Purkinje cells are likely to be involved in other components of the movement, such as turning the head away from the puff. Bearing these points in mind, convergent anatomical and physiological evidence suggest with reasonable certainty that capacity for expression is satisfied for LTD.

Testing Necessity of Convergence

Several experiments have addressed this criterion by demonstrating that the tone is conveyed to the cerebellum via mossy fiber inputs, and that the puff is conveyed to the cerebellum via activation of climbing fibers (Figure 2). Briefly, lesions of mossy fiber and climbing fiber inputs produce effects equivalent to omission of the tone and the puff, respectively, whereas stimulation of these pathways can substitute respectively for tone and puff presentation to support normal eyelid conditioning. Moreover, recording studies have shown that mossy fibers can be activated by auditory stimuli.

These findings point to the gr→Pkj synapses as a site of convergence of tone and puff pathways and highlight the capacity for LTD-mediated changes in Purkinje cell activity to influence the expression of conditioned responses. It has not yet been possible to record from granule cells during eyelid conditioning. However, the clear evidence that mossy fibers are activated by tones and that mossy fibers excite granule cells makes tone activation of granule cells very likely. In support, several studies have shown changes in Purkinje cell simple spike activity during tone presentations, consistent with the idea that granule cell inputs during the tone must be different from background. The evidence for climbing

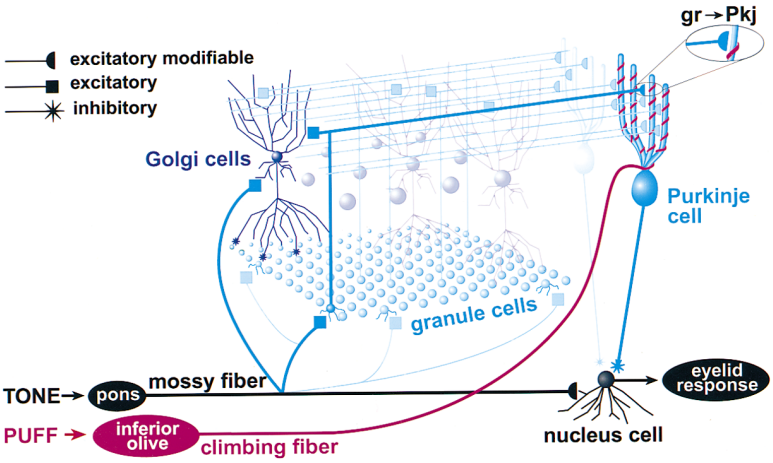

Figure 2. The Anatomy of Eyelid Conditioning

This schematic illustrates the basic relationships between the anatomy of the cerebellum and the pathways involved in eyelid conditioning (Mauk and Donegan, 1997). Tones and puffs are conveyed to the cerebellum via mossy fiber (black) and climbing fiber (red) pathways. Increased output of the interpositus nucleus elicits the conditioned eyelid response. Two cerebellar pathways appear to be involved in response expression: a relatively direct pathway (black) and a less direct pathway through the cerebellar cortex (blue). Key features related to LTD at gr→Pkj synapses are: (1) Capacity for expression. Since Purkinje cells inhibit nucleus cells, an LTD-mediated decrease in Purkinje cell activity could contribute to response expression. (2) Necessity of convergence. Tone and puff pathways converge at the gr→Pkj synapses. (3) Necessity for expression. Putative plasticity at both gr→Pkj synapses and mossy fiber synapses in the nucleus (triangles) complicate analysis of LTD's role. (4) Sufficiency for induction. Granule cell activity is influenced by interactions with Golgi cells (purple), which may affect the required temporal properties of LTD induction.

fiber activation by the puffs is more direct; several groups have shown that puff presentations elicit climbing fiber–mediated complex spikes in Purkinje cells. Thus, it seems fairly safe to conclude that necessity for convergence is satisfied.

Testing Necessity for Learning

Of the four, necessity for learning is by far the most commonly addressed, and most taken-for-granted, criterion. In general, it is relatively easy to make a manipulation (lesion or mutation) and then determine its effect on eyelid conditioning. However, several factors make satisfying this criterion much more demanding than simply testing whether an animal can acquire conditioned responses. For brevity, we will focus on only one such factor; the evidence that eyelid conditioning is mediated by plasticity in both the cerebellar cortex and nucleus.

The classic first step in assessing necessity for learning is to assess the effects of lesions. However, the effects of cerebellar cortex lesions are difficult to determine due to a catch 22. To be informative, lesions must obviously include a sufficiently large portion of the critical region without inadvertently damaging other key structures such as the cerebellar nuclei. Without such assurance, overly large lesions may produce false positives by abolishing responses due to inadvertent damage to other pathways, and small lesions may produce false negatives by sparing responses. Given this potential for misdirection, it is difficult to determine the critical regions of cerebellar cortex without knowing what the effects of a selective cortex lesion are, and it is necessary to make selective lesions to determine those effects.

Pharmacological block of the GABAergic Purkinje cell projections to the interpositus nucleus provides one escape from this dilemma. Temporarily disconnecting the cerebellar cortex output in this way obviates the need to know exactly what regions of cortex may be involved. Recent studies have shown that infusion of the GABA antagonist picrotoxin into the interpositus nucleus of well-trained rabbits reduces the amplitude and abolishes the timing of the conditioned responses (Garcia and Mauk, 1998; Figure 1C). These same effects are produced by lesions of the cerebellar cortex when the damage includes the anterior lobe (Perrett and Mauk, 1995; Perrett et al., 1993). Thus, one effect of removing the appropriate region of cerebellar cortex is disruption of the learned timing of eyelid responses.

These results provide the opportunity to address the necessity of the cerebellar cortex for learning. By assessing the effects of lesions on previously trained responses, it is possible to use short latency as opposed to well-timed responses as an index of sufficiently extensive cerebellar cortex damage, and as an index that the interpositus nucleus and critical input/output pathways are intact. These studies have provided consistent and clear results. Animals with lesions of the cerebellar cortex can neither extinguish previously learned responses nor acquire new responses (Perrett and Mauk, 1995).

These data are consistent with the hypothesis that plasticity in both the cerebellar cortex and interpositus nucleus contribute to the expression of conditioned responses in the intact animal. Plasticity in the cortex appears to be important for the timing of responses, whereas plasticity in the nucleus seems capable of only contributing to the final amplitude of the response. Moreover, the induction of plasticity in the cerebellar nucleus appears to require an intact cerebellar cortex.

With these factors in mind, how do we address the necessity for learning criterion for LTD? The paper by De Zeeuw et al. (1998 [this issue of *Neuron*]) typifies one approach; test a cerebellar-dependent form of learning (VOR adaptation in this case) in mice genetically altered to prevent LTD. In this study, mice with Purkinje cell–specific expression of a broad spectrum PKC inhibitor showed an absence of LTD in vitro and a deficit in VOR adaptation. When learning is completely absent as in this case, necessity for learning is supported with the usual caveats. Perhaps the mutation caused nonspecific or developmental problems. Perhaps the absence of PKC activity in Purkinje cells can block learning in ways that are independent of LTD, etc. This study is exceptional in three ways. The mutation was specific to Purkinje cells, was expressed relatively late in development,

and involved expression of a peptide inhibitor rather than a knockout of a PKC gene, diminishing several caveats. This mutation appeared to cause no gross morphological or physiological problems in the cerebellum but blocked the induction of LTD and prevented adaptation of the VOR.

Similar genetic approaches have been employed for eyelid conditioning. The induction of LTD is also known to require activation of mGluR1 receptors (Linden and Connor, 1993), and mice deficient for these receptors show severe deficits in their ability to acquire conditioned eyelid responses (see Kim and Thompson, 1997). In these animals, and in mutant animals that lack Purkinje cells, the deficits in eyelid conditioning are not complete—the animals learn, but slowly and poorly. The implications of this residual learning remain unclear, in part because the caveats are numerous. Perhaps the spared learning reflects an incomplete block of LTD. For example, the mutation might make LTD induction more difficult such that it is blocked in vitro but not completely absent in vivo. Perhaps the spared learning reflects an ability for plasticity in the interpositus nucleus to occur with an intact cerebellar cortex incapable of LTD. Perhaps the learning is mediated by pathways that are unmasked by the mutation and do not normally operate in intact animals. The key is that attention must be given to the precise nature of the behavioral deficits—particularly to two points. Is the residual learning mediated by the cerebellum? Do the conditioned responses display abnormal learning of response timing, indicating a disrupted contribution of the cerebellar cortex? Answers to these questions would greatly aid in evaluating the impact of genetic studies on necessity for learning for LTD. In any case, these data—like the effects of cerebellar cortex lesions—are consistent with, but do not directly satisfy, the necessity for learning criterion for LTD in eyelid conditioning. A more definitive statement on this criterion awaits further studies, perhaps from mutations that are both Purkinje cell specific, like the mouse used by De Zeeuw et al. (1998), and inducible and/or reversible.

Testing Sufficiency of Induction

This criterion simply reflects the expectation that there should be agreement between the rules for LTD induction and the way tones and puffs activate granule cells and climbing fibers. This expectation has been at the center of an important and ongoing debate. Several authors have noted that sufficiency of induction is violated because the apparent timing of granule and climbing fiber inputs for the induction of LTD are incompatible with the timing of tone and puffs required to promote eyelid conditioning (Schreurs and Alkon, 1993; De Schutter, 1995). Essentially, LTD induction occurs when granule cells and climbing fibers are activated at about the same time. In contrast, eyelid conditioning requires tone onset to precede the puff by 100 ms or more.

However, viewing this timing as a mismatch involves certain assumptions about the way in which granule cells become active during tones. The learned timing of conditioned responses suggests that granule cell activity persists, and varies, throughout the tone. In this case, the timing for LTD induction and the timing of tone and puff required for learning do not need to match. Indeed, the temporal properties for LTD, as they are currently understood, seem adequate to mediate the ability of Purkinje cells to acquire the type of well-timed decreases in activity that could contribute to the expression of appropriately timed conditioned responses (Mauk and Donegan, 1997; Mauk et al., 1997).

These arguments are obviously indirect, but they clearly contradict the expectation that the timing of stimuli required to promote eyelid conditioning should necessarily match the timing of inputs required to induce the underlying plasticity. As such, sufficiency of induction remains an important, unresolved criterion.

Where Do We Stand?

It should be clear from the above discussion that the putative role of cerebellar LTD will continue to be a debated issue. There is neither a smoking gun experiment that establishes a causal link nor are all four of our criteria solidly satisfied. Even so, these criteria can serve as a road map leading to firmer ground, and we are struck by how far the journey has progressed. Consider, for example, the relative status of these criteria for cerebellar LTD relative to hippocampal LTP and spatial learning. For LTP, the only criterion that has been addressed seriously is necessity for learning, with results approximately equivalent to LTD and eyelid conditioning. Thus, despite the work remaining to be done, the evidence linking LTD to motor learning is better established than for many other form of plasticity in the vertebrate nervous system. With more work, we may eventually find that Marr's theory was more prophetic than emetic.

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