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Synaptic Memories Upside Down: Bidirectional Plasticity at Cerebellar Parallel Fiber-Purkinje Cell Synapses

Review

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Information storage in neural circuits depends on activity-dependent alterations in synaptic weights, such as long-term potentiation (LTP) and long-term depression (LTD). Bidirectional synaptic plasticity endows synapses with mechanisms for rapid reversibility, but it remains unclear how it correlates with reversibility in behavioral learning and whether there is a universal synaptic memory mechanism that operates similarly at all types of synapses. A recently discovered postsynaptic form of LTP at cerebellar parallel fiber (PF)-Purkinje cell (PC) synapses provides a reversal mechanism for PF-LTD and enables a fresh look at the implications of bidirectional plasticity in a brain structure that is particularly suitable to correlate cellular to behavioral learning events. Here, we will review recent studies that reveal unique properties of bidirectional cerebellar plasticity and suggest that the induction cascades for cerebellar LTP and LTD provide a mirror image of their counterparts at hippocampal synapses. We will also discuss how PF-LTP helps to explain reversibility observed in cerebellar motor learning.

Bidirectionality is a plasticity feature that has been described at many types of synapses. The presence of both LTP and LTD mechanisms allows synapses to rapidly reverse synaptic gain changes in an activity-dependent way. A fast reversal mechanism differs from passive decay of stored information that is no longer in use, as it provides a "reset" function that can lead to storage of new information. At glutamatergic synapses between hippocampal CA3 and CA1 pyramidal cells (which are widely seen as reference synapses for the study of synaptic memories), LTP and LTD act as mechanisms for information storage and reversal. At cerebellar PF synapses, a reversal mechanism for PF-LTD, which is postsynaptically expressed, was for a long time missing. A form of LTP had been characterized, but it turned out to be presynaptically expressed (Salin et al., 1996) and thus cannot truly reverse LTD. The recent discovery of a postsynaptic form of LTP at cerebellar PF synapses (Lev-Ram et al., 2002; Coesmans et al., 2004) finally provides a candidate mechanism for LTD reversal. Here, we describe the cellular mechanisms underlying the induction of this new form of LTP and its functional implications.

Why is it particularly interesting to study the involvement of LTP and LTD mechanisms in cerebellar learning? There are two main reasons: first, it turns out that the cellular mechanisms underlying (postsynaptic) LTP and LTD at PF synapses differ substantially from their counterparts at hippocampal synapses. In this review, we will point out both differences and similarities between the plasticity mechanisms at both types of synapses. Second, the cerebellum is particularly suitable to the study of the cellular substrates of reversible learning. The cerebellum mediates forms of nondeclarative memory, in which reversibility features can be related to LTP and LTD. As we will show, this advantage rests on a detailed understanding of the underlying cerebellar circuitry and the topographical distribution of the sensory input and the motor output.

Cerebellar Circuitry Involved in Motor Learning

The cerebellar circuitry differs in some important aspects from that of other brain areas. PCs provide the sole output of the cerebellar cortex and form inhibitory synapses with their target cells in the cerebellar or vestibular nuclei. These nuclei constitute the beginning of the motor command chain, whereas the cerebellar cortex can be seen as a controlling side loop (Ito, 1984; Medina et al., 2002b; see also Figure 1). PCs receive two types of excitatory, glutamatergic synapses that are very differently organized, namely from PFs (the axons of granule cells) on the one hand, and from a climbing fiber (CF; originating from the inferior olive) on the other (Figure 1). In the adult brain, each PC receives input from only one CF, which nevertheless provides massive, powerful excitation, as it comprises ~1500 release sites (Silver et al., 1998). CF activation leads to an all-or-none complex spike, which consists of a fast sodium spike, followed by a series of spikelets riding on top of a plateau. The initial sodium spike is elicited in the axon, but does not actively propagate back into the dendrite (unlike in pyramidal cells; Stuart and Häusser, 1994). A hallmark of the CF response is dendritic calcium spike activity (Llinas and Sugimori, 1980), which is reflected in the late complex spike components (for review see Schmolesky et al., 2002). As we will see below, the complex spike-associated calcium transients in PC dendrites (Miyakawa et al., 1992; Figure 3D) play a key role in cerebellar plasticity.

Theoreticians have long viewed the cerebellum as a machine-like structure designed for fast computation and associative learning, a view that was inspired by the remarkable regularity and geometrical structure of its circuitry. Already in 1969, Marr suggested that the PF synaptic input to PCs was under heterosynaptic control and would be potentiated when activated in conjunction with the CF (Marr, 1969). Albus (1971) instead assumed that coactivity with the CF would lead to a depression of PF synapses since the artificial neural network, the *simple perceptron*, with which he compared

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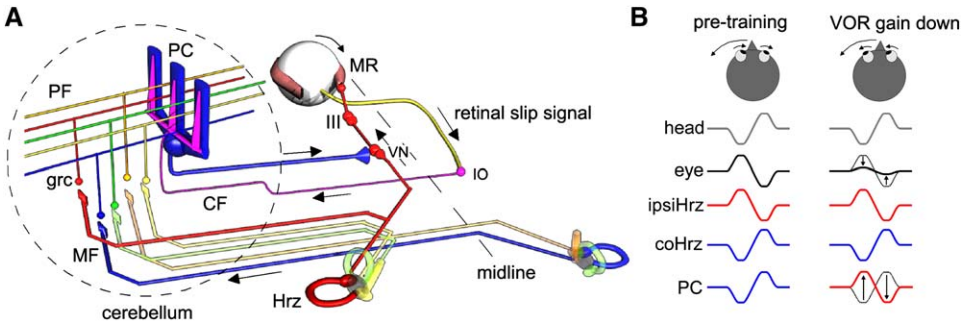

Figure 1. Cerebellar Control of the VOR

(A) Circuit diagram illustrating the information flow through the brainstem and the cerebellar cortex (flocculus). In response to head movement, the horizontal semicircular canal (Hrz) provides input that, via the vestibular nuclei (VN), drives the oculomotor neurons (III) innervating the medial rectus (MR) muscle (red connections). The vestibular nuclei controlling the horizontal VOR component receive inhibitory input from PCs in the H zone of the flocculus, the output neurons of the modulatory side-loop in the cerebellar cortex. MFs provide the cerebellar cortex with vestibular input from the semicircular canals (presumably mainly as secondary vestibular afferents, see Gerrits et al., 1989). This input is conveyed to PCs via granule cells (grc), which provide the PFs. The PCs receive error signals from CFs, which originate from the inferior olive (IO), when a retinal slip occurs. The retinal slip signal is mediated via an indirect pathway (Ito, 1984).

(B) Motor learning in the VOR. In the adapted, normal state (pretraining): when the head turns to the left, the eyes move with the same speed in the opposite direction (pretraining). PCs that control the horizontal VOR fire out of phase with the ipsilateral Hrz input (red, ipsiHrz) and in phase with the contralateral Hrz input (blue, coHrz). This is compatible with the PF input from ipsiHrz being depressed. The resulting disinhibition in the vestibular nuclei allows a high throughput of the ipsiHrz signals to oculomotor neurons. However, when an experimental reduction of the VOR gain is induced by training with image motion in the same direction as the head, PCs lose their correlation with the coHrz firing and instead start to fire in correlation with the ipsiHrz (VOR gain down). This can be interpreted as if the adaption to a lower VOR gain is mediated by a depression of PF input from coHrz (see Ito, 1982, 1984). Note that the two different driving inputs of the H zone PCs (coHrz and then ipsiHrz) may be considered to be the PF receptive fields of these PCs, and the VOR analysis is in this way closely related with the receptive field analysis performed for PCs in the C3 zone below.

the cerebellar cortex, learns to recognize patterns most efficiently through weakening of erroneously activated inputs. From these theories, the first concept of how the cerebellum might contribute to functional adaptation and learning emerged: CFs convey error signals in movement performance and will initiate error correction by promoting depression at simultaneously active PF synapses. In the classic Marr-Albus-Ito theories (Ito, 1984), LTD at PF synapses on Purkinje cells is sufficient to explain cerebellar motor learning. In the meantime, the involvement of a second learning site at mossy fiber (MF) synapses onto cerebellar nuclei neurons has been suggested (Medina et al., 2001, 2002b). The participation of a second learning site offers the computational advantage of task specialization: evidence suggests that the first learning site (cortex) initiates learning, while the second (nuclei) is involved in long-term storage and is more resistant to extinction (Medina et al., 2002b).

VOR Gain Adaptation and the History of PF-LTD

One of the best examples of how detailed knowledge of the underlying circuitry promotes an understanding of the functional consequences of synaptic plasticity is the horizontal vestibulo-ocular reflex (VOR) and its control by the cerebellar flocculus (Figure 1; Ito, 1982). The function of the VOR is to maintain stability of the retinal image when the head turns in one direction by eliciting counterbalancing eye movements in the opposite direction. The reflex component itself consists of vestibular afferent activation of cells in the vestibular nuclei, which in turn activate the oculomotor neurons. Attached to this simple circuitry is a cerebellar side-loop in which vestibular afferents drive Purkinje cells through activation of the mossy fiber (MF)-PF pathway. PCs provide inhibitory input to the vestibular nuclei and are thus able to modulate the VOR gain (Figure 1A). Around 1970, Ito and coworkers began to examine the role of the cerebellar flocculus in VOR control (reviewed by Ito, 1982, 1984). It became apparent that the activation patterns of afferent input fitted the predictions made in the Marr-Albus theory of cerebellar learning (Figure 1; Ito, 1982). MFs, and therefore PFs, are strongly driven by vestibular input. In contrast, the CF input to PCs is activated when there is a slip in the retinal image, providing a perfect example of an error signal (Simpson and Alley, 1974). The subsequent characterization of activity patterns in the circuitry suggested that a depression of synaptic strength at PF synapses, resulting from simultaneous PF and CF activity, played a crucial role in gain adaptation (Figure 1B). LTD was a good candidate mechanism to depress the ipsiHrz PF input, thereby causing a decrease in the firing frequency of PCs and a subsequent disinhibition of the vestibular nuclei neurons. This disinhibition facilitates activation of the vestibular nucleus (VN) by the excitatory ipsiHrz input, resulting in a strong activation of the oculomotor neurons and the high gain that is typical for the VOR when it is in its normal, adapted state (Figure 1B, left). In this state, the PC follows the activity of the coHrz input when the VOR is elicited. If the visual world is now changed experimentally, so that the appropriate response of the reflex is to move the eye at a lower gain relative to the head movement, the coHrz input will start to coincide with the CF input as the reflex initially triggers retinal slip signals. The drive of the PCs in response to the coHrz input will then gradually be depressed by LTD, and the PCs will instead start to follow the ipsiHrz input when the VOR has become adapted to the new condition, i.e., with a low gain (Figure 1B, right). The involvement of CF-induced plasticity was confirmed by the finding that lesions in the

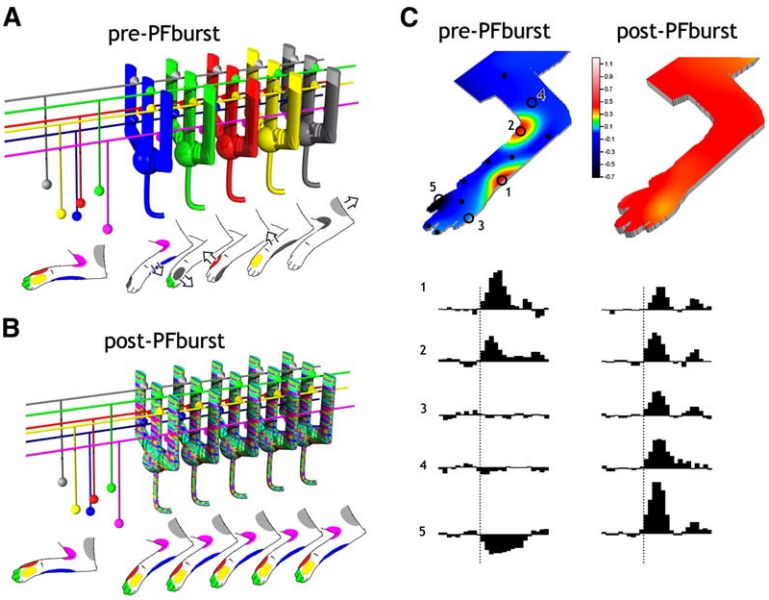

Figure 2. Circuitry Analysis of Receptive Field Properties

(A) Scheme illustrating relationship between the receptive fields of individual grc-PFs and PCs along a beam of PFs in the normal animal (control conditions). The neuronal elements are color coded according to the location of their receptive field. Different PFs have different receptive fields. PCs have synaptic contact with all PFs but are only driven by one of them, which hence defines its PF receptive field. All other PF synapses on the PC are depressed or silent. The location of the CF receptive field and the inhibitory receptive field is the same and is indicated as a dark shaded area on the skin in the forelimb outline below each PC. Small arrows indicate the direction of principal movement controlled by PCs with the given CF receptive field. Note that the PF receptive field lies in the path of the movement, whereas the CF receptive field has a functionally opposite location relative to the movement (for review see Ekerot and Jörntell, 2003).

(B) Scheme as in (A) but for PCs along a beam of PFs after a LTP protocol.

(C) Quantified skin inputs, represented in a contour plot, before (left) and after application of PF burst stimulation (right). Peristimulus histograms below show the net drive from repeated stimulations at skin sites 1 through 5 (stimulus onset at dotted line, bin width 20 ms).

floccular-projecting part of the inferior olive or its afferents blocked adaptation of the VOR (Ito, 1984). The subsequent discovery and characterization of cerebellar LTD revealed that PF and CF coactivation is indeed required for LTD induction, confirming the predictions made earlier (Ito and Kano, 1982; Ito et al., 1982). In these early studies, it was suggested that both gain increases and decreases can be explained by a LTD mechanism ("LTD-only" model; see Ito, 1982, 1984). As we will argue below, adding a synaptic reversal mechanism helps to overcome the limitation of this model that eventually synaptic depression would reach saturation, preventing further learning events to take place.

Receptive Field Plasticity In Vivo Supports a Role for Postsynaptic PF-LTP

A similarly detailed analysis of the organization of in vivo circuits was recently performed for the C3 zone of the cerebellar cortex, which controls limb movement areas of the motor cortex and red nucleus via the anterior interposed nucleus (for review see Ekerot and Jörntell, 2003; Apps and Garwicz, 2005). In this case, the implications for LTP and LTD were deduced from an analysis of receptive fields, without direct observations of behavioral change. However, in analogy with the circuitry analysis of the VOR, in which a change in the receptive field of PCs with a given movement control was correlated with a change in behavior (Figure 1), the receptive fields of PCs are also here considered to provide a "read-out" of the circuitry changes underlying the behaviorally adapted state of the normal, adult animal.

MFs in the C3 zone are driven from small skin areas (Garwicz et al., 1998), which permits the selective, experimental activation of a large number of different MF inputs. The receptive fields of single MF-PFs contribute to the total PF receptive field of a PC, but since PCs have small PF receptive fields resembling those of individual MF-PFs, it is clear that far from all of the available PFs drive the PC (Figure 2A). It is likely that the observed PF receptive fields are the result of LTD and LTP, respectively, at distinct sets of PF synapses (Ekerot and Jörntell, 2001; Jörntell and Ekerot, 2002). The involvement of LTD is suggested by the absence of PF input from the skin area that overlaps with CF receptive fields (Figure 2A), resulting from a depression of PF synapses that were coactive with the CF input. However, LTD cannot be the only plasticity mechanism shaping receptive fields. If it was the only mechanism, PF receptive fields would represent excitatory input from the entire forelimb skin with cut-outs from the skin area that overlaps with the CF receptive fields. In contrast, PF receptive fields are small and well localized and differ substantially between PCs along the same PF beam and between PCs and neighboring interneurons (Ekerot and Jörntell, 2001).

PF receptive fields are small and specific to the postsynaptic properties of PCs, i.e., PCs with similar PF receptive fields also have similar CF receptive fields, similar inhibitory receptive fields, and similar movement control (Ekerot and Jörntell, 2001, 2003). These observations motivated the search for a mechanism mediating postsynaptic potentiation in vivo. To this end, a PF burst stimulation protocol, which also effectively potentiates PF-EPSPs in vitro (Smith and Otis, 2005), was applied (Jörntell and Ekerot, 2002). PF burst stimulation causes a dramatic expansion of receptive fields in those PCs that are contacted by activated PFs (Figures 2B and

2C). In contrast, when the same PF stimulation is applied in conjunction with CF activation, the receptive field is reduced to its previous size (not shown). In addition, receptive field sizes in neighboring interneurons change in the opposite direction (Jörntell and Ekerot, 2002, 2003). These observations seem to imply that the receptive field changes depend on postsynaptic changes. Since a majority of the available PF synapses on PCs are electrically silent or depressed (Wang et al., 2000b; Isope and Barbour, 2002; Brunel et al., 2004; Ito, 2006), it is likely that a recruitment of silent PF synapses by a postsynaptic LTP mechanism is responsible for the observed dramatic increases in PF receptive field size. Intrinsic excitability changes are not likely to be involved since (1) an LTD protocol can reverse the receptive field changes when applied to the same PF beam, but not when it's applied to a different PF beam innervating the PC, and (2) although input from previously "silent" skin areas increased, input from the original receptive field relative to the background activity actually decreased (Jörntell and Ekerot, 2002) and the contour plot of the quantified receptive field is consequently "flattened" (Figure 2C). Altogether, these observations suggest that postsynaptic LTP, rather than another type of cerebellar plasticity, is involved in increases in PF receptive field size.

Postsynaptic LTP as a Reversal Mechanism for PF-LTD

PF-LTD is postsynaptically induced and expressed (for review see Hansel et al., 2001; Ito, 2001, 2002), and therefore a reversal mechanism, triggered by PF activity alone, needs to act postsynaptically as well. This is an important notion, because the first type of PF-LTP described, which can be induced by brief PF stimulation at high frequencies (typically 4–8 Hz for 15 s), is presynaptically induced and expressed and depends on the activation of calcium/calmodulin-sensitive adenylyl cyclase I and the subsequent activation of cAMP-dependent kinase (PKA; Salin et al., 1996; Chen and Regehr, 1997; Storm et al., 1998; Jacoby et al., 2001). Presynaptic LTP could "reverse" cerebellar learning by masking LTD. True reversibility (as opposed to masking) requires shared expression sites. The postsynaptic form of LTP, which was more recently described (Figure 3A; Lev-Ram et al., 2002; Coesmans et al., 2004), has indeed been shown to enable mutual reversibility with PF-LTD (Figure 3C; Lev-Ram et al., 2003; Coesmans et al., 2004). PF-LTD can be induced by paired PF and CF stimulation (Ito et al., 1982), which is typically applied at 1–4 Hz for 5 min (Figure 3B). The CF contribution to PF-LTD induction consists of the large, widespread calcium transients that are evoked by complex spikes (Figure 3D; Konnerth et al., 1992). PF-LTP can be induced by the same PF stimulation, when CF stimulation is omitted (Lev-Ram et al., 2002; Coesmans et al., 2004). The observation that the polarity of postsynaptic PF plasticity depends on the activity of the CF input is remarkable, considering that the CF provides a qualitatively different, heterosynaptic input.

The signaling cascades involved in LTP and LTD induction at excitatory synapses onto neocortical and hippocampal CA1 pyramidal neurons have been studied in detail. At these types of synapses, the induction of both LTP and LTD depends on a postsynaptic calcium transient, but the required features of the calcium transients differ. For LTP induction, a higher calcium threshold amplitude has to be reached than for LTD induction (Figure 3E; Bear et al., 1987; Cummings et al., 1996; Hansel et al., 1997; see also Yang et al., 1999, for kinetic aspects). The idea of a synaptic modification threshold was proposed in 1982 by Bienenstock, Cooper, and Munro (Bienenstock et al., 1982), after whom this two-threshold mechanism was termed the "BCM rule." A physiological correlate of these calcium thresholds can be found in spike-timing-dependent plasticity (STDP), in which coincidence of synaptic activity with backpropagating action potentials (APs) evokes a large calcium signal and leads to LTP, whereas unpaired stimulation causes lower calcium signals and LTD or no change (Magee and Johnston, 1997; Markram et al., 1997; Koester and Sakmann, 1998). In STDP, the coincidence intervals between EPSPs and APs are crucial: APs following EPSPs lead to LTP, whereas APs preceding EPSPs can cause LTD (Markram et al., 1997). In line with this observation, spine calcium transients are larger when APs follow EPSPs than when they precede (Koester and Sakmann, 1998).

Bidirectional Cerebellar Plasticity as a Mirror Image of Hippocampal Plasticity

CF activity evokes all-or-none complex spikes and concurrently large, widespread calcium transients in PC dendrites (Figure 3D; Miyakawa et al., 1992). Calcium transients measured in spines reach supralinear amplitudes when the CF and PF inputs are coactivated (Wang et al., 2000a). Despite the resulting larger calcium transient, paired PF and CF stimulation induces PF-LTD, whereas PF stimulation alone causes LTP (Lev-Ram et al., 2002; Coesmans et al., 2004). These observations suggested a calcium-threshold mechanism for bidirectional PF plasticity operating "inversely" to the BCM rule. Subsequently, inverse calcium thresholds have indeed been demonstrated: PF-LTP is calcium dependent, but a higher calcium threshold has to be reached for LTD than for LTP induction (Figure 3E; Coesmans et al., 2004). In this study, the calcium signaling requirements were not examined using an imaging approach, but by experimentally manipulating the calcium transient using the calcium chelator BAPTA and photolysis of the caged calcium compound DMNP-EDTA, respectively (Coesmans et al., 2004). Remarkably, the calcium transients observed by two-photon laser scanning microscopy after paired stimulation are largest when PF activity precedes CF activity by 50–200 ms, which also is the optimal timing window for LTD induction (Wang et al., 2000a) and, in addition, correlates well with the delay considered to be required for VOR learning in a simulation study (Raymond and Lisberger, 1998).

The differences in the calcium-dependent initiation of LTP- and LTD-induction cascades at cerebellar and hippocampal synapses, respectively, should be seen in the context of accompanying differences in the physiological conditions promoting calcium influx. Mature Purkinje cells lack functional N-methyl-D-aspartate (NMDA) receptors (Crepel et al., 1982) and lack dendritic sodium conductances at a density required to support spike backpropagation (Stuart and Häusser, 1994). Thus,

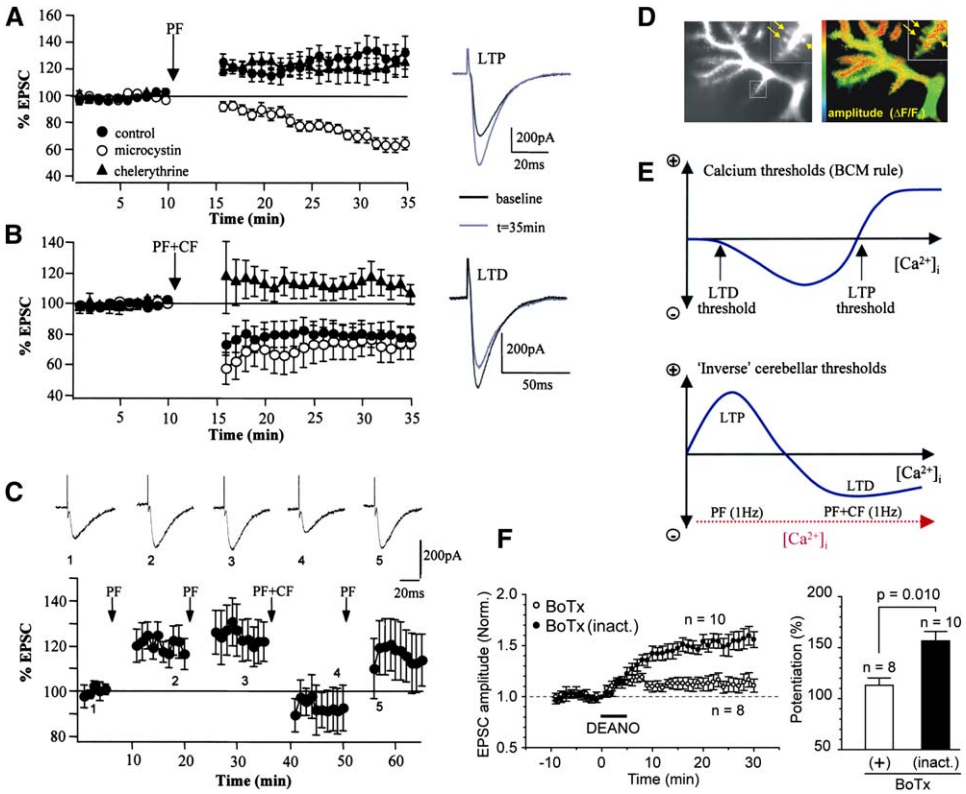

Figure 3. Cellular Mechanisms of PF-LTP Induction

(A) PF-LTP is blocked by the PP1/2A inhibitor microcystin LR (10 μM), but not by the PKC inhibitor chelerythrine (10 μM). Control LTP, filled circles (n = 11); microcystin LR, empty circles (n = 7); chelerythrine, filled triangles (n = 7). Traces: EPSCs before and after LTP induction (control).

(B) PF-LTD (control: n = 8) is blocked by chelerythrine (n = 5), but not by microcystin LR (n = 9). Symbols are the same as in (A). Traces: EPSCs before and after LTD induction (control).

(A) and (B) are modified from Belmeguenai and Hansel (2005). Copyright 2005 by the Society for Neuroscience.

(C) Saturated LTP can be reversed by application of the LTD protocol (n = 6).

(D) CF-evoked calcium signaling in a PC. The fluorescent calcium indicator Oregon Green BAPTA-1 was loaded into a PC and imaged by two-photon excitation while a complex spike was evoked. (Left) Fluorescence image of PC dendrite. (Right) Pseudocolor-coded map of peak amplitudes (blue to red is ΔF/F = 0% to 80%). Inset shows magnified portion demonstrating calcium transients in spines. Unpublished data kindly provided by Thomas Knöpfel (RIKEN Brain Science Institute, Japan).

(E) Calcium threshold models for LTP and LTD induction. (Top) In pyramidal cells, there is a higher calcium threshold amplitude for LTP than for LTD induction (BCM rule). (Bottom) Bidirectional plasticity at cerebellar PF synapses is governed by a calcium threshold mechanism that operates inversely to the BCM rule.

(C) and (E) are modified from Coesmans et al. (2004). Copyright 2004 by Elsevier.

(F) DEANO-evoked PF-LTP is prevented by the exocytosis inhibitor botulinum toxin (BoTx; 100 nM). (Left) Intact BoTx blocks LTP (open circles, n = 8), while heat-inactivated BoTx (closed circles, n = 10) does not. (Right) Summary bar graphs. (F) is taken from Kakegawa and Yuzaki (2005). Copyright 2005 by the National Academy of Sciences, USA.

two important elements of pre- and postsynaptic coincidence detection in pyramidal cell dendrites are missing in Purkinje cells, which seem to rely on coincident complex spike activity as a functional analog.

Can the mirror image-like arrangement of LTP- and LTD-induction cascades at PF synapses, as compared to their hippocampal counterparts, also be observed downstream of the calcium transients? As we will describe below, the inverse induction cascades can be seen down to the level of factors that control the cycling of AMPA receptor subunits. However, a picture emerges, which shows that it all depends on the type of AMPA receptor subunit.

At glutamatergic synapses, the crucial factor in determining synaptic efficacy is the phosphorylation state of AMPA receptors, which can mediate biophysical changes at the individual receptor level and/or can modify the insertion/internalization balance of AMPA receptors (for review see Bear and Linden, 2000; Song and Huganir, 2002). Key molecules in hippocampal LTP are the α isoform of calcium/calmodulin-dependent kinase II (αCaMKII; Lisman, 1989; Silva et al., 1992; Pettit et al., 1994; Lledo et al., 1995), protein kinase C (PKC; for review see Malenka and Siegelbaum, 2000), whose ζ isoform is upregulated during LTP maintenance (Hrabetova and Sacktor, 1996), and PKA (Esteban et al., 2003). On the other hand, LTD induction depends on the activation of protein phosphatases (Mulkey et al., 1993; Thiels et al., 1998; Morishita et al., 2001). CaMKII and protein phosphatase 1 (PP1) have been suggested to act as a kinase/phosphatase switch, regulating the phosphorylation state of AMPA receptors and/or their auxiliary proteins (Lisman, 1989; Lisman and Zhabotinsky, 2001; Malleret et al., 2001). Notably, PP1 itself is not calcium

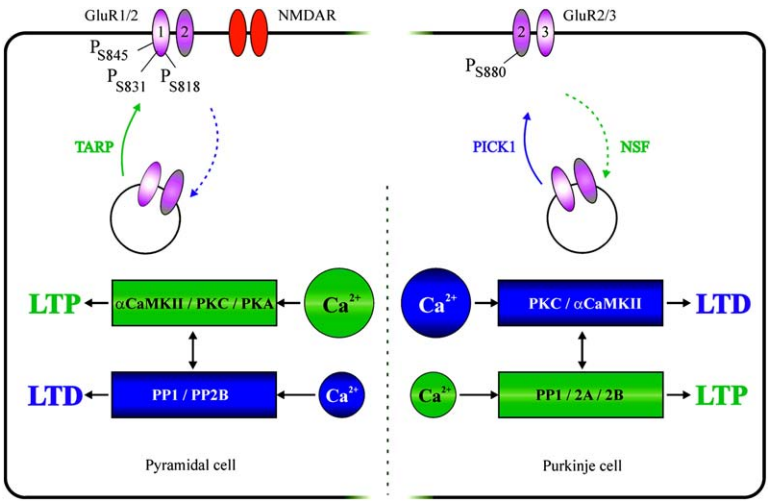

Figure 4. Comparison of LTP and LTD Induction Casacades at Hippocampal (Left) and Cerebellar Synapses (Right)

LTD induction cascades are shown in blue, and LTP induction cascades in green. For simplicity, no synaptic terminals are shown, and one schematic postsynaptic compartment is used to illustrate LTP and LTD induction and expression cascades in both pyramidal cells and Purkinje cells. Functional NMDA receptors (red) are present in pyramidal cells, but not in Purkinje cells. Other calcium sources (voltage-dependent calcium channels/internal stores) present in both types of neurons are not displayed. Note that PKA and PP1/PP2A are not directly calcium-activated. PKC has been implicated in hippocampal LTP, but also in the internalization of GluR2 subunits. The dashed arrows indicate that question marks remain behind the functions of the indicated processes (GluR1 endocytosis in LTD/GluR2 insertion in LTP). The dashed line separating the "pyramidal cell" from the "Purkinje cell" does not continue all the way to the top, as PICK1-controlled GluR2 endocytosis (right side) occurs in both types of neurons. TARP: transmembrane AMPA receptor regulatory protein, e.g., stargazin. Other abbreviations are used as explained in the text.

dependent, but acts in tandem with the calcium/calmodulin-dependent PP2B (also calcineurin; Lisman, 1989).

The kinase/phosphatase switch at cerebellar PF synapses operates in reverse order, consistent with the inverse calcium dependencies as described above. PF-LTD induction requires the activation of PKC (Linden and Connor, 1991; De Zeeuw et al., 1998; Leitges et al., 2004) and, as recently shown using αCaMKII knockout mice, also requires the activation of αCaMKII (Hansel et al., 2006). PF-LTP, in contrast, depends on the activation of PP1, PP2A, and PP2B (Figure 3A; Belmeguenai and Hansel, 2005), but does not depend on CaMKII (Kakegawa and Yuzaki, 2005; Hansel et al., 2006) or PKC activity (Figure 3A; Belmeguenai and Hansel, 2005). The cellular mechanisms promoting LTP seem to counteract those promoting LTD (similar to the kinase/phosphatase switch characterized in the hippocampus), as inhibition of phosphatases facilitates LTD induction (Ajima and Ito, 1995; Eto et al., 2002; Launey et al., 2004). These observations suggest that the LTP- and LTD-induction cascades at cerebellar and hippocampal synapses provide a mirror image of each other, not only with regard to their calcium dependencies but also with regard to kinase and phosphatase activation requirements (Figure 4).

AMPA Receptor Trafficking Reveals Differences to Hippocampal Plasticity

At different types of excitatory synapses, plasticity is mediated by conductance changes of AMPA receptor-gated ion channels and/or changes in the synaptic density of AMPA receptors, both of which are regulated by phosphorylation events (for review see Song and Huganir, 2002; Collingridge et al., 2004; Boehm and Malinow, 2005). AMPA receptors are heteromeric complexes of the four homologous subunits GluR1 to GluR4. In hippocampal CA1 pyramidal cells, the majority of AMPA receptors consist of GluR1/GluR2 and GluR2/GluR3 heteromeric complexes. In GluR1/GluR2 heteromers, GluR1 subunits dominate the trafficking behavior (for review see Song and Huganir, 2002). In contrast, Purkinje cells only weakly express GluR1 subunits (Baude et al., 1994), and GluR2/GluR3 heteromers constitute the majority of AMPA receptors. This lower degree of AMPA receptor diversity in Purkinje cells might explain why it currently seems easier to understand the role of AMPA receptor cycling in synaptic plasticity in the cerebellum than in the hippocampus.

PF-LTD is not associated with changes in AMPA receptor properties, such as receptor conductance, kinetics, or agonist affinity (Linden, 2001), but is rather mediated by GluR2 endocytosis (Wang and Linden, 2000). This internalization requires PKC-dependent phosphorylation of GluR2 at Ser-880 (Chung et al., 2003). The clathrin-mediated endocytosis of GluR2 involves unbinding of GluR2 from the glutamate-receptor-interacting protein GRIP1 (Dong et al., 1997) and binding to protein interacting with C-kinase 1 (PICK1) (Xia et al., 1999; Chung et al., 2000), promoting subsequent GluR2 internalization (Xia et al., 2000). PKC-mediated phosphorylation of Ser-880 disrupts the binding of GluR2 to GRIP1. Whereas PF-LTD is mediated by GluR2 endocytosis, PF-LTP induction involves the membrane insertion of GluR2 (Figure 3F; Kakegawa and Yuzaki, 2005) via GluR2 interaction with N-ethylmaleimide-sensitive factor (NSF).

In the hippocampus, in contrast, GluR1 subunit trafficking plays a dominant role. The phosphorylation events involved in CA1 hippocampal LTP induction are complex: phosphorylation of GluR1 at the PKA phosphorylation site Ser-845 seems to act as a "priming" step for GluR1 membrane insertion (Esteban et al., 2003). Synaptic targeting of GluR1/GluR2 receptors ultimately requires CaMKII-mediated phosphorylation of a family of small transmembrane AMPA receptor regulatory proteins (TARPs), such as stargazin (for review see Nicoll et al., 2006). It remains unclear what role a previously reported phosphorylation of GluR1 at the CaMKII/PKC phosphorylation site Ser-831 plays that

has been observed after LTP induction (Lee et al., 2000). Ser-831 phosphorylation does not affect receptor trafficking (Hayashi et al., 2000), but might increase the receptor conductance (Derkach et al., 1999). In a recent study, it was shown that a third GluR1 phosphorylation site is involved in LTP induction. A PKC-mediated phosphorylation at Ser-818 is required for GluR1 insertion and LTP (Boehm et al., 2006). As LTP is correlated with increased kinase activity and phosphorylation of GluR1, it has been suggested that LTD is associated with phosphatase activity and dephosphorylation of GluR1. Experimental evidence indeed supports this hypothesis. Dephosphorylation at Ser-845 occurs during LTD (Lee et al., 2000) and may promote internalization of AMPA receptors (Lee et al., 2003). Accordingly, PKA-mediated phosphorylation at Ser-845 is required for AMPA receptor reinsertion and recovery from depression (Ehlers, 2000). Inhibition of PP2B blocks GluR1 internalization, suggesting that phosphatase activity is required for GluR1 endocytosis and LTD (Beattie et al., 2000). A crucial role for GluR1 in LTD expression was furthermore supported by the finding that NMDAR-dependent LTD is blocked in mutant mice, in which phosphorylation at Ser-831 and -845 is prevented by mutations to alanine ("phospho-free mice"; Lee et al., 2003). These data suggested that hippocampal LTP and LTD are dominated by trafficking of GluR1/2 heteromers, whereas GluR2/3 insertion might function as a constitutive delivery mechanism, which is not driven by synaptic activity (Shi et al., 2001). Both LTP and LTD are indeed present in GluR2-deficient mice (Jia et al., 1996).

During the last years, however, evidence accumulated supporting a more active role of GluR2 subunits. The mechanisms underlying GluR2 endocytosis operate similarly as in Purkinje cells. GluR2 phosphorylation at Ser-880 leads to LTD induction by clathrin-mediated GluR2 endocytosis at both hippocampal (Seidenman et al., 2003; Man et al., 2000) and cerebellar synapses (Chung et al., 2003; Wang and Linden, 2000).

A recently promoted hypothesis gains weight, which states that hippocampal LTP is mediated by membrane delivery of AMPA receptors with long cytoplasmic termini (e.g.GluR1-, GluR2L-, and GluR4-containing AMPA receptors), whereas LTD is mediated by the endocytosis of AMPA receptors with short cytoplasmic termini (GluR2-containing AMPA receptors; Malinow, 2003; McCormack et al., 2006). At both types of synapses, NSF-GluR2 binding promotes GluR2 insertion (Lüscher et al., 1999; Steinberg et al., 2004), but whereas such insertion mediates LTP at PF synapses, it remains unclear what the function of GluR2 membrane delivery is at hippocampal synapses.

Taken together, the picture of a mirror image-like arrangement of induction cascades between CA1 hippocampal and cerebellar plasticity emerges very clearly when comparing the calcium signaling requirements and the kinase/phosphatase involvement (Figure 4). The observed inverse induction cascades indicate that synaptic memories are governed by different mechanisms at different types of glutamatergic synapses. When it comes to AMPA receptor cycling, the mirror image-like arrangement can be partially found as well. For example, high-amplitude calcium transients and kinase activity promote GluR1 membrane delivery (and LTP) in pyramidal cells while promoting GluR2 endocytosis (and LTD) in Purkinje cells. It remains to be determined what the relative roles of endocytosis of GluR1/GluR2 heteromers and GluR2/GluR3 heteromers, respectively, are in hippocampal LTD. This missing piece of the puzzle will help to understand whether hippocampal and cerebellar plasticity share mechanisms of LTD induction (GluR2 endocytosis) and thus how complete the mirror image really is.

Reversibility as a Feature of Cerebellar Motor Learning

As described above, bidirectional PF plasticity can control the PF input strength to PCs over a tremendous range. When depressed PF inputs were potentiated experimentally, receptive fields expand by several thousand percent (Jörntell and Ekerot, 2002). In contrast, subsequent application of the LTD protocol results in a reduction of the PF receptive field back to its original size. In this in vivo plasticity paradigm, specific roles can be assigned to PF-LTD and -LTP, respectively. Theoretical assumptions predicted that LTD should occur most frequently at PF synapses activated from skin areas that fully or partially overlap with CF receptive fields. Subsequently, it was indeed demonstrated that PF receptive fields in PCs avoid the CF receptive field. However, PF receptive fields are not entirely shaped by CF receptive fields, as there is no correlation between the intensity of PF input and the distance from CF receptive fields. This and other observations led to the suggestion that PF-LTP plays a key role in establishing PF receptive fields in PCs. The experimental induction of LTP results in large and unspecific PF receptive fields in all PCs located along the beam of stimulated PFs due to a massive recruitment of silent PF synapses (Figure 2). In contrast, PF-LTP is likely to act more specifically in the behaving animal. This interpretation is supported by the observation that naive PCs that were not experimentally tetanized typically have one or two small PF receptive field components (Figure 2A) and that specific receptive field components can be added to PCs by localized skin stimulation (Jörntell and Ekerot, 2002).

The cerebellar control of the VOR gain can also be interpreted along these lines. Input from the ipsilateral horizontal semicircular canal (ipsiHrz), which drives the reflex, provides strong MF input to the flocculus. Nevertheless, these MFs do not appear to activate PCs (Figure 1B), suggesting that the ipsilateral MF-PF input to PCs is depressed in the naive state (Ito, 1982, 1984), thus promoting a high gain of the reflex. The PF input from the contralateral side (coHrz) activates PCs more efficiently and might well be in a potentiated state resulting from postsynaptic LTP. In this model, floccular PCs receive two different, antagonistic sets of PF input (reviewed by Ito, 1982), of which only the coHrz input is strongly connected to the PCs. Following this assumption, LTD at previously potentiated PF synapses (activated by the coHrz input) could result from gain mismatch and subsequently enhanced CF activity. At the same time, postsynaptic LTP might occur at PF synapses activated from the ipsiHrz input, whose activity is unlikely to coincide with CF activity. This will cause a

strengthened ipsiHrz input and a decrease in the VOR gain. Repeated up-and-down changes in VOR can be easily performed by this mechanism. This model would hence complement the LTD-only model (Ito, 1982, 1984) and at the same time eliminate previous problems that the LTD-only model had in explaining repeated VOR gain reversals. In the LTD-only model, it was proposed that LTD at one type of PF synapses (ipsiHrz) would lead to an increase in VOR gain, whereas LTD at other types of PF synapses (coHrz) could lead to a gain decrease. The problem with this model is that as the VOR gain is repeatedly adjusted up and down, the flocculus would soon run out of PF synapses left to depress. If PF-PC synaptic plasticity changes are bidirectional, so that functionally antagonistic PF inputs are changed reciprocally, this limitation of the model is eliminated.

A novel view of VOR gain adaptation, which also involves both LTD and LTP (and thus a reversal mechanism), but restricted to ipsiHrz PF synapses, was proposed by Boyden and Raymond (2003). Parameters such as passive decay kinetics, generalization, and reversibility led the authors to the conclusion that, in contrast to the LTD-only model, the plasticity mechanism involved in decreasing the VOR gain is different from that for increasing the VOR gain (Boyden and Raymond, 2003; Boyden et al., 2004). When examining properties of reversibility, these authors found that complete reversal was reached by gain-down training after gain-up training. In contrast, gain-up training after gain-down training led to an incomplete reversal (Boyden and Raymond, 2003), and it was suggested that the asymmetric reversibility was caused by an involvement of both post- and presynaptic PF-LTP in the downregulation of the VOR gain. Presynaptic LTP causes an upregulation of transmitter release and would thus be able to mask, but not to reverse, the postsynaptic reduction in AMPA receptor responsiveness mediated by LTD. The postsynaptic form of PF-LTP can, however, account for the true reversal component, as both LTD and this form of LTP mediate synaptic plasticity by regulating the membrane trafficking of the AMPA receptor subunit GluR2 (Wang and Linden, 2000; Kakegawa and Yuzaki, 2005) at the same PF synapses. A couple of limitations of this "masking versus true reversibility" discussion needs to be mentioned: whereas a shared expression site (postsynaptic) and a shared synaptic target molecule (GluR2) are a required condition for true reversibility, a complete and entire reversal can only be reached if LTP is associated with a dephosphorylation of GluR2 at Ser-880, the site phosphorylated by PKC during LTD (Chung et al., 2003). Whether this is the case remains unknown at this point. Another aspect that needs to be considered is that pre- and postsynaptic components of LTP might differ in their spatial specificity.

A third example of cerebellar motor learning is associative eyeblink conditioning. Here, pairing of a conditioned and an unconditioned stimulus leads to the acquisition of a conditioned response. In associative eyelid conditioning, the CF input conveys information related to the unconditioned stimulus, while the PF input conveys information related to the conditioned stimulus (Thompson and Kim, 1996; Mauk and Donegan, 1997; Yeo and Hesslow, 1998; Carey and Lisberger, 2002). As PF-LTD can be induced by paired PF and CF stimulation, there is an obvious correlation between the activity requirements for LTD and this form of cerebellar motor learning. The need for heterosynaptic CF activity can be explained by its role in conveying the error signal. A long-standing observation in cerebellar motor learning is that repetitive application of the conditioned stimulus alone can cause an extinction of the conditioned response. The similarity to the observations made in vitro is striking: PF stimulation alone can induce PF-LTP, suggesting that learning at both the cellular and the behavioral level can be reversed by isolated PF activity. A requirement for such isolated PF activity in vivo is an inhibition of inferior olive activity below background levels by cerebellar projections (Medina et al., 2002a). It should be furthermore noted that there are features in the acquisition and extinction phases as well as in the timing of the learned responses that suggest that other forms of cerebellar plasticity, for example in nuclear cells (Medina et al., 2001) or in PF synapses on interneurons (Jörntell and Ekerot, 2002, 2003; Rancillac and Crépel, 2004), might also be involved (see De Zeeuw and Yeo, 2005).

Apart from indications arising from circuitry analysis, how solid is the experimental proof that synaptic plasticity provides a cellular correlate of learning? To date, one of the best experimental approaches to examine this relationship is to use genetically altered mice in which a key molecule, supposed to be involved in LTP or LTD, is targeted. The advantage of this approach is that the learning effect can be studied at the synapse level on the one hand and at the behavioral level on the other. The cerebellum belongs to the brain areas in which the most convincing correlations were obtained so far, which is largely due to the use of the PC-specific promotor L7. PC-specific mGluR1 deletion impairs LTD and causes motor coordination deficits (Ichise et al., 2000). PC-specific expression of a PKC peptide inhibitor (L7-PKCI) impairs LTD and VOR gain conditioning (De Zeeuw et al., 1998) as well as eyeblink conditioning (Koekkoek et al., 2003). Similar observations were made for signaling factors required for PF-LTD induction that were not discussed so far: conditional knockout mice with a PC-specific ablation of cGMP-dependent kinase I (PKGI) show reduced LTD and an impairment of VOR gain adaptation (Feil et al., 2003). Furthermore, in mutant mice lacking GluRδ2, the orphan glutamate receptor that is predominantly expressed in PCs, LTD induction is blocked (Kashiwabuchi et al., 1995) and eyeblink conditioning is affected (Kishimoto et al., 2001). While these studies provide strong support for the widespread hypothesis that LTD mediates forms of cerebellar motor learning (for review see De Zeeuw and Yeo, 2005), there are also examples of failures to establish such relationship: using a pharmacological approach it was shown that T-588, a drug that affects calcium release from internal stores, blocks PF-LTD in vitro (Kimura et al., 2005) but does not impair associative eyelid conditioning (Welsh et al., 2005). Studies are on the way to examine the effects of disrupting PF-LTP on motor learning. These studies are crucial to test how cerebellar LTP mediates extinction in eyeblink conditioning and may provide additional insights into how synaptic plasticity contributes to learning and memory.

Is Postsynaptic LTP a Learning Mechanism or Merely a Reversal Mechanism?

The recent discovery of a postsynaptically expressed form of LTP at PF synapses helps to explain reversal phenomena observed in behavioral paradigms but also poses new questions: Is LTP just a means to erase information that was previously stored by PF-LTD, or is LTP as much a learning mechanism as LTD (and, consequently, LTD as much a reversal mechanism as LTP)? Do we have to abandon the view that PF-LTD is the main cellular correlate of cerebellar motor learning?

It is well established that an overwhelming majority of PF synapses are silent (Wang et al., 2000b; Ekerot and Jörntell, 2001; Isope and Barbour, 2002; Ito, 2006), which would seem to indicate that LTD is the dominating plasticity process.This reasoning would be underlined if the PF synapse is active, nonsilent in the "default" state (in the early developmental stages, before the animal reaches an adapted state). The role of LTD during development and learning in the adult would then be to trim down the number of active PF synapses and in this way establish the specific input-output associations that are required to fine-tune movements. During relearning (i.e., for example recalibration of the VOR gain), some of the previously depressed PF synapses (ipsiHrz) might be restored to the default, active state by LTP, whereas part of the previously active PF synapses (coHrz) might now become depressed instead.

The alternative view is that both LTP and LTD, acting on different sets of PF synapses with antagonistic functions, are required to wire in some PF synapses and remove others in order to establish specificity in learning. Reversibility is readily achieved in this model, as argued for in the preceding section. This view would be underlined if the default state of PF synapses during development is silent, because LTP would then be the original driving process required in order to obtain any kind of learning. Accordingly, an important open issue for both views is the state of PF synapses (silent or nonsilent) at the developmental time period when cerebellar-dependent behavioral adaptations begin.

Is PF-LTP more than a reversal mechanism for PF-LTD? Maybe at this point the best answer is that it depends on the learning situation. In learning paradigms, which allow for bidirectional adaptation (e.g., VOR gain adaptation; PF receptive field changes) there is no obvious reason to assign a "learning" tag to one particular direction of change. We believe that this statement holds true, independently of the initial activity state of a synapse early during development (default state). Does this assumption limit the value of PF-LTD as a cellular correlate of motor learning? Certainly not. Despite the open questions about the general relationship between synaptic plasticity and learning, PF-LTD still provides the most convincing example available showing that alterations in synaptic strength can mediate behavioral learning. PF-LTD, with its specific requirements for induction and with its functional implications, still characterizes the cerebellum as a giant switch board for associative learning.

It also has to be kept in mind that several additional forms of Purkinje cell plasticity were discovered during the last years, including a presynaptic form of PF-LTP, LTD at the CF input to PCs, LTP of inhibitory inputs ("rebound potentiation"), and forms of activity-dependent alterations in intrinsic excitability (reviewed by Hansel et al., 2001). It still remains an open question as to which particular functions these types of plasticity have and how they interact with each other, particularly with bidirectional postsynaptic PF plasticity. A more detailed understanding of the different forms in which plasticity emerges as a network feature will likely also help us to better understand the relationship between synaptic and behavioral learning.

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