Cerebellar Long-Term Depression: Characterization, Signal Transduction, and Functional Roles

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Ito, Masao. Cerebellar Long-Term Depression: Characterization, Signal Transduction, and Functional Roles. Physiol Rev 81: 1143–1195, 2001.—Cerebellar Purkinje cells exhibit a unique type of synaptic plasticity, namely, long-term depression (LTD). When two inputs to a Purkinje cell, one from a climbing fiber and the other from a set of granule cell axons, are repeatedly associated, the input efficacy of the granule cell axons in exciting the Purkinje cell is persistently depressed. Section I of this review briefly describes the history of research around LTD, and section II specifies physiological characteristics of LTD. Sections III and IV then review the massive data accumulated during the past two decades, which have revealed complex networks of signal transduction underlying LTD. Section III deals with a variety of first messengers, receptors, ion channels, transporters, G proteins, and phospholipases. Section IV covers second messengers, protein kinases, phosphatases and other elements, eventually leading to inactivation of N-methyl-D-aspartate-sensitive glutamate receptors that mediate granule cell-to-Purkinje cell transmission. Section V defines roles of LTD in the light of the microcomplex concept of the cerebellum as functionally eliminating those synaptic connections associated with errors during repeated
exercises, while preserving other connections leading to the successful execution of movements. Section vii examines the validity of this microcomplex concept based on the data collected from recent numerous studies of various forms of motor learning in ocular reflexes, eye-blink conditioning, posture, locomotion, and hand/arm movements. Section vii emphasizes the importance of integrating studies on LTD and learning and raises future possibilities of extending cerebellar research to reveal memory mechanisms of implicit learning in general.

I. INTRODUCTION
(HISTORICAL BACKGROUND)

Studies of the cerebellum have a long history well documented in the monographs of Jansen and Brodal (247) and Moruzzi and Dow (127). The elaborate structural organization of the cerebellum analyzed by Jansen and Brodal (247) encouraged researchers toward exploration of its functional meanings. The enormous data of classic lesion experiments compiled by Dow and Moruzzi (127) suggested characteristic functional features of the cerebellum as enabling us to learn to move smoothly and accurately even at high speeds and without visual feedback. These earlier studies paved the way for the modern cerebellar research to proceed in the directions to uncover the elaborate neuronal mechanism of the cerebellum and to define its roles in the entire nervous system function. Brief history presented here overviews the progress of cerebellar research made during the past four decades toward these directions.

A. Dissection of Neuronal Network in 1960s

Until around 1960, synaptic plasticity was a concept of only theoretical importance, since there was no experimental evidence for it. Hebb (195) postulated that, in an assembly of mutually interconnected neurons, synapses activated synchronously, both presynaptically and postsynaptically, are strengthened in their transmission efficacy. Eccles (133), investigating posttetanic potentiation in the spinal cord, assumed that presynaptic activation alone is sufficient to potentiate synaptic transmission. Based on a set theory that includes possible combinations of presynaptic and postsynaptic events, Brindley (70) proposed the presence of 10 different types of synaptic plasticity in the central nervous system, including the Hebb and Eccles types. It is noteworthy that Brindley included long-term depression (LTD) under the designation “habituation,” giving equal weight to potentiation and habituation. He also predicted synaptic plasticity in inhibitory synapses, which was not known to exist until very recently (see sects. vC5 and vC8). To demonstrate the computational capability of a neuronal assembly, Rosenblatt (454) constructed a three-layered artificial neuronal network capable of learning, called the “Simple Perceptron,” which incorporated synaptic plasticity in one layer.

In the 1960s, the neuronal circuit of the cerebellum, involving five types of neurons [Purkinje cells (PCs), basket, stellate, Golgi and granule cells], was dissected in detail by morphological and microelectrode techniques, as summarized by Eccles et al. (134). PCs were revealed to receive two distinct excitatory inputs: climbing fibers (CFs) and axons of granule cells (GAs) (135–137) (Fig. 1). CFs originate from the inferior olive, while GAs relay mossy fibers (MFs) originating from the brain stem and spinal cord. Basket, stellate, and Golgi cells were identified as inhibitory neurons. The PCs providing the sole output pathway of the cerebellar cortex were defined as exclusively inhibitory upon their target neurons in the vestibular and cerebellar nuclei (244–246).

Convergence of numerous GAs and a single CF to each PC is a characteristic unique feature of neuronal circuitry in the cerebellum (Fig. 1) well known since Cajal (74). Brindley (69) was the first who viewed this structure as representing the Hebbian type of synaptic plasticity. Because CF signals via the numerous junctions between a CF and a PC (see sect. II4) could regularly excite PCs, synchronous activation of the CF and GAs results in a conjunction of presynaptic and postsynaptic excitation, which might in turn lead to long-term potentiation (LTP) of the GA-PC synapses. Marr (359) adopted this view in developing his epochal network theory of the cerebellar cortex. In view of the stable operation of the system, however, Albus (15) suggested that the conjonction results in a depression instead of potentiation and conceived the neuronal circuit in the cerebellar cortex as a pattern separator like the Simple Perceptron.

B. Exploration of Synaptic Plasticity in 1970s to 1980s

In the 1970s, two lines of evidence suggested the presence of a synaptic plasticity in the cerebellum. We observed that in the flocculus, an evolutionarily old part of the cerebellum, MF pathways arising from the vestibular organs and CF pathways arising from the retinas converge onto PCs (157, 224, 351). This unique pattern of convergence suggests that a synaptic plasticity occurs in the flocculus as causal to the remarkable adaptability of the vestibuloocular reflex (VOR), and the observation made in PCs of rabbits flocculus favored Albus’ hypothesis (128, 166, 167, 230). Gilbert and Thach (170) found that, in a monkey adapting to compensate for a sudden
change in arm load, PCs changed their discharge patterns in the manner predicted from Albus' synaptic plasticity assumption (sect. VI A5).

Nevertheless, earlier efforts devoted to demonstrating the occurrence of synaptic plasticity by conjunctively stimulating GAs and CFs failed to reveal a significant change in GA-PC transmission, which may be attributed to the following two major reasons. The first reason appears to be the small extracellular field potentials representing GA-PC transmission. LTP had successfully been detected in the hippocampus by recording synaptic events extracellularly (57); however, because the field potentials in the cerebellar cortex are 10 times smaller than those in the hippocampus, minor changes could have been undetected. A later study using an averaging technique revealed a depression (by 25%) in the field potentials of the in vivo cerebellum (234) and cerebellar slices (88). The second reason could be the vulnerability of LTD to various factors that might block its induction (see sects. iii and iv).

In the early 1980s, an analysis of the firing index of PCs in in vivo cerebella of rabbits revealed the presence of LTD (138, 241). When the stimulus for MFs or GAs was critically set at a threshold for exciting a PC, LTD was sensitively reflected in the lowering of the probability of firing in the PC. Later, LTD was also detected by recording GA-induced events in PCs either extracellularly (234) or intracellularly (459) and was eventually established as a distinct type of synaptic plasticity near the end of the 1980s (228). Different types of LTD were also reported to occur in the hippocampus and cerebral neocortex (65; see also Ref. 26).

C. System Approach in 1970s to 1980s

Although neuronal network theories developed by Marr (359), Albus (15), and others help us to understand computational mechanisms of elaborate neuronal networks in the cerebellum, control system theories help to understand how the elaborate networks of the cerebellum are utilized for controlling movements. Considering the unique way of involvement of the cerebellum in control of VOR, the author suggested that the cerebellum makes feed-forward control possible by replacing the feedback loop and that this replacement is effected due to a learning mechanism referring to “control error” signals conveyed by CFs (223, 225). That CF signals represent errors was also suggested from two other viewpoints. Miller and Oscarsson (375) proposed that the inferior olive acts as a comparator for command signals from higher centers and the activity these signals evoke at lower levels. Albus (15) postulated that CFs convey teacher’s signal informing PCs about their misperformance in conducting pattern recognition like the Simple Perceptron. Current evidence for the error representation by CFs is reviewed in section VI A.

Through the 1970s and 1980s, various simple forms of motor learning involving the cerebellum such as adaptation in ocular movements, eye-blink conditioning, locomotion, and hand/arm movements were established as model systems for studying mechanisms of motor learning. Thach (515) applied microelectrode techniques to the cerebellum of awake animals and collected signals of individual PCs correlated to these motor tasks that the animals performs. A morphological study of the cerebellum by Voogd and colleagues (178, 179) led to a
remarkable finding that, in addition to the classic lobular structure, the cerebellum consists of a longitudinal compartmental structure including seven major zones (A, B, C1, C2, C3, D1, and D2). Oscarsson (422) electrophysiologically defined a small, functionally uniform longitudinal area, called microzone, as a module of the cerebellar cortex.

Efforts were devoted earlier to understand global functional meanings of the unique anatomical architecture of the cerebellum such as the interconnection between the cerebellum and the cerebral cortex via thalamus, red nucleus, and pontine nucleus (17, 145, 223, 496). The unique cerebrocerebellar communication loop was interpreted as representing an internal model simulating the limb and its spinal motor centers (223, 227) (see sect. vE). These initial efforts are rewarded by the remarkable development of computational approach to the cerebellum in the 1990s (275, 276, 372, 558).

D. Discovery of Signal Transduction and Cognitive Function in 1990s

In the 1990s, owing to marked advancements in cellular physiology, biochemistry, and molecular biology, the complex signal transduction mechanisms of LTD have become a major research theme in neuroscience, as reviewed in sections III and IV. The advancement in knowledge of LTD signal transduction mechanisms provides new tools for investigating functional roles of LTD in various forms of cerebellar learning, as reviewed in section VI. In the 1990s, a new development in cerebellar studies was the expansion of cerebellar roles to cognitive functions. Such roles were proposed based on anatomical connections of the cerebellum with the cerebral association cortex (309, 310), and the author supported this view by analogies in movement and thought from a control system viewpoint (229). Experimental evidence for the involvement of the cerebellum in certain mental functions now accumulates in studies applying noninvasive measurements and advanced clinical examinations to a variety of human mental activities including language (146), attention (16), cognitive affective syndromes (466), fear and anxiety caused by threats of pain (436), thirst sensation and fear for air hunger (426), and motor relearning (221).

This review focuses on the major results of investigations conducted during the past two decades on the characterization of cerebellar LTD, its signal transduction mechanisms, and roles in cerebellar functions. Based on analyses of the available data, the review aims to clarify the targets of LTD studies in the forthcoming decades, in particular, the relationship of LTD with permanent memory and roles of LTD in not only physical but also mental activities.

II. CHARACTERIZATION OF CEREBELLAR LONG-TERM DEPRESSION

A. Induction of LTD

1. GA and CF synapses on PCs

GAs consist of segments ascending from the granular layer to the molecular layer and the bifurcating parallel fiber (PF) branches that run along the molecular layer (Fig. 1). A recent study revealed that PFs extend for 2–3 mm to each side of the bifurcation point, much longer than previously thought. Functional implication of this long PF projection is discussed in section vB2. Previously, PFs were assumed to provide the sole GA inputs to PCs, but Llinás (334) proposed a substantial contribution of the ascending axons to GA-PC transmission. Functional implication of the ascending segments of GAs is discussed in section vB1.

While each PC was reported to receive 60,000–80,000 synapses on their dendritic spines, generally, one synapse from one PF (424), more recent data in rats indicate that each PC receives as many as 175,000 PF synapses (405, 406). In contrast, each PC comes into contact with only one CF via numerous discrete synaptic junctions formed on stubby dendritic spines (424). Multiple innervation of a PC by CFs occurs during early development or in abnormal conditions such as genetic deficiency (see sect. vB4). The number of junctions formed between a CF and a PC has been calculated as ~300 in the frog cerebellum (335).

In the rat cerebellum, the number of synaptic junctions formed per 100-μm length of PC dendrites is 11.45 for GAs and 1.7 for CFs (412). This may suggest that as many as 26,000 synaptic junctions are formed between a CF and a PC in rat. Since, however, CFs do not reach peripheral portions of PC dendrites (424), this would give an overestimate.

2. Electrical signals that induce LTD

When recorded extracellularly in vivo, PCs generate two different types of spikes. Simple spikes discharge at a rate of 50–100 Hz, and complex spikes, in a form of short burst of spikes, occur at irregular, low rates around 1 Hz (515). Stimulation of GAs elicits simple spikes (Fig. 2A), whereas stimulation of CFs evokes complex spikes (Fig. 2B). When recorded intracellularly, PCs produce Na+ spikes and Ca2+ spikes (337, 338). The extracellularly recorded simple spikes are Na+ spikes generated in the somatic region and passively spread into the dendrites (Fig. 2C). This earlier notion has been supported by dual patch recording from somata and dendrites (500), and also using sodium-binding benzofuran isophtalate (SBFI) as a specific indicator for Na+ (77); the changes in intracellular sodium concentration ([Na+]i) associated...
with antidromically or intrasomatically evoked Na\(^+\) spikes were confined to the cell somata. CF responses are large excitatory postsynaptic potentials (EPSPs) due to the numerous synapses made by a single CF on the PC dendrites, superposed with somatic Na\(^+\) spikes followed by smaller Ca\(^{2+}\) spikes (337, 338) (Fig. 2D).

GA impulses evoke two pharmacologically distinct types of synaptic potentials in PCs. One is mediated by DL-\(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolone-propionate (AMPA)-selective glutamate receptors (see sect. III B1) (Fig. 2E) and the other by metabotropic glutamate receptors (mGluRs) (sect. III B3) (Fig. 2F). AMPA-EPSPs are fast and distinctly evoked by individual GA impulses, whereas mGluR-EPSPs are slow and observable after a brief tetanus of GAs (8 pulses at 50 Hz) in the presence of an AMPA receptor antagonist (39, 40). The size of the AMPA-mediated excitatory postsynaptic currents (AMPA-EPSC) generated by a single GA-PC synapse was estimated in cerebellar slices to be 2–60 pA (34).

LTD in GA-PC transmission is indicated by persistent reduction of the firing index of a PC in response to GA stimulation in extracellular recording, the initial rising slopes of GA-evoked AMPA-EPSPs in intracellular recording (Fig. 3), or the size of GA-evoked AMPA-EPSCs (Fig. 4A) or spontaneously arising miniature EPSCs (mEPSCs) (Fig. 4C), in whole cell clamping.

3. Conjunctive, homosynaptic, and heterosynaptic LTD

The major subject of this article is the special type of LTD induced at GA-PC synapses when GAs are repeatedly activated in conjunction with the CF converging onto the same PC. In view that the LTD is induced in those GA-PC synapses involved in conjunctive stimulation, it is homosynaptic, but in the sense that the LTD requires activation of CFs, it is heterosynaptic. It may be called conjunctive LTD, as needed.

GA-PC synapses also exhibit homosynaptic LTD when a relatively large set of GAs is repetitively stimulated without involving CFs (188). However, as long as GAs are moderately stimulated, LTD occurs only after GA-CF conjunctive stimulation (265, 381). Homosynaptic LTD also occurs in CF-PC transmission during stimulation of CFs at 5 Hz for 30 s (184), but not during 1-Hz CF/GA stimulation used for inducing conjunctive LTD.
Functional meanings of these types of homosynaptic LTD is discussed in section V, C1 and C4. When conjunctive or homosynaptic LTD occurs in a set of GA-PC synapses, LTD is also induced in the neighboring GA-PC synapses (445, 539). Functional meanings of this heterosynaptic type of LTD are discussed in section V C2.

4. Stimulus parameters

The optimal condition for inducing conjunctive LTD in the cerebellum in vivo is a 100-pulse stimulation of CFs and GAs at 4 Hz with a CF-to-GA stimulus interval of 125–250 ms (139, 265). In cerebellar slices, simultaneous GA/CF stimulation at 1 Hz for 5 min (300 pulses) was optimal in the presence of a GABA$_A$ antagonist (picrotoxin) (265). In the absence of a GABA$_A$ antagonist, 600 pairings at 1 Hz of GA and CF stimuli within ±250-ms intervals were required for effectively inducing LTD, while 100 pairings were sufficient in the presence of the GABA$_A$ antagonist (bicuculline) (88).

Although stimulation of GAs simultaneous with or after CF stimulation LTD is usually used for inducing LTD, whether a GA stimulus preceding a CF stimulus is effective in inducing LTD or not is often questioned. This question has been addressed in studies in which the GA/CF stimulation intervals were systematically changed (88, 139, 265). However, the results of such studies are inconclusive, because GA/CF stimulation intervals cannot be determined uniquely during repetitive GA/CF stimulation; for example, at 1-Hz stimulation, a GA stimulus preceding a CF stimulus by 100 ms follows the preceding CF stimulus with a 900-ms delay. Such a second-order effect may be reduced if the stimulus frequency is lowered. However, this strategy is not realistic because at a frequency lower than 1 Hz, conjunctive stimulation becomes less effective in inducing LTD (265). Unless an appreciable LTD can be induced by the conjunction of one GA stimulus and one CF stimulus, it is difficult to define a GA/CF time relationship.

To natural stimuli, GAs respond repetitively, and CFs often respond in the same way. Hence, there could be probabilities of overlap between a series of GA and CF impulses even when the MF- and CF-activating natural stimuli are not critically timed. It should also be kept in mind that GA and CF impulses produce complex signal transduction processes in PCs, as reviewed in sections III and IV, some of which could be sufficiently long lasting to provide a conjuction between GA-induced and CF-induced signal transduction. Schreurs et al. (468) successfully induced LTD in rabbit cerebellar slices by applying a series of 8 pulses (79 s, 100 Hz) to GAs and immediately thereafter 3 pulses (20 Hz) to CFs, 20 times at 30–40-s intervals. Wang et al. (539) also induced LTD in rat cerebellar slices by applying three to eight pulse stimuli to GAs followed by a single pulse stimulus to CFs within 50–200 ms. These GA-CF timing relationships are consistent with behavioral timing in motor learning (sect. VI, A2, B2, and C1). Although the reason why our previous study on rat cerebellar slices applying 15 pulses at 50 Hz to GAs and immediately thereafter 3 pulses at 50 Hz to CFs, repeated every 20 s for 10 min, failed to induce LTD (265) is unclear, the stimulus conditions for LTD induction need to be further explored in connection with stimulus parameters and pharmacological environments, in which cerebellar tissues are placed.

5. Interaction with postsynaptic inhibition

Induction of GA/CF-induced LTD in the cerebellum in vivo was effectively blocked when the inhibitory
postsynaptic potentials (IPSPs) were concomitantly induced in PCs by stimulation of off-beam PFs (138). This effect is apparently related to the shortening or abolition of long-lasting plateau potentials, which represent \( \text{Ca}^{2+} \) currents evoked by CF impulses. Imaging of intracellular \( \text{Ca}^{2+} \) concentration ([Ca\(^{2+}\)]\(_i\)) with fura 2 demonstrated that IPSPs strongly reduced the CF-associated increase of [Ca\(^{2+}\)]\(_i\) in PC dendrites (76). In cerebellar slices, this complication is usually avoided by blocking IPSPs with a GABA\(_A\) antagonist (picrotoxin or bicuculline). However, even in the absence of a GABA\(_A\) antagonist, GA stimulation with intermittent tetanic high-frequency effectively induced LTD (265, 484). In the cerebellum in vivo, LTD induction was not disturbed unless relatively large IPSPs were evoked by electrical stimulation (138). These observations refute the suggestion that the LTD induced in cerebellar slices treated with a GABA\(_A\) antagonist is not a physiological phenomenon.

**B. Features as Synaptic Plasticity**

1. **Postsynaptic origin**

   Because the glutamate sensitivity of PCs undergoing LTD was persistently depressed after the conjunction of CF stimulation and iontophoretic application of glutamate, GA/CF-induced LTD has been ascribed to a reduction in the efficacy of postsynaptic glutamate receptors mediating GA-PC transmission (241). That a reduced form of LTD (sect. II B3) can be induced in cultured PCs in the absence of presynaptic elements confirms that LTD occurs entirely postsynaptically. Metabotropic glutamate receptors subtype 4 (mGluR4) are distributed in the molecular layer along the presynaptic membrane of GAs (284, 363). Mice deficient in mGluR4 exhibited impairment of the GA-induced paired-pulse facilitation and posttetanic potentiation in PCs, whereas LTD was not impaired (428). This indicates that LTD induction does not involve the
mGluR4-regulated presynaptic mechanism for maintaining synaptic efficacy during repetitive activation. GAs express cannabinoid type 1 receptors (CBR1s) and their mRNAs, and selective CBR1 agonists markedly depress GA-PC transmission (313). CBR activation reduced transmitter release at GA-PC and CF-PC excitatory synapses by modulation of presynaptic voltage-gated channels, and also at inhibitory synapses in PCs by not only presynaptic voltage-gated channel modulation but also suppression of the vesicle release mechanisms (505). CBR agonists also partially inhibited GA/Ca$^{2+}$ spike-induced LTD (313), but how the presynaptic CBR1s are linked with LTD induction is unclear at present.

2. Input specificity

When the vestibular nerve on one side was stimulated in conjunction with stimulations of CFs at the inferior olive, LTD was observed in the flocculus PCs only in their responses to stimulation of that nerve, but not of the other vestibular nerve (241). When two PF beams were differentially stimulated at a distance of 100–200 $\mu$m in vivo cerebellum (138, 256) or 300 $\mu$m in cerebellar slices (88) in the direction perpendicular to the PF beams, LTD was induced only in the PF beam involved in conjunctive stimulation with CFs. In cultured PCs, a reduced form of LTD (see below) occurred only on the part of the dendrite exposed to stimuli in the absence of presynaptic elements (324). These observations are indicative of the input specificity of LTD in PCs, but according to the recent studies introduced below, the input specificity of LTD does not strictly hold for those synapses located within ~100 $\mu$m from stimulated GA-PC synapses.

When conjunctive LTD was induced by stimulating a PF beam, test stimulation of a second PF beam at a distance of 36–109 $\mu$m from the first beam revealed spread of LTD to neighboring synapses despite the fact that they were not involved in the conjunctive stimulation (445). The spread still occurred even when the stimulation of the first PF beam was lowered to involve only ~20 PF fibers. Spread of LTD to neighboring synapses has also been demonstrated by locally applying glutamate to PC dendrites (539). Glutamate was released by uncaging with 3- to 5-$\mu$m diameter ultraviolet (UV) light spot. It was thus found that glutamate sensitivity of a PC dendrite was reduced for 100 $\mu$m from the activated synapses. The spread of LTD could be mediated by a chemical signal moving from the conjunctively activated synapses to their neighborhood, either extracellularly or intradendritically. Such a signal may be provided by one of the complex signal transduction processes underlying LTD such as nitric oxide (NO) (see sect. V A2). Functional implication of the reduced input specificity of conjunctive LTD is discussed in section V C2.

3. Reduced forms of LTD

In the cerebellum in vivo, iontophoresis of glutamate or quisqualate, but not kainate or aspartate, to PCs, replacing GA stimulation, effectively induced a persistent reduction in sensitivity of PCs to glutamate, when combined with CF stimulation (241, 256). In cerebellar slices, replacement of CF stimuli by application of depolarizing pulses, which cause the entry of Ca$^{2+}$ into PCs through voltage-gated channels, induced LTD when combined with GA stimulation (GA/depolarization-pairing at 1 Hz for 15 min) (104, 105). To obtain LTD with GA/depolarization-pairing, however, GA stimuli stronger than with GA/CF conjunction are required, suggesting an additional contribution of CF stimulation to Ca$^{2+}$ entry (445). A protocol using 20 pairs of a brief GA tetanus combined with a subsequent 100-ms depolarization is also effective in inducing LTD (151).

Various chemical stimuli, related to signal transduction for LTD as reviewed in sections III and IV, induce LTD when combined with GA stimulation. For example, NO donors (484), membrane-soluble cGMP analogs (485), and protein phosphatase inhibitors (12) are effective. The simplest procedure for inducing LTD is to apply quisqualate (574) or to increase the extracellular K$^+$ concentration (101). The grease-gap method was used for determining the chemical sensitivity of PCs in a cerebellar slice trimmed into a wedgelike form (39, 235, 236, 237, 574). Persistent reduction in AMPA-induced potentials of PCs was induced by AMPA application following perfusion of 8-bromo-cGMP, trans-1-aminocyclopentyl-1,3-dicarboxylate (trans-ACPD), a mGluR agonist, or sodium nitroprusside, a NO donor (236).

In cultured PCs devoid of both GAs and CFs, a reduced form of LTD is induced by a combination of glutamate (or quisqualate) pulses and membrane depolarization (328, 329) (Fig. 4B). The LTD then is detected as a reduced sensitivity of PCs to glutamate or AMPA. This form of LTD occurs in very simplified preparations devoid of dendritic spines, indicating that LTD induction does not require such morphological specialization (407). In PCs cocultured with granule cells, mEPSCs can also show LTD (395) (Fig. 4C).

4. Time course

GA/CF-induced LTD in cerebellar slices usually develops progressively over an hour (265, 267) (Fig. 3). In contrast, glutamate/depolarization-induced LTD in cultured (322) or freshly isolated (407) PCs or GA/depolarization-induced LTD in PCs cocultured with granule cells (542) reaches a steady peak within a few minutes (Fig. 4B). LTD in cultured PCs occurs in two phases. The short-term depression (STD) declines after the peak at ~5 min and recovers in 30 min and normally is replaced by the late-phase depression. Inhibitors of phospholipase
(PL) A2 (see sect. viE2) abolished only the late phase and left the STD in isolation (322). Apparently, STD is lacking in GA/CF-induced LTD, which would correspond to the late phase of glutamate/depolarization-induced LTD. STD might arise from the use of large depolarizations to replace CF stimuli.

The observation time for LTD in cerebellar slices and in the cerebellum in vivo is usually 30 min to 1 h, and occasionally for 2–3 h. In certain special cases, longer times have been reported. The quisqualate-induced persistent reduction in AMPA sensitivity of PCs, as revealed by the grease-gap method, was monitored for 12 h without recovery (235). The amplitude of mEPSCs in cultured PCs was persistently reduced after 5-min conjunctive application of 50 mM K⁺ and 100 µM glutamate, and this reduction lasted for 36 h and recovered to the original level after 48 h (395) (Fig. 4D). When the hemispheric area of the lobule simplex (HVI) of rabbit cerebellum was sliced 24 h after the rabbit had been trained for eye-blink conditioning, sequentially applied GA and CF stimuli failed to induce LTD, which occurred in slices dissected from control rabbits (469). This suggests that LTD underlying the eye-blink conditioning (sect. viB2) persists for at least 24 h and precludes eliciting another LTD. An increased excitability of the PC dendrites in the rabbit lobule HVI was detected after eye-blink conditioning, even after 1 mo (467). This learning-specific excitability increase was presumably caused by changes in a K⁺ current, possibly mediated by an Iₐ-like current, but its relationship to LTD is presently unclear.

III. SIGNAL TRANSDUCTION: INITIAL PROCESSES

Section iii deals with the initial steps of signal transduction for LTD involving first messengers, receptors, ion channels, G proteins, and phospholipases. Readers may also refer to recent review articles (53, 110, 312, 327).

A. First Messengers

In addition to glutamate that plays a major transmitter role in GA-PC synapses and probably also in CF-PC synapses, NO, corticotropin-releasing factor (CRF), and insulin-like growth factor I (IGF-I) are released from GAs or CFs and act on PCs as first messengers (Fig. 5).

1. GA-released glutamate

GAs contain and, upon stimulation, release glutamate (see Ref. 227). Because the specific antagonist for AMPA-
selective glutamate receptors, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), blocks GA-PC transmission (298, 429), glutamate has been established as a transmitter from GAs. Release of glutamate from GAs in GA-PC transmission is controlled by Ca$^{2+}$ entering PFs via multiple types of Ca$^{2+}$ channels (377). Release of glutamate from GAs is also regulated by mGluR4s, CR1Rs (sect. II), and adenosine receptors coupled with adenylyl cyclase, cAMP, and protein kinase A (PKA) (sect. IV).

Glutamate transporters take up the glutamate released into the synaptic gap. Among the five structurally distinct types of glutamate transporters so far identified, EAAT4 is abundantly expressed in PCs and is concentrated on the extrajunctional membrane of dendritic spines in contact with the GAs (510). GLAST, on the other hand, is highly expressed in the Bergman glial processes that ensheath GAs to PC synapses (308). Since the time courses of GA-evoked EPSCs in PCs are normal in both GLAST-deficient (548) and EAAT4-deficient (512) mutant mice, these transporters do not appear to be the primary factors determining the kinetics of GA-evoked EPSPs.

2. GA-released NO

Nitric oxide synthase (NOS) is of three types: neuronal (nNOS), epithelial (eNOS), and inducible (iNOS). The nNOS is abundant in the granule cells of the cerebellum (289, 291, 450); eNOS is also expressed in the granule cells at a lesser level (125). NOS immunoreactivity is also strong in basket cells, but not observed in PCs (141). The mRNAs harvested from single neurons using micropipettes encoded NOS from granule cells, which were absent in PCs (101). Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d), which is a critical co-enzyme of NOS, is abundant in the granular and molecular layers but not in PCs (494, 562). The molecular structure of nNOS, which is abundant in the brain, contains recognition sites for NADPH and calmodulin, as well as phosphorylation sites resembling those of cytochrome P-450 reductase (68). In cerebellar slices, NO is released by activation of GAs (483). The NO release from GAs is potentiated by the tetanic GA stimulation, as well as by activation of PKA in GAs (283).

The earlier assumption that NO might be released from CFs, or within PCs stimulated by CF signals, was based on the following observations. Rats treated with 3-acetylpromazine (3-AP) to lesion CFs showed a substantial reduction in electrical stimulus-induced NO release (484) or a K+-induced increase in cGMP concentration presumably caused via NO activation of guanylyl cyclase (sect. III) (494) in cerebellar slices. These effects are, however, transient and are not directly related to the loss of CFs; the cGMP levels in the rat cerebellum were depressed once with a peak on day 7 and thereafter returned to the control level in 14–20 days (421; see also Ref. 382).

There is substantial evidence showing that NO plays a role in LTD induction. 1) Bath application of a NO donor, sodium nitroprusside (484), or its infusion into PCs (109), effectively induced LTD. 2) The release of NO from its caged form within PCs induced LTD when combined with depolarization-induced Ca$^{2+}$ entry (314). 3) Bath application of NOS inhibitors readily blocked LTD (103, 484). As would be expected from the location of NOS in GAs, the postsynaptic application of a NOS inhibitor did not block LTD (109). 4) Targeted disruption of the nNOS gene in mice resulted in the virtual loss of NOS activity in the cerebellum (217), and accordingly, GA/depolarization-conjunction did not induce LTD in cerebellar slices obtained from these mice (316). Photolytic uncaging of NO and cGMP inside PCs did not recover the loss of LTD, suggesting that prolonged absence of nNOS might lead to alteration of the signaling pathway downstream of cGMP. Thus the requirement of NO in LTD induction in cerebellar slices is evident, but it is to be noted that this is not the case in cultured PCs. NOS donors, scavengers, or inhibitors did not affect the reduced form of glutamate/depolarization-induced LTD in cultured PCs (326), and LTD in cultured PCs derived from nNOS-deficient mice was indistinguishable from that in cultures from wild-type mice (328).

In cerebellar tissues, sensitivity of NOS to Ca$^{2+}$ is regulated by protein kinase C (PKC) (418). The compound 6R9–5,6,7,8-tetrahydro-L-biopterin (H$_4$B), a cofactor of NOS, binds to NOS to stabilize it against phosphorylation by PKC (420). Cerebellar tissues from those mice partially deficient in H$_4$B had significantly lower cGMP levels compared with the control (66). Yet, the increase in cGMP levels following application of a NO donor was normal, suggesting that impairment of cGMP synthesis in H$_4$B-deficient mice was due to decreased NO synthesis (see sect. III).

The compound NO is unique compared with the usual neurotransmitters, for it is a short-lived gas diffusing into the vicinity of its source. Calculations based on the diffusion constant suggest that NO diffuses over 14 m in 10 ms (539). Since 1 $\mu$m of the molecular layer contains 7.24 $\times$ 10$^8$ dendritic spines of PCs (406), a spherical space of the molecular layer, 14 $\mu$m in radius, would contain 4,136 spine synapses. Hence, NO released from one site in the molecular layer would influence 4,000 spine synapses by diffusion of NO in 10 ms. The mediator for heterosynaptic LTD is yet to be identified, but NO could be a candidate for it (445).

3. CF-released amino acid

Aspartate and homocysteate were previously proposed as candidate transmitters released from CFs on the
basis of the results of uptake and release experiments (534, 555), but only weak immunoreactivity to either aspartate or homocysteate was found in CFs (577). On the other hand, evidence suggests that glutamate is a transmitter from CFs. 1) CFs exhibit glutamate-like immunoreactivity at a significant level (577). 2) CF-PC transmission is effectively blocked by AMPA-specific antagonists such as CNQX (298). However, caution is needed because the excitatory action of homocysteate is also blocked by CNQX (572), and PCs express distinct aspartate receptors sensitive to glutamate antagonists (573). 3) Blocking glutamate transporters in PCs by infusion with d-aspartate through a whole cell clamp micropipette prolonged the decay of CF-evoked EPSPs (506). Because CF-EPSPs are normal in EAAT4-deficient mice, another transporter may be responsible for this effect (512). 4) A transmitter released from CFs activates both AMPA receptors and glutamate transporters expressed by the Bergman glial cells (130). Interestingly, CF transmitter at the same time presynaptically inhibited GABA-mediated inhibitory synapses on PCs (463).

Despite these lines of supportive evidence, the possibility of glutamate being a CF transmitter remains to be confirmed since the specific release of glutamate from CFs has not been proven.

4. CF-released CRF

CRF-like immunoreactivity has been observed in all divisions of the inferior olivary nucleus and at all levels, from the cells of origin of the olivocerebellar afferents in the inferior olivary neurons to their CF terminals on PCs (106, 425). A KCl challenge induces CRF release from rat cerebellar slices (55). CRF plays a permissive role in the LTD induction in cerebellar slices (sect. \( \mu B7 \)). However, because CRF is likely to be absent in tissue cultures devoid of CFs, CRF may not be an indispensable factor for the glutamate/depolarization-induced reduced form of LTD in cultured PCs.

It is noted that besides the CFs, CRF is also distributed densely in the paraventricular nucleus of the hypothalamus, central nucleus of amygdala, and locus coeruleus, all of which are involved in stress responses. The functional meaning of the presence of CRF in CFs may be interpreted in connection with the role of CFs in motor learning (sect. \( \nu A \)), since motor learning can usually be accomplished by repeated exhausting exercises. Motor learning may well be a kind of stress in a wide sense (298).

5. CF-released IGF-I

IGF-I, a basic peptide, is known to modulate cell growth and differentiation and to act as a modulator at various synaptic sites. Several lines of evidence indicate the presence of IGF-I in CFs. The inferior olive exhibits IGF-I immunoreactivity (4) and expresses IGF-I mRNAs (59, 551). Electrical stimulation of the inferior olivary significantly increased the IGF-I levels in the cerebellar cortex (81). Chemical and surgical lesions of the olivocortical pathway produced a drastic decrease in cerebellar IGF-I levels (523). IGF-I antisense oligonucleotide injected into the inferior olivary induced a significant reduction in the size of dendritic spines on PCs (412). There is now evidence that IGF-I plays a role in LTD induction (sect. \( \mu B7 \)). PCs have been reported to exhibit strong IGF-I immunoreactivity in the PC cell somata, dendrites, dendritic spines as well as axons, and in the rough endoplasmic reticulum (3, 4, 481). Relationships of the intracellularly localized IGF-I immunoreactivity to LTD induction are unclear for the present.

B. Receptors

Activation of the four types of receptors by the first messengers released from GAs and CFs trigger diverse signaling processes in the membrane of PCs such as the activation of ion channels, G proteins, and associated phospholipases (Fig. 5). In addition, NO released from GAs diffuses into PCs and reacts with an enzyme. A unique feature of PCs, distinct from most other neurons including cerebellar granule cells, is the absence of N-methyl-D-aspartate (NMDA) receptors. Nevertheless, adult rat PCs intensely express the mRNAs for the subunit NR1, and the NR2A mRNA weakly (13). NR2D mRNAs are expressed in PCs transiently during the first week after birth. Mouse cerebellum also exhibited NR2A and NR2B immunoreactivity in PCs (519). These subunits apparently do not form NMDA receptors in adult PCs.

1. AMPA receptor in GA-PC synapses

AMPA receptors mediate the fast GA-PC transmission and are the final target of the signal transduction for LTD (sect. \( \nu E \)). AMPA receptors in mature PCs include mainly GluR2 and GluR3 and also GluR1 subunits (465). The mRNAs harvested from individual PCs encode the following five subunits: the flip and flop versions of GluR1 and GluR2 as well as GluR3 flip, with GluR2 being the most abundant (304). Receptors labeled with antibodies against GluR2/3 or GluR2 are localized in the synaptic zone of dendritic spines, with a decrease in receptor density in the outer 20% of the synapse (431). GluR1 is also present in the postsynaptic membrane of GA-PC synapses (43).

Activation of AMPA receptors appears to be one of the requirements for LTD induction, because the combination of GA/Ca\(^{2+}\) spikes failed to induce LTD when applied in the presence of CNQX (198). Nevertheless, because LTD can be induced without stimulating AMPA receptors in cases such as conjunctive activation of
mGluR with membrane depolarization (108, 198) (sect. mB3), activation of AMPA receptors may not be an indispensable requirement for LTD induction.

Although AMPA receptors are typical ionotropic receptors associated with ion channels, evidence has been presented that AMPA receptors are also associated with chemical signal transduction like the metabotropic receptors. The GluR1 subunit in AMPA receptors of cortical neurons is associated with G protein (541). Activation of AMPA receptors in cerebellar neurons results in activation of protein tyrosine kinase (PTKs) (193), which also has a role in LTD induction (sect. ivC3).

2. δ2-Receptor in GA-PC synapses

The δ2 subtype of glutamate receptors is selectively expressed in PCs, while low levels of δ1 subtype are found widely in the adult brain (22, 344). Immunogold labeling revealed the presence of δ2- subunits of glutamate receptors in GA-PC synapses with a distribution pattern similar to AMPA receptors (305, 431). In the dendritic spines of PCs, δ-receptors are anchored to actin filaments via spectrin, an actin-binding protein (205, 206).

A role for δ2-receptors in LTD induction is suggested by two major findings. 1) Treatment of cultured PCs with an antisense oligonucleotide against the δ2-subunit mRNA blocked LTD induction but had no appreciable effect on the basic physiological or morphological properties of PCs (208, 250). 2) PCs in cerebellar slices (269) and tissue cultures (209) derived from δ2-deficient gene-knockout mice did not exhibit LTD.

The δ2-receptors do not form functional glutamate-gated ion channels. However, when an A (alanine)-to-T (threonine) point mutation is introduced at position 654, the end of its third transmembrane segment, as occurs in lurcher mutant mice, and when a Q (glutamine)-to-R (arginine) mutation is also induced at the so-called Q/R site in the second transmembrane segment, the modified homomeric δ2-receptors display ion channel activity including a moderate Ca2+ permeability in the absence of ligand binding (293). A factor(s) that may activate δ2-receptors, a ligand, a receptor subunit or associated messengers have yet to be found for understanding how δ2-receptors contribute to LTD induction.

3. mGluR in GA-PC synapses

Eight subtypes of the mGluR family are classified into three groups: I (mGluR1 and -5), II (mGluR2 and -3), and III (mGluR4, -6, -7, -8) (98, 401). Group I subtypes are characterized by their stimulating phosphatidylinositol hydrolysis and Ca2+ release from intracellular stores, whereas the group II and III subtypes negatively regulate cAMP formation. Several of these subtypes have multiple splice variants. So far, four isoforms of mGluR1 (a-d) have been detected in PCs (49, 177). Cultured PCs exhibit immunoreactivity for mGluR1a in both somatic and dendritic regions (173, 410). In the mouse cerebellum, 80% of all PC dendritic spines express mGluR1a immunoreactivity. The expression was retained even when presynaptic GAs were lost by intoxication with methylazoxymethanol (504). In the dendritic spines of PCs, both mGluR1a and mGluR1b are localized in the perisynaptic areas outside the postsynaptic densities (346, 362, 431). While mGluR1a dominates within 60 nm from the edge of the postsynaptic densities, mGluR1b distributes more evenly to over 360 nm (362).

The involvement of mGluR1 in LTD induction has been demonstrated in the following experiments. 1) LTD induction in cerebellar slices was blocked by an antagonist of mGluR1, (RS)-a-methyl-4-carboxyphenylglycine (MCPG) (185). 2) Antibodies inactivating mGluR1 blocked the reduced form of LTD in cultured PCs (487). 3) LTD was impaired in mGluR1-deficient gene-knockout mice (6, 100). The transgenic mice whose mGluRs were rescued only in the cerebellum restored normal LTD (220). 4) Activation of mGluRs by the agonists trans-ACPD or 1S,3R-aminocyclopentyl-dicarboxylate (1S,3R-ACPD) induced LTD when combined with GA tetanus (102) or depolarization-evoked Ca2+ spikes, even in the presence of CNQX (108, 198).

Activation of mGluR1 evokes mGluR-EPSPs in PCs (39, 40). The mGluR-EPSPs induced by repetitive stimulation of GAs (usually 8 pulses at 50 Hz) have a slow time course when observed in the presence of an AMPA antagonist (peak time, 200–300 ms). Even though mechanisms for generating mGluR-EPSPs have not yet been identified, Tempia et al. (514) suggest that it is due to activation of a nonspecific cation channel. In fact, generation of mGluR-EPSPs is associated with an increase in intradendritic Na+ concentration (288). An alternative possibility is the activation of an electrogenic Ca2+/Na+ exchanger that extrudes Ca2+ released from intracellular stores by the activation of mGluR1s (sect. ivA2). However, because KB-R7943, a selective inhibitor of the Ca2+/Na+ exchanger, only partially depressed the mGluR agonist-evoked inward currents, mGluR-EPSPs may have only a minor contribution from the Ca2+/Na+ exchanger (210).

4. Glutamate receptor in CF-PC synapses

In CF-PC synapses, all three subunits of the AMPA receptors (GluR2, GluR3, GluR1) and mGluR1 are distributed in a similar manner to GA-PC synapses (43). The δ2-receptors are expressed in both GA-PC and CF-PC synapses during developmental stages, but after postnatal day 21 and in adulthood, δ2-receptors are scarce in CF-PC synapses, while they are predominant in GA-PC synapses (579, 431). The report that L-homocysteic acid generates substantial responses in cultured PCs (572) may draw
attention because of the earlier proposal that L-homocysteate might be a transmitter (sect. maA3), but these responses are mediated by AMPA receptors.

Receptors in CF-PC synapses are blocked by CNQX (29) similarly to AMPA receptors in GA-PC synapses (290). However, these two types of synapses differ in their sensitivity to another antagonist, a synthetic analog of Joro spider toxin, 1-naphthyl-acetyl-spermine (NAS). NAS blocked the GA-PC synapses in cerebellar slices but not the CF-PC synapses (11). It is noted that, in cultured hippocampal neurons, NAS differentially blocked AMPA receptors associated with a strong inward rectification and permeability to Ca\(^{2+}\), but not those associated with a slight outward rectification and low Ca\(^{2+}\) permeability (294). In recombinant glutamate receptors expressed in Xenopus oocytes, NAS differentially blocked AMPA receptors composed of GluR1, GluR3, GluR4, or GluR1/3, but not those composed of GluR1/2, GluR2/3, or GluR6 (56). These results would suggest that the AMPA-type receptors in the CF-PC synapses may have a different subunit composition from those in GA-PC synapses.

5. Guanylyl cyclase

The soluble form of guanylyl cyclase is a heme-containing protein involved in the enzymatic conversion of GTP to cGMP. Soluble guanylyl cyclase is localized in PCs, particularly in the primary dendrites (27), and the mRNA for soluble guanylyl cyclase is expressed in the granule cells and PCs (543). Soluble guanylyl cyclase acts as a receptor for NO and, in the NO-activated form, synthesizes cGMP. In cultured PCs, immunofluorescence associated with a monoclonal antibody recognizing both \(\alpha\)- and \(\beta\)-subunits of soluble guanylyl cyclase was shown to increase in response to NO generated by a NO donor (525). Intracellular application of the potent and selective inhibitor of soluble guanylyl cyclase, 1H-[1,2,4]oxadiazole[4,3-a] uinoxalin-1-one (ODQ), has been shown to effectively block LTD induction (62).

Carbon monoxide (CO) is an activator of guanylyl cyclase and is formed by the catalytic action of inducible type 1 and constitutive type 2 heme oxygenase. The heme oxygenase-2 mRNA is densely distributed in the granular cells and PCs, similarly to the mRNA for soluble guanylyl cyclase, which may suggest that CO replaces NO in activating soluble guanylyl cyclase (533). Nevertheless, this possibility is doubtful because of the low enzymatic activity of the soluble guanylyl cyclase-CO complex (212). In primary cultures of olfactory neurons, zinc-protoporphyrin-9 (ZnPP), a heme oxygenase inhibitor, was shown to deplete endogenous cGMP (533). However, the specificity of ZnPP to heme oxygenase is questionable because ZnPP may act directly on soluble guanylyl cyclase or may deplete intracellular \(L\)-arginine that is required for NO synthesis (347, 419). Another potent activator of guanylyl cyclase is arachidonic acid (524) (sect. nB5).

6. CRF receptor

The CRF type 1 receptor (CRFR1) and its mRNAs are present on the somata and dendrites of PCs (437). CRFR1 is positively coupled with adenylyl cyclase through \(G_s\) proteins (42), but there is no evidence indicating that adenylyl cyclase, cAMP, or PKA has any role in LTD induction (sect. nC5). There is evidence that PKC is activated via CRF receptors in cerebellar tissues (382).

Involvement of CRFR1s in LTD induction is indicated by the finding that CRF antagonists, \(\alpha\)-h CRF and astrepsin, blocked LTD induction (382). For LTD induction, however, CRF release driven by CF impulses does not appear to be required each time because the CRF antagonists effectively block the LTD induced by GA/Ca\(^{2+}\) spike conjunction without stimulating CFs. CRF released spontaneously from CF may play a permissive role in LTD induction.

7. IGF-I receptors

PCs express receptors for IGF-I (60, 162, 551). Involvement of IGF-I in LTD induction has been suggested in a microdialysis experiment where glutamate applied to rat cerebellar cortex induced the release of GABA from the cerebellar nucleus (82). The glutamate-induced GABA release was persistently inhibited when IGF-I, but not the basic fibroblast growth factor (bFGF), was also applied to the cerebellar cortex (81). Electrical stimulation of the inferior olive in conjunction with glutamate application to the cortex also inhibited subsequent glutamate-induced GABA release from the nuclei. A PKC inhibitor and a NOS inhibitor blocked the effect of glutamate/IGF-I conjunction in depressing the glutamate-induced GABA release. A phorbol ester, a PKC activator, or \(L\)-arginine, a NO donor, when applied in conjunction with glutamate, mimicked the effect of IGF-I in depressing the GABA release.

More direct evidence for the involvement of IGF-I and its receptors in LTD induction has recently been reported (542). Application of IGF-I or insulin that also binds to IGF-I receptors effectively induced LTD in cultured PCs as represented by a reduction of AMPA currents, but not NMDA currents, and this effect of IGF-I was blocked by an antibody or an inhibitor of IGF-I receptors. How the activation of IGF-I receptors results in LTD induction is not clear for the present. Because IGF-I stimulates production of 1,2-diacylglycerol (DAG) (295), it could in turn activate PKC as required for LTD induction (sect. ni, B1 and C1). Because partial peptides of dynamin and amphiphysin that interfere with endocytosis blocked the IGF-I-induced LTD (541), this form of LTD may be caused by internalization of AMPA receptors by endocytosis, as suggested to occur in the final stage of signal transduction.
transduction for LTD (sect. ivE3). Insulin-induced LTD also reduced the number of GluR2-containing AMPA receptors in tissue-cultured hippocampal neurons, and this form of LTD is caused by clathrin-dependent endocytosis (356).

C. Ion Channels

Ions enter neurons in general through voltage-sensitive as well as ligand-gated channels. Synaptic transmission in GA-PC and CF-PC synapses induces movement of ions across PC membrane.

1. \( \text{Ca}^{2+} \) channels

Because PCs do not develop NMDA receptors associated with \( \text{Ca}^{2+} \) channels (see above), \( \text{Ca}^{2+} \) entry to PCs is mostly mediated by voltage-sensitive \( \text{Ca}^{2+} \) channels, occurring during either \( \text{Ca}^{2+} \) spikes or current-induced membrane depolarization (315, 373, 377, 513). A significant transient entry of \( \text{Ca}^{2+} \) in PC dendrites occurs during \( \text{Ca}^{2+} \) spikes and associated plateau potentials, but some \( \text{Ca}^{2+} \) signals also occur in somata (263, 315, 373). \( \text{Ca}^{2+} \) entry is induced not only during CF-evoked \( \text{Ca}^{2+} \) spikes, but also during AMPA-EPSPs induced by GA stimulation of below the threshold of the generation of \( \text{Ca}^{2+} \) spikes. This \( \text{Ca}^{2+} \) entry is confined to a compartment consisting of a small number of branchlets of terminal spiny dendrites (142) or even in individual dendritic spines (112). The dendritic \( \text{Ca}^{2+} \) signals depended on the size of GA-evoked EPSPs so that 20–30 GAs must be activated within the local dendritic area to produce a detectable increase in the postsynaptic \( \text{Ca}^{2+} \) concentration. \( \text{Ca}^{2+} \) entry is indispensable for LTD induction (sect. ivA1).

Six types of voltage-dependent \( \text{Ca}^{2+} \) channels have so far been identified (termed T, L, N, P, Q, and R). These channels are heteromultimeric complexes composed minimally of one main subunit, \( \alpha \), serving as both pore and voltage sensor, and auxiliary \( \beta \) and \( \delta \)-subunits. PCs are immunoreactive to \( \alpha_{1A} \) in the somata as well as the entire length of dendrites (553). The high-threshold P channel was originally described in PCs (339). The high-threshold Q channels were first described in cerebellar granule cells as distinct from P channels in its inactivation kinetics and sensitivity to \( \omega \)-agatoxin IVA, a spider toxin (442). P and Q channels appear to be generated by alternative splicing of the \( \alpha_{1A} \)-subunit gene (61). The funnel web spider toxin (FTX), a specific P channel blocker, abolished the \( \text{Ca}^{2+} \) spikes in PCs (529). The \( \omega \)-agatoxin IVA, which blocks Q type of \( \text{Ca}^{2+} \) channels, abolished the depolarization-induced \( \text{Ca}^{2+} \) spikes, the spike-associated steep increase in the dendritic \([\text{Ca}^{2+}]_d\), and the steady intrasomatic increase in \([\text{Ca}^{2+}]_i\) concentration (546). Thus P/Q channels are responsible for the generation of dendritic \( \text{Ca}^{2+} \) spikes. The N and L types of high-voltage-activated \( \text{Ca}^{2+} \) channels are also expressed in PC dendrites (552), but evidence supporting the possibility that N or L types of \( \text{Ca}^{2+} \) channels play significant roles in PC functions is limited (546).

The presence of low-voltage-activated (also called low-threshold or T type) \( \text{Ca}^{2+} \) channels has been controversial. The results of electrophysiological recordings and optical \( \text{Ca}^{2+} \) measurements suggest the presence of the T type of \( \text{Ca}^{2+} \) channels in the PC dendrites (392, 546). Of the three T-channel-related \( \alpha \)-subunit genes (\( \alpha_{1G}, \alpha_{1H}, \) and \( \alpha_{1I} \)), \( \alpha_{1G} \)-mRNAs were found to be strongly expressed in PCs (509). The \( \alpha_{1E} \)-subunit of another low-voltage-activated/fast-inactivating type of \( \text{Ca}^{2+} \) channel was also reported to be present in the fine dendrites of PCs (571). The blockade of low-voltage-activated \( \text{Ca}^{2+} \) channels by \( \text{Ni}^{2+} \) delayed the onset of generation of depolarization-induced \( \text{Ca}^{2+} \) spikes, suggesting that the T-type \( \text{Ca}^{2+} \) channels or those containing the \( \alpha_{1E} \)-subunit generate transient inward currents that facilitate the generation of \( \text{Ca}^{2+} \) spikes (546).

2. \( \text{Na}^+ \) and \( \text{K}^+ \) channels

\( \text{Na}^+ \) enters PCs via glutamate-gated cation channels, voltage-sensitive \( \text{Na}^+ \) channels, and \( \text{Ca}^{2+}/\text{Na}^+ \) exchangers. An increase in \([\text{Na}^+]_i\) was observed in both dendrites and somata after GA stimulation (77). However, the increase in the somatic \([\text{Na}^+]_i\) was observed only when regenerative spikes were detected in the somata and therefore would represent \( \text{Na}^+ \) entry through voltage-sensitive \( \text{Na}^+ \) channels. The increase in the dendritic \([\text{Na}^+]_i\) evoked by GA stimulation was blocked by CNQX and should be associated with AMPA receptors.

In cultured PCs, multiple types of voltage-sensitive \( \text{K}^+ \) channels having different single-channel conductances have been identified in both PC somata and dendrites (180). An excitability increase in PC dendrites, presumably due to changes in a \( \text{K}^+ \) current, has been detected even 1 mo after eye-blink conditioning (468).

D. G Proteins

Guanine nucleotide-binding G proteins are coupled with a variety of metabotropic receptors. Activation of metabotropic receptors results in conversion of the inactive form of G protein coupled with GDP to its active form coupled with GTP. Because the G protein itself is a GTP-ase, the active form of G protein coupled with GTP will be ultimately converted back to its inactive form coupled with GDP. In the heterotrimeric structure of G proteins, \( \beta\gamma \)-subunits are bound to an \( \alpha \)-subunit maintaining it in a low-activity state. When metabotropic receptors are activated, \( \beta\gamma \)-subunits are released leaving the activity of the \( \alpha \)-subunit high.

There are \( \sim 20 \) different species of G proteins exerting different effector actions. \( G_s \) is a family of G proteins...
that activates adenyl cyclase, whereas \( G_\text{i} \) inhibits adenyl cyclase or activates cGMP-phosphodiesterase. A new class of G proteins, the \( G_\text{q} \) family, has recently been identified and found to be associated with group I mGluRs (sect. \( \mu B3 \)) and involved in PLC activation (sect. \( \mu C4 \)). Of the four isoforms of \( G_\text{ao} \) subunits (\( G_{\text{ao}11}, G_{\text{ao}14}, G_{\text{ao}15}, G_{\text{ao}16} \)), the highest transcriptional rate of \( G_{\text{ao}} \) was observed in PCs and hippocampal pyramidal cells. That of \( G_{\text{ao}11} \) was also noted in hippocampal pyramidal cells, but the other two were scarce in the central nervous system (511). The isoforms \( G_{\text{ao}} \) and \( G_{\text{ao}11} \) share a similar amino acid sequence (88%), receptor specificity, and ability to activate PLC-\( \beta \). Immunoreactivity for an antibody against the COOH terminus common to \( G_{\text{ao}} \) and \( G_{\text{ao}11} \) is abundant in the dendrites of PCs (352). With immunogold labeling, \( G_{\text{ao}11} \) immunoparticles are distributed in the perijunctional zones within 600 nm from the edge of the postsynaptic densities, corresponding to the perijunctional distribution demonstrated for mGluR1 (511) (sect. \( \mu B3 \)).

Certain bacteria toxins are used to disrupt G protein activities by ADP-ribosylation of the \( \alpha \)-subunit of G protein (30). Cholera toxin blocks the \( G_\text{ ao} \) family, and pertussis toxin blocks the \( G_\beta \) family. The \( G_\text{ ao} \) family, however, is resistant to both of these toxins. The action of mGluR1s to release Ca\(^{2+} \) from intracellular stores in PCs (sect. \( \mu D2 \)) is insensitive to pertussis toxin (575), as would be expected because this action is mediated by \( G_\text{ao} \) protein. However, quisqualate-induced reduced form of LTD, recorded by the grease-gap method, was blocked by pertussis toxin (236), suggesting the involvement of another type of G protein, probably \( G_\beta \), in LTD induction.

Hydrolysis-resistant analogs of GTP such as guanosine 5’-O-(2-thiodiphosphate) (GDP\( \beta S \)) and guanosine 5’-O-(3-thiotriphosphate) (GTP\( \gamma S \)) are also used to interfere with G protein activities by persistently activating effector systems. Photolytic release of GTP\( \gamma S \) in PCs produces a large inward current followed by a small outward current, mimicking the response to an mGluR agonist (\( \mu S3R-ACPD \)) (sect. \( \mu B3 \)) (501). On the other hand, prolonged intracellular infusion of either GDP\( \beta S \) or GTP\( \gamma S \) prevented the \( \mu S3R-ACPD \)-induced generation of the inward current (210). In mice deficient in \( G_{ao1} \), CF-PC transmission was functional, but CFs remained to multiply innervate PCs (414) and LTD was lacking (381).

E. Phospholipases

1. PLC

PLC is a family of enzymes that hydrolyze the membrane phosphatidylinositol 4,5-bisphosphate (PIP\(_2 \)) producing two second messengers: DAG (see sect. \( \mu D1 \)) and inositol trisphosphate (IP\(_3 \)) (see sect. \( \mu D2 \)). Among its five isoforms of \( \alpha, \beta, \gamma, \delta, \) and \( \epsilon \), three (\( \beta, \gamma, \) and \( \delta \)) are phosphoinositide specific, and PLC-\( \beta \) alone is associated with G proteins (199). The activation of metabotropic receptors leads to the activation of PLC-\( \beta \) via the activation of \( G_{q1} \). The four isoforms of PLC-\( \beta \) have recently been cloned, of which PLC-\( \beta 3 \) mRNA is specifically expressed in PCs, and PLC-\( \beta 4 \) mRNA is most highly expressed in PCs, whereas PLC-\( \beta 1 \) and -2 are localized in other areas of mouse and rat brain (456, 545). PLC-\( \beta 4 \) is distributed equally in both the rostral and caudal cerebellum, whereas PLC-\( \beta 3 \) is abundant in the caudal compared with the rostral cerebellum. In other words, PLC-\( \beta 4 \) functions dominantly in the rostral cerebellum, while PLC-\( \beta 3 \) and PLC-\( \beta 4 \) function to a similar extent in the caudal cerebellum. In PLC-\( \beta 4 \)-deficient mice, the activation of mGluR1s, which normally induces an increase of \( [Ca^{2+}]_i \), in the cerebellum, resulted in no such effect in the rostral cerebellum and only a small increase in the caudal cerebellum (501). PLC-\( \beta 3 \) and PLC-\( \beta 4 \) are activated differently from each other by subunits of \( G_\beta \) proteins (199, 248); \( \alpha \)-subunit activates PLC-\( \beta 4 \) more potently than PLC-\( \beta 3 \), whereas \( \beta \gamma \)-subunit activates PLC-\( \beta 4 \) more potently than PLC-\( \beta 3 \) (501).

The PLC-mediated pathway, presumably involving \( G_{q1} \) and PLC-\( \beta 3 \)-4, plays a crucial role in LTD induction, since its downstream effects, both activation of PKC by DAG and release of \( Ca^{2+} \) from intracellular stores by IP\(_3 \), are required for LTD induction (sect. \( \mu B1 \) and \( \mu B2 \)). A PLC inhibitor actually greatly attenuated the mGluR agonist-induced \( [Ca^{2+}]_i \) increase (410), which was also blocked in mice deficient in the PLC-\( \beta 4 \) gene (264). On the other hand, PLC does not seem to contribute significantly to the generation of mGluR-EPSPs because PLC inhibitors, PKC inhibitory peptides, or heparin sodium, a nonspecific inhibitor of IP\(_3 \) receptors, have no significant effects on the mGluR-EPSPs (514) or mGluR agonist-induced inward currents (210). This is consistent with the report that photolytic release of IP\(_3 \) from its caged form did not induce an inward current (501).

2. PLA\(_2 \)

PLA\(_2 \) is a superfamily of enzymes that hydrolyzes ester bonds of the membrane phospholipids, thereby releasing unsaturated free fatty acids such as arachidonic and oleic acids on one hand and lysophosphatidylethanolamine on the other. Platelet-activating factor (PAF) is also a product of PLA\(_2 \) activity (sect. \( \mu E2 \)). PLA\(_2 \) is classified into two distinct forms: secreted and cytosolic (cPLA\(_2 \)). cPLA\(_2 \) is rapidly activated by increased concentrations of cytosolic \( Ca^{2+} \), which cause the translocation of cPLA\(_2 \) from the cytosol to membranes. The 85-kDa cPLA\(_2 \) (cPLA\(_{2 \alpha} \)) is widely distributed in the central nervous system. In the cerebellum, its mRNAs are abundant in the granular and PC layers including the PC cytoplasmas, but only moderately contained in the molecular layer (285).
Of the other two novel types of cPLA₂, i.e., cPLA₂β (114 kDa) and cPLA₂γ (61 kDa), cPLA₂β is most strongly expressed in the cerebellum compared with other parts of the central nervous system and various organs (433). The PLA₂ immunostained in the PC somata and dendrites had a molecular mass slightly above 100 kDa (271) and may be equivalent to cPLA₂β.

The involvement of PLA₂ in LTD induction was shown in cultured PCs, in which inhibitors of PLA₂, manoolide and mepacrine (equivalent to quinacrine), blocked the late phase of glutamate(depolarization-induced LTD (322). The role of PLA₂ in LTD induction may be considered in connection with its link to type 1 mGluRs, from neurons (23, 129, 343). A PLA₂ activating protein (PLAP) is known to intercalate signal transduction from mGluRs to PLA₂. A 30-kDa PLAP isolated from Aplysia ganglia is also located in rat cerebral cortex and is activated by PKC (75). In addition to the above postulated mGluR-G protein-PLA₂ pathway, a possibility has also been raised that cPLA₂ is activated by mitogen-activated protein kinases (MAPKs) by phosphorylation at serine-505 (320) (sect. IV C). PLA₂ is also activated by PKC (99), but this is probably mediated through activation of MAPK by PKC (320).

mGluRs are thus linked to LTD induction via both the PLC pathway involving PKC and IP₃ (sect. iv, B2 and C1) and the PLA₂ pathway. PKC may activate PLA₂ either directly or indirectly via activation of MAPK. Arachidonic acid, a PLA₂ product, is known to exert a long-lasting activation on PKC-γ, but not PKC-α or -β (479) (see sect. ivC). If PKC involved in LTD induction is γ isoform, a possibility arises that PLC and PLA₂ mutually activate and provide a mechanism of self-regeneration required for the initial development of synaptic plasticity (see sect. ivF). However, because PKC-γ-deficient mice retain LTD (2), this possibility does not seem to be realistic (see sect. ivC).

IV. SIGNAL TRANSDUCTION:
FURTHER PROCESSES

After the initial processes of signal transduction described in section iii, intracellular ion concentrations are set high, and a number of second messengers are produced to activate protein kinases and phosphatases. There are still a number of factors that may contribute to LTD induction, yet mechanisms of their action have not yet been well understood. The final process of LTD induction is inactivation of AMPA receptors mediating GA-PC synapses. Eventually, a complex network of chemical reactions accounts for signal transduction for LTD.

A. Ion Concentrations

1. Ca²⁺ concentration

During current-induced depolarization from −70 to 0 mV, Ca²⁺ concentration attained 30 μM in PC dendrites and 5 μM in PC somata on the average of a number of measurements (349). Two lines of observations have demonstrated the requirement of Ca²⁺ for LTD induction: 1) LTD induction was blocked after intracellular injection of Ca²⁺ chelators, such as EGTA (400, 151) or BAPTA (297); and 2) activation of Ca²⁺ channels by current step-elicited membrane depolarization induced LTD when combined with GA stimulation (104, 297). Membrane depolarization by itself is not a factor required for LTD induction, because an increase in [Ca²⁺], caused by photolysis of nitr 5, a photolabile Ca²⁺ chelator, effectively induced LTD when combined with the iontophoretic application of glutamate in cultured PCs (270). When the GA-evoked Ca²⁺ entry is sufficiently large, it can replace the CF-evoked Ca²⁺ spikes so that GA stimulation alone can induce homosynaptic LTD (188, sect. vC1).

[Ca²⁺], is regulated by buffering by Ca²⁺ binding proteins and diffusion. PCs contain three types of endogenous Ca²⁺ binding proteins, namely, calbindin-D28K, parvalbumin, and calrectin (556). All PCs in various animal species display strong immunoreactivity to calbindin-D28k. All PCs in chicks and rats (451, 452) also display immunoreactivity to parvalbumin, but in monkeys, a significant portion of parvalbumin-negative PCs occur among the positive PCs (149). PCs in chicks and rats show no immunoreactivity to calrectin, or its mRNAs, but in monkeys about half of PCs display weak calrectin immunoreactivity, and in humans, calrectin immunoreactivity appears in PCs after 21 wk of gestation and increases thereafter (570). In rat cerebellum, calrectin immunoreactivity appeared in PFs and their varicosities that formed synapses with unlabeled PC dendritic spines, suggesting a role of calrectin at the presynaptic sites of GA-PC synapses (21). Mice deficient of calbindin-D28k, or both calbindin-D28k and parvalbumin, exhibited abnormal morphology of dendritic spines of PCs (532). Calbindin-D28k null mutant showed motor discoordination, and their PCs displayed marked changes of synthetically evoked postsynaptic Ca²⁺ transients, their fast decay component having larger amplitudes in null mutant than in wild-type mice (7). The existence of two types of Ca²⁺ buffer in cultured PCs has recently been revealed: a high-affinity, cooperative, and mobile Ca²⁺ buffer, resembling calbindin-D28k, and a mobile, low-affinity Ca²⁺ buffer (349).

2. Na⁺ concentration

An important role of Na⁺ in inducing LTD was suggested in an experiment using cultured PCs. The quisqual-
ate depolarization-induced LTD was blocked by replacing extracellular Na\(^+\) with Li\(^+\), Cs\(^+\), tetraethylammonium, or N-methyl-d-glucamine, or by pairing quisqualate pulses with step depolarizations to near the membrane equilibrium potential of Na\(^+\), thus preventing Na\(^+\) entry via Na\(^+\) channels (330). How Na\(^+\) influx contributes to LTD induction has not yet been clarified, but it is probable that Na\(^+\) ions are required for activation of certain enzymes. It is noted that PCs are equipped with a powerful Na\(^+\)-K\(^-\) ATPase sodium pump, which constantly hyperpolarizes PC membrane (165). The sodium pump activity and ouabain binding to cerebellar homogenates in rat increase with development (385).

**B. Second Messengers**

After the activation of the receptors and enzymes by the first messengers and subsequent activation of ion channels, G proteins and phospholipases as aforementioned, a number of second messengers are produced and activate various signal transduction cascades (Fig. 5). According to the general scheme of metabotropic receptor-initiated signal transduction, signals received by metabotropic receptors are transferred to a target molecule via G proteins and at the same time to one or more effector molecules that regulate second messengers acting upon the target molecule.

1. **DAG**

The substance DAG, generated by PLC from membrane PIP\(_2\), is a major activator of PKC. DAG has been assumed to take part in LTD induction by activating PKC (sect. ivC). Direct evidence for the involvement of DAG in LTD induction was derived from the observation that application of exogenous synthetic DAG (oleoylacetlyglycerol and dioleoylglycerol) to cultured PCs in combination with depolarizing pulses and test AMPA pulses induced LTD (408).

DAG is under regulatory influences of DAG kinase (DGK), which phosphorylates DAG to generate phosphatidic acid. DGK is hence considered to attenuate the PKC activity. DGK may also initiate the resynthesis of phosphatidylinositols that have been cleaved by PLC. The phosphatidic acid produced by DGK may well be a second messenger itself. Nine isoforms of DGK have been identified, and their activity is found not only in the membrane, but also in the nucleus and the cytoskeleton (530). DGK\(_\alpha\) is predominant in PCs, while DGK\(_\zeta\) distributes in the cerebral and cerebellar cortices (174). DGK in the cerebellum and cerebrum is activated by arachidonic acid (sect. ivB5).

2. **IP\(_3\)**

The activation of mGluRIIs results in the production of IP\(_3\) via G\(_{\alpha_q}\) and PLC-\(\beta\). In the cerebellum, this was previously demonstrated by an increase in phosphoinositide turnover in PCs and the molecular layer following the activation of mGluRIIs by trans-ACPD (219). Serotonin also stimulated IP\(_3\) production in the cerebellum (462). IP\(_3\) 5-phosphatase hydrolyzes IP\(_3\) to inositol 1,4-bisphosphate (IP\(_2\)), and IP\(_3\) 3-kinase phosphorylates IP\(_3\) to 1,3,4,5-tetrakisphosphate (IP\(_4\)). In the adult rat cerebellum, IP\(_3\) 3-kinase activity is comparable to that in the cerebral cortex and hippocampus, while 5-phosphatase activity is 5- to 10-fold higher than in other parts of the brain (194). mRNA for type 1 IP\(_3\) 5-phosphatase is localized in PCs (115). Immunoreactivity to IP\(_3\) 3-kinase is strong in the dendrites (353, 383), particularly in dendritic spines of PCs and basket cells (560). IP\(_3\) 3-kinase mRNA is highly expressed in PCs (354).

Receptors specific to IP\(_3\) are abundantly present in PCs (350). IP\(_3\) receptors constitute a family of Ca\(^{2+}\) channels involved in mobilization of intracellular Ca\(^{2+}\) stores, of which three different gene products (I-III) have been isolated (427). Immunoreactivity to IP\(_3\) receptors is detected mainly in the endoplasmic reticulum, but not in the mitochondria or the cell membrane (455). A morphological counterpart of IP\(_3\) receptors is small dense projections observed on the cytoplasmic surface of smooth endoplasmic reticulum in PCs, which appear to be composed of four subparticles, surrounding a central channel (254). IP\(_3\) receptors are present in particularly high concentrations within stacks of the endoplasmic reticulum cisternae, which may be massively formed as an adaptive response to the high concentration of IP\(_3\) receptors (508). The intracellular Ca\(^{2+}\) stores in the cerebellum appear to consist of the three microsomal components, i.e., IP\(_3\) receptors, membrane fragments endowed with Ca\(^{2+}\) pumps, and a calcium-binding protein, calsequestrin (535). It has recently become apparent that, when intracellular Ca\(^{2+}\) stores are depleted, specific store-operated Ca\(^{2+}\) channels in the plasma membrane open to cause capacitative Ca\(^{2+}\) entry so as to prevent exhaustion of the intracellular Ca\(^{2+}\) stores (374, 438). It has also been known that IP\(_3\) receptors interact with many cytoskeletal proteins and their modulators and that the sequential process from activation of mGluRIIs to activation of IP\(_3\) receptors is facilitated by a protein called Homer (526) or Cupidin (490), which cross-links mGluRs with IP\(_3\) receptors.

IP\(_3\) activates its receptors and thereby opens associated Ca\(^{2+}\) channels, leading to the release of Ca\(^{2+}\) from intracellular stores located mainly in the endoplasmic reticulum. IP\(_3\) also releases Ca\(^{2+}\) from cerebellar microsomes to a lesser degree than IP\(_3\) (251). Repetitive GA stimulation produced a transient local increase in dendritic [Ca\(^{2+}\)]\(_i\) with early and late components. The early component is associated with AMPA receptors, because its time course corresponds to the fast EPSPs, and it is blocked by CNQX. The late component is associated with mGluR1-IP\(_3\)-mediated Ca\(^{2+}\) signals, because it is blocked
by MCPG and also by heparin sodium (147, 507). Application of a series of weak stimuli to GAs induced IP$_3$-mediated Ca$^{2+}$ signals discretely restricted to individual dendritic spines, whereas that of stronger stimuli increased Ca$^{2+}$ concentration in dendritic spines as well as, to a lesser degree, in adjacent dendritic shafts (147, 539). Ca$^{2+}$ signals induced by photolysis of caged IP$_3$ by a beam of 3–5 mm in size spread to 12.3 $\mu$m in a half-width along the dendritic branchlet (147).

The following observations indicate that the Ca$^{2+}$ thus released are involved in LTD induction. 1) Depletion of Ca$^{2+}$ from the intracellular stores by thapsigargin, an inhibitor of Ca$^{2+}$-ATPase on the endoplasmic reticulum, blocked the induction of mGluR/Ca$^{2+}$ spike-induced LTD in cerebellar slices (198) and glutamate/depolarization-induced LTD in tissue cultures (292). However, it is noted that thapsigargin did not block GA/Ca$^{2+}$-spike induced LTD (198). LTD induced by using sparse activation of GAs was blocked by thapsigargin, whereas LTD induced by dense activation of GAs was insensitive to thapsigargin (539). This is in accordance with the fact that the sparse GA stimulation enhances Ca$^{2+}$ signals confined in spines, while strong GA stimulation induces Ca$^{2+}$ into dendritic branchets via voltage-sensitive Ca$^{2+}$ channels. 2) Heparin sodium, an inhibitor of the IP$_3$ receptor, blocked glutamate/depolarization-induced LTD in tissue cultures. 3) The combination of AMPA application and depolarization, which alone did not induce LTD, induced LTD when applied in conjunction with photolysis of caged IP$_3$ (270). Combination of photolysis of caged IP$_3$ and large depolarizing pulses also induced LTD (280). 4) A specific antibody against the IP$_3$ receptor blocked the LTD induction, and mice with a disrupted IP$_3$ receptor type 1 gene completely lacked LTD (222). 5) IP$_3$-mediated Ca$^{2+}$ signaling in dendritic spines of PCs, and concomitantly LTD, were absent in those mice and rats with mutations in myosin-Va, in which the endoplasmic reticulum failed to enter the dendritic spines (380). The loss of LTD was rescued by photolysis of a caged Ca$^{2+}$ compound. 6) In mGluR1-deficient knockout mice, the photolytic release of IP$_3$ is sufficient to rescue GA/Ca$^{2+}$ spike-induced LTD which otherwise failed, and this LTD is sensitive to the presence of a PKC inhibitor (111). It is notable that a strong and persistent Ca$^{2+}$ signal produced following the photolytic release of caged IP$_3$ in peripheral dendrites of a PC was sufficient to evoke persistent depression in GA-evoked EPSP (147).

However, it is noteworthy that in cultured or freshly isolated PCs, IP$_3$ is not required for inducing LTD by glutamate/depolarization conjunction (408). 1) Glutamate pulses did not cause a significant increase in [Ca$^{2+}$], Ca$^{2+}$ transients produced by glutamate/depolarization conjunction and those produced by depolarization alone were not significantly different. 2) A potent and selective IP$_3$ receptor channel blocker, xestospongin C, did not affect the LTD induction. 3) Exogenous synthetic DAG successfully induced LTD, even while it activates PKC, but does not induce Ca$^{2+}$ release from intracellular stores. Mechanisms for maintaining [Ca$^{2+}$] and their involvement in LTD might be altered in isolated PCs.

3. Ryanodine receptor

Ryanodine receptors provide another Ca$^{2+}$-releasing mechanism. While several subtypes of ryanodine receptors are present in PCs, the skeletal muscle type (a-form, but not $\beta$-form) of ryanodine receptor is specific to PCs in the nervous system (302, 423). Ryanodine receptors are present in the PC somata, dendrites, and axons, but in contrast to IP$_3$ receptors, they are not found in the dendritic spines (143). Although IP$_3$ receptors are involved in IP$_3$-induced Ca$^{2+}$ release, ryanodine receptors function as intracellular channels for Ca$^{2+}$-induced Ca$^{2+}$ release (182). The transient increase in [Ca$^{2+}$]$_i$ in the PC somata and dendrites after the addition of caffeine is presumably mediated by ryanodine receptors, since it was blocked by ruthenium red, a ryanodine receptor inhibitor (257). Cyclic ADP ribose (cADPR) acts on the nonskeletal type of ryanodine receptor Ca$^{2+}$ channels (371). If the level of cADPR is regulated by cGMP as observed in sea urchin eggs (160), it is possible that ryanodine receptors are activated via the NO-cGMP pathway as part of the signal transduction for LTD induction (48).

In the GA/CF conjunction, the CF-induced increase in [Ca$^{2+}$]$_i$ in the PC dendrites may be amplified by the Ca$^{2+}$ released from the ryanodine-sensitive Ca$^{2+}$ stores under the influence of NO released from GAs. The CF-induced elevation in the levels of Ca$^{2+}$ within the dendrites may spread into the spine and sensitize IP$_3$ receptors to the IP$_3$ generated via the mGluR1 activation due to GA impulses; IP$_3$ receptors within the spine may thus function as a molecular coincidence detector (48). In fact, ryanodine receptors are likely to have a role in the induction of LTD by glutamate/depolarization conjunction in cultured PCs, because ryanodine, which keeps ryanodine receptor-associated Ca$^{2+}$ channels open, and ruthenium red, which inhibits ryanodine receptor, blocked the LTD induction (292).

4. cGMP

The compound NO activates synthesis of cGMP in cerebellar neurons (163). The cGMP is produced from GTP by the catalytic action of guanylyl cyclase (sect. mB5) and is degraded to GMP by phosphodiesterase. Calmodulin-dependent phosphodiesterase is highly expressed in the PC somata and dendrites (32). Interestingly, degeneration of CFs by a 3-AP treatment resulted in a selective reduction in phosphodiesterase expression in PCs, without affecting other calmodulin-binding proteins in PCs and without changing phosphodiesterase activity.
in the cerebral cortex. Phosphodiesterase activity seems to be regulated trans-synaptically via CFs.

Pharmacological data indicate a role for endogenous cGMP in LTD induction, because inhibition of phosphodiesterases produces an effect equivalent to intradendritic injection of cGMP, and either of these induced LTD when associated with GA stimulation (186, 187). Although immunocytochemical studies failed to reveal the presence of cGMP in PCs (117, 494), this may have been due to the strong phosphodiesterase degradation of cGMP.

5. Arachidonic acid and oleic acid

The enzyme PLA₂ catalyzes the release of cis-unsaturated free fatty acids such as arachidonic and oleic acids from membrane phospholipids. Involvement of arachidonic/oleic acids in signal transduction for LTD induction has been demonstrated by showing that the depressant effect of manoalide and quinacrine, inhibitors of PLA₂, on LTD is counteracted by coapplication of arachidonic acid or oleic acid (322).

The free fatty acids act as second messengers on diverse target molecules. For example, arachidonic acid is known to stimulate DGK, thereby downregulating the level of DAG in neuronal membranes, which may in turn downregulate PKC activity (443). However, arachidonic acid and its metabolites, or oleic acid, are also known to strongly stimulate PKC-γ (479). Arachidonic acid may activate guanylyl cyclase (524). Even though the relevance of glutamate transporters to LTD induction is unclear, the following reports may be noteworthy. Arachidonic acid enhanced the inward current generated in PCs by an excitatory amino acid transporter, and PLA₂ inhibitors blocked this effect (271). Arachidonic acid decreased glutamate uptake mediated by the type EAAT1 transporter but increased that by the type EAAT2 transporter (576). Arachidonic acid also activated the transporter-mediated proton currents (527).

The roles for diverse metabolites of arachidonic acid, collectively termed eicosanoids (434, 488), in LTD induction have yet to be investigated. Among them, the prostaglandin D₂-binding protein and NAPD-linked 15-hydroxy-prostaglandin D₂ dehydrogenase were found to be localized in PCs of the pig cerebellum (547), but the presence of these proteins has not been confirmed in other animal species.

C. Protein Kinases and Protein Phosphatases

Second messengers specifically activate certain protein kinases, which in turn phosphorylate various substrate proteins. The phosphorylating action of protein kinases is counterbalanced by the dephosphorylating action of protein phosphatases.

1. PKC

Stimulation of a cell by diverse types of first messengers activates the PKC family of serine-threonine protein kinases, which in turn phosphorylate a variety of substrates. In general, inactivated PKC is present mainly in the cytosol, whereas its hydrophobic activators are present on the membrane. PKC activation results in the translocation of PKC to the membrane, cytoskeletal elements, nuclei, and other subcellular components (384). More than 10 subspecies of PKC have been identified in mammalian tissues (413). Four classical PKCs (α, β₁, βⅡ, and γ) are activated by Ca²⁺, phosphatidylycerine, and DAG or phorbol esters; this activation is enhanced and prolonged by cis-unsaturated fatty acids and lysophosphatidylcholine (489). The other four novel subspecies of PKC (δ, ε, η, θ) are Ca²⁺ independent, and four atypical subspecies (ζ, τ, λ, μ) are Ca²⁺ and DAG independent.

The subspecies PKC-γ is present in PCs but not in other types of neurons in the cerebellum, while the β and βⅡ subspecies are predominantly expressed in the granular and molecular layers, respectively (28). High-resolution immunogold analysis revealed that PKC-γ is highly expressed on the dendrosomatic plasma membrane forming two pools, one associated with the membrane and the other located within 50 nm of the plasma membrane (78). PKC-γ also occurs in dendritic spines of PC, particularly abundant in and near the postsynaptic density. PKC-γ appears to have a developmental role, i.e., in the elimination of multiple CF innervations of PCs to attain the typical single CF innervation onto each mature PC (258). PKC-γ mRNA is highly expressed in the dendrite-rich neuropil of the mouse cerebellum at postnatal day 14, compared with adult mice, suggesting a role in synaptogenesis (390).

The subspecies PKC-δ is abundant in PCs located in the visual- and vestibular-receiving areas in the posterior lobules (90, 203, 204). In lobules VI–IX, the PKC-δ-containing PCs form distinct parasagittal bands, suggesting a PKC-δ role in the specific circuit connection between PCs and subcortical neurons. The immunoreactivity for PKC-δ is prominent in the PC somata and is also occasionally observed in the dendrites, axons, and their terminals (370). PKC-δ immunoreactivity is associated with the rough endoplasmic reticulum, but not detected on the cell membrane or PC dendritic spines (78). PKC-ε is present in the PC somata and PKC-ζ in the PC somata and dendrites (554). PKC-η and PKC-θ are present in the PC somata, and PKC-λ is also in the PC somata, probably associated with a 200-kDa neurofilament component, whereas PKC-τ is absent in PCs (35).

The PKCs obtained from the cerebellum have also been classified into types I, II, III, corresponding to the γ, β₁/βⅡ, and α genes, respectively (479). Type I is unique in its activation by cis-unsaturated fatty acids, to which
types II and III are almost inert. When attenuation of voltage-gated K⁺ channels was taken as an index of activation of PKC, perforated patch recording in tissue cultures revealed that PKC in PCs was sensitive to both DAG and oleic acid, the sensitivity being blocked by a PKC inhibitor, while PKC in granule cells was insensitive to oleic acid (331). These results are consistent with the differential distribution of type I (γ) PKC in PCs and type II (β₁, βII) PKC in granule cells.

Bath application of phorbol esters, activators of PKC, induced sustained depression of glutamate-induced responses in PCs (105). Phorbol ester-induced LTD in cultured PCs mutually occluded the quisqualate/depolarization-induced LTD (325). Various PKC inhibitors have been shown to block LTD induction (103, 151, 186, 325). No LTD occurred in PCs of transgenic mice expressing the pseudosubstrate PKC inhibitor, PKC-[19–31] (118). However, LTD remains intact in PCs of PKC-γ-deficient mice (86). It is noted that in PKC-γ-deficient mice, hippocampal LTP was greatly impaired, while hippocampal LTD remained normal (2). The PKC subspecies involved in LTD induction in PCs may not be γ or, even if it is, it may be replaced by another subspecies in PKC-γ-deficient PCs. The PKC involved in LTD induction may not be β₁, βII, δ, λ, or ε because of their limited localization (see above). The possibility may be either α, η, θ, or ζ, but in view of the dependence of the LTD induction on Ca²⁺⁺ (sect. IV.B1) and mGluR-DAG cascade (sects. IV.B3 and IV.B1), PKC-α is the most likely candidate.

2. Protein kinase G

Protein kinase G (PKG) is a dimeric cGMP-binding protein of molecular weight 150,000 consisting of two identical subunits (mol wt 75,000), each of which contains two cGMP-binding sites (476). PKG equally catalyzes the phosphorylation of serine or threonine residues, while PKA shows a preference for serine. PKG types I and II have different catalytic and regulatory properties (161). Type I PKG is highly concentrated in PCs throughout their somata, dendrites, and axons, whereas type II PKG is widely distributed in the brain (141). Immunoreactivity for PKG is observed in all mature PCs of rats after postnatal day 4, but until postnatal day 3, PCs form PKG-positive and -negative clusters, apparently related to the compartmentalization of the cerebellum (543).

The role of PKG in LTD induction has been demonstrated by showing that a PKG inhibitor, KT5824, effectively blocked the quisqualate-induced persistent reduction of glutamate sensitivity of PCs (236) and GA/CF-induced LTD (186). PKG may contribute to LTD induction by phosphorylating AMPA receptors as demonstrated with partial peptides of AMPA receptors (403) (sect. IV.E1). Alternatively, PKG may facilitate LTD induction through the inhibitory action of PKG-phosphorylated G-substrate on protein phosphatases (sect. IV.C7). Some other possible implication of PKG in LTD induction is noteworthy. 1) PKG activates ADP-ribosyl cyclase to produce cADPR that acts to release Ca²⁺⁺ from the ryanodine-sensitive Ca²⁺⁺ stores (sect. IV.B3). 2) PKG may prevent the thrombin-stimulated production of IP₃ and thereby reduce the release of Ca²⁺⁺ from intracellular stores, as demonstrated in Chinese hamster ovary (CHO) cells transfected with PKG (457). 3) PKG may mediate the action of CO to induce a long-lasting change in 3-Na⁺⁺-K⁺⁻ ATPase localized in PCs, because this action is absent in PC-deficient mice, mimicked by 8-bromo-cGMP, and blocked by inhibition of PKG (409). This action of CO is probably mediated by mGluRs because it is mimicked by glutamate and mGluR agonists.

3. Protein tyrosine kinases

Protein tyrosine kinases (PTKs), which transfer the γ-phosphate of ATP to tyrosine residues, are classified into receptor-coupled and non-receptor-coupled types. The receptor-coupled PTKs are associated with neurotrophin and growth factor receptors and regulates cellular survival, growth, and differentiation, whereas the non-receptor-coupled PTKs are components of the intracellular signaling cascade (357). PTK activity is high in the cerebellum, and PCs express neuronal isoforms of c-src PTK, pp60c-src (+) (502), and pp62c-yes, another member of the src subfamily (578). PCs are also rich in tyrosine phosphatase, which reverses tyrosine phosphorylations catalyzed by PTKs (317).

The involvement of PTKs in LTD induction has been suggested because the two structurally distinct PTK inhibitors, lavendustin and herbimycin A, blocked GA/Ca²⁺⁺ spike-induced LTD (63). Herbimycin A also prevented depression of CA-EPSPs produced by intracellular infusion of a PKC activator, (-)-indoctanil V. These results indicate that PTKs, operating in association with PKC, are required for LTD induction. PTKs may interact with PKC directly, but an indirect interaction via the G protein-PLC-DAG-PKC pathway is also possible, because PTK inhibitors block Gq/11 protein-coupled receptor-mediated formation of IP₃ (528). However, this possibility has to be considered with caution since inhibitors for tyrosine phosphatases also blocked the IP₃ formation.

A src family nonreceptor PTK, Lyn, has been shown to be associated with AMPA receptors (193). Lyn PTK is activated through AMPA receptors independent of Ca²⁺⁺ and Na⁺⁺ influxes, and it in turn activates MAPK in cerebellar primary cultures. Evidence was given to the possibility that activation of MAPK translocates into the nucleus and regulates expression of brain-derived neurotrophic factor (BDNF) gene, suggesting yet another pathway in LTD induction (sect. IV.D4). A possibility is also entertained that certain receptor PTKs are involved
in LTD induction through their association with IGF-I receptors (sect. mB7).

4. MAPK

The MAPK has been implicated in signal transduction cascades for cell proliferation, cell differentiation, and early embryonic development, as well as in synaptic plasticity (93). MAPK immunoreactivity is prominently located in cell bodies and dendrites of PCs (148). In cultured PCs, glutamate/K\(^+\)-conjunctive stimulation induced LTD and at the same time activated MAPK, and both of these effects were blocked by a specific inhibitor of MAPK kinase (MAPKK or MEK), PD98059, or an anti-active MAPK antibody (274).

How MAPK is activated in PCs is unknown, but some possibilities may be pointed out. MAPK could be activated by Ca\(^{2+}\) influx that leads to activation of a small G protein (Ras), and a Ras-dependent signaling pathway could in turn activate MEK and MAPK (453). Or, Ca\(^{2+}\) influx could first activate PKC that in turn activates the MAPK cascade. It is also possible that PAF activates MAPK and MEK, as demonstrated in CHO cells (215). How MAPK contributes to LTD induction is also unknown, but some possibilities may be raised. Since PD98059 attenuated the mGluR1 agonist-induced inward current, it is possible that MAPK contributes to LTD induction by acting on mGluR1s (274). Or, MAPK may contribute to LTD induction by activating cPLA\(_2\) by phosphorylation (320), or MAPK and/or its downstream kinases could affect LTD induction by phosphorylating AMPA receptors. Another possibility is that MAPK translocates into the nucleus and regulates the expression of the BDNF gene (193).

5. PKA

Although there is no evidence indicating that PKA is involved in the induction of LTD (237), it plays a role in GA-PC transmission on the presynaptic side in connection with GA-LTP. Ca\(^{2+}\)/calmodulin-sensitive type I adenyl cyclase, which produces cAMP and thereby activates PKA, is highly expressed in granule cells (91). The activation of adenyl cyclase in the GAs appears to be involved in the induction of LTP in GA-PC synapses by the tetanus of GAs for two reasons: 1) PKA inhibitors blocked LTP (324, 461), and 2) forskolin, an activator of PKA, induced potentiation of GA-PC transmission by increasing the probability of transmitter release (87). Mice deficient in type I adenyl cyclase lacked GA-evoked LTP (490).

6. Ca\(^{2+}\)/calmodulin-dependent protein kinase

Ca\(^{2+}\)/calmodulin-dependent protein kinases (CaMKII) are serine/threonine protein kinases expressed at high concentrations preferentially in neurons. Of the two isoforms of CaMKII, \(\alpha\) and \(\beta\), \(\alpha\) is predominant in the forebrain (\(\alpha/\beta\) ratio, 3:1), while \(\beta\) is predominant in the cerebellum (\(\alpha/\beta\) ratio, 1:4) (376). In the cerebellum, CaMKII\(\alpha\) is specifically concentrated in the PC somata and dendrites (537). CaMKII\(\beta\) appears to function as an F-actin targeting molecule for localizing CaMKII\(\alpha/\beta\) heterooligomers to the dendritic spines (480). Arachidonic acid and its metabolites inhibit CaMKII (435).

Although CaMKII has been implicated in hippocampal LTP induction (446), evidence is scarce for its involvement in cerebellar LTD induction. The CaMK inhibitors KN-93 and KN-62 potentiated glutamate-induced currents in cultured PCs (268). Specific roles of CaMKII in PCs have yet to be identified.

7. Protein phosphatases

Two major types of serine/threonine-specific protein phosphatases, PP1 and PP2, have been identified. PP1 specifically dephosphorylates the \(\beta\)-subunit of phosphorylase kinase and is inhibited by nanomolar concentrations of proteins, termed inhibitor-1 and inhibitor-2. PP2 dephosphorylates the \(\alpha\)-subunit of the phosphorylase kinase preferentially and is unaffected by either inhibitor-1 or inhibitor-2. PP2 comprises three enzymes: PP2A, PP2B, and PP2C (95). PP2A, in a manner similar to PP1, does not require cations for its activity and is sensitive to okadaic acid, whereas PP2B, called also calcineurin, is Ca\(^{2+}\)/calmodulin dependent and much less sensitive to okadaic acid. PP2C is Mg\(^{2+}\) dependent and insensitive to okadaic acid.

The PP1 consists of isoforms having different catalytic units. Strong immunoreactivity for a catalytic unit of PP1, PP1\(\gamma\)l, but not PP1\(\beta\) or PP1\(\alpha\) was observed in PCs including the fine dendritic branches and spines (191). Immunoreactivity for PP2A (\(\alpha\) and/or \(\beta\)) is predominant in the cerebellum (493). The G substrate specifically contained in PCs becomes a potent inhibitor of PP1 as well as PP2A when phosphoprylated by PKG (116). The molecular structure of G substrate has recently been determined (144, 183). The cloned G substrate inhibits PP2A more sensitively than PP1 (144).

Bath application of calyculin A or okadaic acid, or intracellular injection of microcystin LR into PCs, all being potent inhibitors of PP1/2A, induced LTD when combined with GA stimulation (12). Similarly, glutamate-induced currents in cultured PCs were markedly reduced during repeated application of glutamate pulses in the presence of calyculin A (268). These effects are presumably due to inhibition of the dephosphorylating action of protein phosphatases antagonizing phosphorylating action of protein kinases (C and/or G). The observation in the cerebellum is contrasted to the finding in the hip-
pocampus, in which protein phosphatase inhibitors inhibit LTD induction (394).

D. Other Factors

1. Protein synthesis

Not only somata but also dendrites contain a number of different mRNAs and the translational machinery including ribosomes, polyribosomes, and elongation factor 2, as well as the endoplasmic reticulum and cisternae of the Golgi apparatus. Protein synthesis appears to occur in both somata and dendrites of neurons in association with synaptogenesis and synaptic plasticity.

The requirement of protein synthesis for the induction of LTD has been demonstrated by using translational inhibitors (anisomycin, puromycin, and cycloheximide). mRNA cap analog has also been used for preventing translation (218, 267). The translational inhibitors depress the late phase of LTP in hippocampal neurons (152, 153, 411). In cultured PCs, translational inhibitors continuously applied immediately after conjunctive stimulation depressed LTD only after 45 min (323). A current view is, therefore, that protein synthesis serves specifically for long-term maintenance of synaptic plasticity.

However, another role of protein synthesis has recently been proposed because translational inhibitors abolish the entire LTD including its early phase in PCs of cerebellar slices (267). In cerebellar slices, a 5-min pulse application of a translational inhibitor depressed protein synthesis, determined by methionine incorporation, to one-half or less quickly and persistently over 30 min. When applied around the time of conjunctive stimulation, a 5-min pulse application of translational inhibitors blocked LTD induction quickly and persistently, indicating that LTD induction requires a quickly turned over protein(s). Because a translational inhibitor pulse no longer affects LTD induction when applied with a delay of 15 min or more after the onset of conjunctive stimulation, the protein appears to play its role in the LTD induction only in a narrow time window within 15 min from the onset of conjunctive stimulation. A 5-min pulse application of a transcriptional inhibitor (actinomycin D or DRB) also effectively blocked LTD induction when it proceeded conjunctive stimulation 30 min or more (267).

Another recent finding that 5-min pulse applications of translational inhibitors depress mGluR-EPSPs quickly and persistently (266) provides a means to follow changes in the concentration of rapidly turned over protein(s) in PCs. Because the peak amplitudes of mGluR-EPSPs were progressively depressed over 30 min after a 5-min pulse application of a transcriptional inhibitor, transcription appeared to be persistently inhibited so that mRNAs were gradually depleted during the 30-min delay time (266).

Rapid turning over of a protein has been demon-

estrated to occur in neuronal dendrites after stimulation of mGluRs (549), and a role of a rapidly turned over protein in the mGluR-mediated or neurotrophin-evoked LTD induction in hippocampal neurons has also been reported (218, 255). Expression of dendritic mRNA increased following LTP (447). A challenging theme is to identify the protein that is quickly turned over in synaptic regions of PCs to play roles in the LTD induction.

2. Gene regulation

A sequential bath application of 8-bromo-cGMP and AMPA to a cerebellar slice, which induced LTD-like persistent reduction in AMPA sensitivity of PCs (236), resulted in enhanced expression of the immediate early genes, c-Fos and Jun-B, in PCs (402). Application of AMPA to the surface of the cerebellum in vivo in conjunction with electric CF stimulation resulted in the expression of Jun-B in PCs (561). GA/CF conjunction in the cerebellum in vivo also resulted in Jun-B expression, which was blocked by a NOS inhibitor (563). Jun-B with c-Fos forms an AP-1 complex, which acts as a transcriptional factor (388), but the mRNAs that are products of this transcriptional factor remain to be identified.

The cAMP response element-binding protein (CREB) is a nuclear protein regulating the transcription of genes with a CRE site in their promoter. When synaptic activation causes a high \([Ca^{2+}]_{i}\), CREB is phosphorylated at its transcriptional regulatory residue, serine-133, by involving CaMKIV. When transfected with a dominant inhibitory form of CREB that prevents DNA binding of endogenous CREB, or with dominant-negative constructs of CaMKIV, PCs failed to develop the late phase of LTD, just as they did under the influences of translational inhibitors (5). CREB and CaMKIV are, therefore, suggested to contribute to the late phase of LTD. Genes encoding \(\alpha\) and \(\beta\) poly-petides of CaMKIV are highly expressed in cerebellar granule cells, but their expression in PCs is unclear (458). CaMKIV/Gr-deficient mice exhibited impaired neuronal CREB phosphorylation and \(Ca^{2+}/CREB\)-dependent gene expression, and cultured PCs derived from these mice lacked the late phase of LTD (211). CaMIV null mice exhibited significant reduction of mature PCs in number and the expression of carbindin 28K in individual PCs (446). These effects make it difficult to simply ascribe the observed locomotor defect to the possible lack of the late phase of LTD.

3. Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein specifically expressed in astrocytes. In the cerebellum, it is highly expressed in the Bergman glial cells, a subset of astrocytes. GFAP-deficient mice exhibited normal cerebellar architecture and synaptic transmission but failed to produce GA/CF-induced LTD...


4. BDNF and tissue plasminogen activator

While BDNF has been implicated in LTP induction in the hippocampus (255), evidence is scarce regarding its involvement in cerebellar LTD induction. Nevertheless, when quisqualate application induced LTD, a significant increase in BDNF mRNA expression occurred in cerebellar tissues with a peak 4 h after the application, suggesting that BDNF plays a role in the later phases of LTD (574). Even though the major source of the BDNF mRNA increase was located in the granule cell fraction, the PC fraction also contained BDNF mRNA, which was increased by quisqualate application. The BDNF genes appear to be regulated by MAPK (sect. IV C4).

Tissue plasminogen activator (tPA) has been implicated in development and regeneration of neurons. Expression of mRNAs for tPA have been reported to increase in PCs within 1 h after rats were trained for a complex motor task (478). However, no evidence is yet reported to indicate a role of tPA in LTD induction.

E. Inactivation of AMPA Receptor

The complex signal transduction initiated by CF/GA-conjunctive stimulation or equivalent reduced form of stimulation eventually results in the inactivation of AMPA receptors mediating GA-CF transmission. Various possible mechanisms of the inactivation have so far been examined.

1. Phosphorylation

Protein phosphorylation has been shown to be a major mechanism for regulating ligand-gated ion channels such as nicotinic acetylcholine receptor, GABA_A receptor, as well as ionotropic glutamate receptors. AMPA receptors have a large extracellular NH_2-terminal domain, one membrane hairpin loop, three transmembrane domains, a large extracellular loop between transmembrane domains TM3 and TM4, and an intracellular COOH-terminal domain. AMPA receptors can be phosphorylated at their serine, threonine, or tyrosine residues. Several phosphorylation sites have been located in GluR1–4 and -6 subunits. In the COOH-terminal domain of GluR1, serine-845 and serine-831 are phosphorylated by PKA and PKC, respectively (449). The serine-831 of GluR1 is also phosphorylated by CaMKII (38, 355). As related specifically to LTD, the serine-880 residue of GluR2 subunit is phosphorylated by PKC (92, 365).

A synaptic cytoskeletal protein includes a PDZ domain (named after three proteins containing the motif, PSD-95, Dig-A and ZO-1) containing modular protein-protein interaction motifs that specifically bind the COOH terminus of membrane-associated proteins. Glutamate receptor-interacting protein (GRIP), AMPA receptor binding protein, and protein interacting with C kinase 1 (PIK1) are synaptic PDZ domain-containing proteins, which anchors the COOH terminus of GluR2/GluR3 subunits of AMPA receptors (126). It has recently been demonstrated that phosphorylation by PKC drastically reduces the affinity for GRIP at serine-880 of the GluR2 COOH terminus (38, 365, 559). Peptides, reproducing the phosphorylated and dephosphorylated GluR2 COOH-terminal PDZ binding motif, attenuated the glutamate/desorption-induced LTD in cultured PCs (559). These findings suggest that unbinding of AMPA receptors from cytoskeletal proteins primes AMPA receptors for further steps of LTD induction (see below).

Attention has been paid also to serine-696 of GluR2 subunit, because a partial peptide that contained phosphorylated serine-696 (12P3) labeled the PC dendrites persistently following inactivation of AMPA receptors by combined chemical stimuli (8-bromo-cGMP plus AMPA, phorbol ester plus AMPA, calyculin A plus AMPA, or quisqualate alone) in cerebellar slices (403, 404). The immunoreactivity was localized at the dendritic spines synapsing with GAs and was inhibited by a Ca^{2+} chelator (BAPTA-AM), a PKC inhibitor (calphostin C), or an mGluR antagonist (MCPG). However, because the serine-696 site is located in the extracellular loop between TM3 and TM4 domains (319, 503), it is unclear how it can be phosphorylated by PKC and PKG located intracellularly or in the membrane, unless the membrane topology of the GluR2 subunit is different in PCs or an activity-dependent translocation occurs dynamically between TM3 and TM4 domains. Or, alternatively, phosphorylation at serine-696 may occur before the intradendritically transported GluR2 subunits are incorporated into the subsynaptic membrane.

2. Desensitization and modulation

Desensitization is a phenomenon in which a receptor is inactivated during prolonged activation by agonist molecules, and its rate is regulated by phosphorylation. In outside-out patches obtained from rat PCs, GluR desensitization developed fast with a similar time constant in patches from dendrites (5.37 ms) and somata (5.29 ms) (192). The possibility that LTD is due to a persistent desensitization occurring in phosphorylated AMPA receptors is supported by an observation using aniracetam,
which is known to markedly reduce desensitization of AMPA receptors. Aniracetam prolonged the decay of the GA-evoked EPSC (time constant of decay from 11.74 to 15.98 ms) and compensated for the reduction in EPSCs during GA/Ca\(^{2+}\)-induced LTD (197). However, the decay time course of GA-evoked EPSC in normal perfusates was not modified during LTD. This seeming contradiction may be reconciled by assuming that the fraction of AMPA receptors desensitized during LTD does not contribute to the EPSC, while the other AMPA receptors maintain normal kinetics and desensitization properties.

Another challenge to the desensitization hypothesis is the observation that the activation of AMPA receptors is not always required for LTD induction. LTD can be induced by combined activation of mGluRs with a Ca\(^{2+}\) spike in the presence of CNQX (198) or by photolysis of caged IP\(_3\) in the PC dendrites (147). A reconciling explanation may be that desensitization is an initial step that can be skipped if further changes in AMPA receptors such as declustering or internalization (see next section) are directly triggered by application of sufficiently strong stimuli.

In addition to desensitization, it is possible that the properties of AMPA receptors are modulated by the action of PLA\(_2\) on the phospholipid moiety of the membrane-containing AMPA receptors (52). In synaptoneurosomes obtained from rat hippocampus, PLA\(_2\) affected the \(^{3}\text{H}\)AMPA binding to the AMPA receptor bimodally; at low concentrations, PLA\(_2\) inhibited binding and at high concentrations enhanced it (84). A PLA\(_2\) inhibitor prevented the depolarization-induced increase in \(^{3}\text{H}\)AMPA binding to AMPA receptors (47), and an activator of PLA\(_2\) increased the AMPA receptor affinity (46) in rat brain synaptoneurosomes.

3. Internalization of AMPA receptors

Internalization by endocytosis provides a mechanism of removing receptors from synaptic membranes (64, 213, 348). Internalization of AMPA receptors by endocytosis was induced by activating cultured hippocampal neurons with AMPA and visualized by labeling with an antibody against GluR1 (80). Furthermore, the decrease in the number of clustered AMPA receptors was observed at synapses on cultured hippocampal neurons, concurrent with induction of LTD (79). Quisqualate/depolarization-induced LTD in cultured PCs was blocked by peptides of dynamin and amphiphysin, which interfere with the clathrin endocytic complex and which also blocked IGF-I-induced attenuation of AMPA receptor-mediated currents (542). The LTD-induction protocol of phorbol ester plus AMPA application resulted in internalization of AMPA receptors in cultured PCs (364). These observations suggest that endocytic internalization of the AMPA receptors unbound from the GRIP/ABP is the final step of LTD expression in PCs is. PICK1, however, also appears to play a role in internalization (559).

F. Chemical Network for LTD

The diverse signal transduction processes implicated in LTD induction in PCs are summarized in Figure 5. They can be roughly classified into the following seven major pathways: 1) GA glutamate→AMPA receptor→Na\(^{+}\)/Ca\(^{2+}\) influx; 2) GA glutamate→mGluR1a→G\(_{q/11}\) protein→PLC relayed by DAG→PKC (2") and IP\(_3\)→IP\(_3\) receptor→Ca\(^{2+}\) release (2’’); 3) GA glutamate→mGluR1a→G protein→PLA\(_2\)→arachidonic/oleic acids; 4) GA NO→guanylyl cyclase→cGMP/PKG probably relayed by G substrate→protein phosphatase; 5) CF amino acid transmitter→glutamate receptors→Na\(^{+}/Ca^{2+}\) influx; 6) CF CRF→G protein→PKC; 7) CF IGF-I→internalization. GFAP and BDNF may provide other inputs to PCs, and PTKs, MAPK, immediate early genes, and a yet-unidentified rapidly turned over protein(s) also participate in LTD induction. There are a number of interconnections between the different pathways. For example, interaction is expected to occur from 2’ and 3 to 4 since guanylyl cyclase can be activated by PKC as well as by arachidonic acid (524). A loop connection has been suggested to subserve self-regeneration, which rapidly builds up the initial phase of synaptic plasticity before it is stabilized in a certain structural change (51, 333). However, there is yet no evidence for such a self-regeneration in LTD induction (see sect. vB5).

GA/CF- or GA/Ca\(^{2+}\)-spike-induced LTD in cerebellar slices involve all of these pathways, and interference with any of them results in the blockade of LTD induction. However, reduced forms of LTD do not necessarily require all of them. For example, glutamate/depolarization-conjunction induces LTD in cultured PCs without involving 2’, 4, or 6. LTD can be induced even by a single procedure, which, however, triggers complex signal transduction processes. K+-induced depolarization causes LTD (101), but in this case, transmitters are released from depolarized presynaptic terminals and Ca\(^{2+}\) and Na\(^{+}\) enter depolarized PCs. Quisqualate alone also induces LTD (236, 574), but it activates both AMPA receptors and mGluRs, and again allows the entry of Ca\(^{2+}\) and Na\(^{+}\) into PCs during membrane depolarization. Photolysis of caged IP\(_3\) alone within a PC dendrite also induce LTD (147). In this case, though, it is expected that a large increase in [Ca\(^{2+}\)]\(_i\) in turn activates a number of Ca\(^{2+}\)-dependent enzymes such as PKC and PLA\(_2\), which would catalyze reactions identical to those resulting from activation of mGluRs. The final event in the signal transduction for LTD appears to be phosphorylation of AMPA receptors in GA-PC synapses, which would in turn result in reduced efficacy of the AMPA receptors due to their desensitization.
and/or modulation and eventually in their removal from postsynaptic membrane by internalization. Any stimulus will induce LTD if it leads to sufficient phosphorylation and/or modulation of the AMPA receptors. To this end, Ca\(^{2+}\), indispensable for activating a number of enzymes including PKC and PLA\(_2\), is the key factor for LTD induction.

Some important properties may emerge from the elaborate chemical networks of signal transduction described in sections III and IV. For example, integration of signals across multiple time scales, generation of distinct outputs depending on input strength and duration, and self-sustained regeneration (51). Such integration of signals across multiple time scale could provide an explanation how CF signals delayed after GA signals still induce LTD (sect. II A3), as proposed theoretically (216, 472, 495). Nonlinear accumulation of signals may occur in the network, which would also explain the frequency dependence and requirement for certain numbers of repetition in LTD-inducing stimulation (sect. II A4). These emergent properties may provide a safety device that prevents an incidental occurrence of LTD when it is unnecessary and which enables LTD to occur robustly whenever the conditions for its occurrence are satisfied. A possibility is also raised that an adaptive increase of the learning rate occurs due to much longer time constants of the chemical events underlying LTD than electrical signals; this “learning” of the learning rate would robustly improve the learning capability of the cerebellum (471).

V. MODELS FOR FUNCTIONAL ROLES OF LONG-TERM DEPRESSION

As reviewed in sections III and IV, research in recent years has been fruitful in elucidating cellular and molecular mechanisms of LTD. However, different approaches are required for identifying roles of LTD in cerebellar functions. These are 1) behavioral studies of animals in which LTD is manipulated, either pharmacologically or genetically; 2) recording of signals from cerebellar neurons correlated to behavior by not only electrophysiological but also by optical recording and brain imaging techniques; 3) computational modeling of the cerebellum as a network and a system incorporating LTD as an essential component and examination of its operation by computer simulation. In the initial stages of research, we usually move in the order from the experimental (1 and 2) to theoretical (3), but at an advanced state of research, the order may be reversed. Section V thus focuses on the microcomplex model of the cerebellum, and section VI examines it in the light of the evidence accumulated in recent experimental studies of various forms of motor learning.

A. Corticonuclear Microcomplex

1. Structure

The corticonuclear microcomplex (hereafter referred to as a microcomplex) has been proposed as the functional module of the cerebellum, which has a skeleton structure such that a cortical microzone is paired with a small distinct group of neurons in a cerebellar or vestibular nucleus (227). The MF afferents arising from various precerebellar nuclei supply excitatory synapses to granule cells in the microzone and also to the nuclear neurons via collaterals. Another set of afferents to the microcomplex is relayed by a small distinct group of inferior olive neurons, whose axons end as CFs on PCs and also supply excitatory synapses to the nuclear neurons via collaterals (Fig. 6). Figure 6 further illustrates three accessory circuits attached to a microcomplex.

![Neuronal circuit structure in the cerebellum. CC, cerebellar cortex; NN, cerebellar and vestibular nuclei; IO, inferior olive; PN, precerebellar nucleus; RN, red nucleus (parvocellular part); MF, mossy fiber; CF, climbing fiber; PC, Purkinje cell; GR, granule cell; PF, parallel fiber; BC, basket cell; SC, stellate cell; GO, Golgi cell; open triangle, excitatory synapse; solid triangle, inhibitory synapse. [From Ito (232). Copyright 2000 Elsevier Science.]](http://physrev.physiology.org/)
namely, nucleocortical and nucleoolivary projections and dentatorubroolivary triangle (see sect. vD).

A typical microcomplex structure is found in the flocculus associated with the VOR system (Fig. 7). Several microzones have been identified in the flocculus by microstimulation, which evoked either horizontal, vertical, or rotatory eye movement presumably through their connections to a component of VOR subserving eye movements in these directions (399, 464, 531). These microzones receive CF inputs related to respective VOR components (176, 240). Another microcomplex has been dissected from the C3 zone of the cerebellum (249). It is connected to motoneurons innervating a finger via the anterior interpositus and red nucleus and receives CF signals from the surface of the same finger via the cutaneous afferents, dorsal column nucleus, and inferior olive.

The microzones defined in the paravermis (422) and in the flocculus (Fig. 7) may have a size of \( \sim 10 \text{ mm}^2 \). In rats, one microzone of this size contains \( \sim 10,000 \) PCs and 274 times more granule cells (190). The human cerebellum is \( \sim 50,000 \text{ mm}^2 \) wide so that it may contain as many as 5,000 microzones as its functional unit (227).

2. Operation

Four basic notions constitute the operational principle of a microcomplex (Fig. 6). 1) MF signals passing to a microzone are relayed by granule cells and in turn excite PCs and other cortical neurons, eventually evoking simple spikes in PCs. Thus, in each microcomplex, simple spike discharges of PCs driven by MF signals would produce a unique functional state due to concerted activities of excitatory and inhibitory synapses on PCs, such as visualized in a computer simulation study (114). 2) The MF
signals also drive the nuclear neurons, which generate output signals of the microcomplex under inhibitory influences of PCs. 3) CFs convey error signals to a microcomplex regarding the operation of the neural system that includes the microcomplex. The error signals are generated by various neuronal mechanisms in diverse preliminary structures. 4) CF error signals induce LTD in the conjointly activated GA-PC synapses (learning rule) and thereby modify the operation of the microcomplex until the error signals are minimized. CF signals evoke complex spikes in PCs, and hence induce conducting impulses in PC axons, which eventually evoke IPSPs in the nuclear neurons. However, CFs normally fire irregularly at a slow rate around 1 Hz, compared with the high firing rate of simple spikes around 50 Hz. Furthermore, the effects of the IPSPs in nuclear neurons are counteracted by the EPSPs evoked via collaterals of olivocerebellar fibers. Therefore, the author suggests that the major role of CF signals in PCs is to induce LTD, but not to influence the nuclear neurons via impulse traffic (227).

The above-postulated operation of the microcomplex has been reproduced by computer simulation using elaborate neuronal network models of the microcomplex including inhibitory neurons in the cerebellar cortex (156, 471, 473).

B. Roles of GAs

1. Ascending segment of GA

The structure of a microcomplex is complicated by the presence of two types of GA-PC synapses, one formed with the ascending segments of GAs and the other formed with PFs (sect. A1). A recent study shows that PC dendritic spines contacting the ascending segments of granule cell axons are located exclusively on the smallest diameter, distal regions of the PC dendrites, while PF synapses are on the intermediate and large diameter regions of the spiny branchlets (181). The ascending segments form ~20% of the GA-PC synapses.

Two modes of activation of PCs by GAs have been reported as corresponding to these two segments. When PFs on the surface of the cerebellum are stimulated, a narrow beam of PFs conduct impulses over a distance of 1 mm, which excite PCs, as visualized using voltage-sensitive dyes (94, 536). However, stimulation of MFs in the white matter of an isolated perfused guinea pig cerebellum induced a circular, nonpropagating patch of synapses form regions of the spiny branchlets (181). The ascending segment synapses may have a higher transmission efficacy than PF-PC synapses. The ascending GA-PC synapses are located in the distal PC dendrites, while CF terminals do not reach (424) and CF-induced Ca²⁺ spikes do not reliably spread (378). For these two reasons, it may appear that these synapses produce the homosynaptic LTD, which does not require CF signals and which plays other roles than error-driven LTD-based learning (sect. A2). The PF-PC synapses located in the intermediate or larger diameter regions of the spiny branchlet could be the site of GA/CF-induced LTD. So far, the two types of synapses have been mixed in experiments on LTD, and it is desirable to examine them separately to define their respective functions in a microcomplex.

2. Length of PFs

Another complication of the microcomplex structure concerns with the length of PFs that exceeds the width of a microzone. In contrast to the width of microzones as narrow as 0.3 to 1 mm [cat (249, 422), rabbit (399)], the length of PF branches from their bifurcation to their terminal has been estimated to be as long as 2–3 mm [chicken and monkey (303), rat (190, 432)].

Physiological data, however, indicate that excitation of PCs via PFs does not spread more than a distance of ~1.5 mm from the site of stimulation (164, 536). In Heck’s (196) experiment on the rat and guinea pig cerebellum in vitro, 11 electrodes were linearly aligned at 130-µm intervals and placed in the granular layer. Stimuli travelling from electrode to electrode at a velocity comparable with the conduction velocity of PFs elicited population spikes of PFs, which linearly increased with the distance and were saturated for distances longer than 1.0 mm. This again suggests that the PF spikes are conducted for a distance of 1 mm but no more. These data may suggest that the very peripheral segments of PFs do not generate conducting action potentials, but there could still be electrotonic spread of membrane depolarization for a certain distance, which could cause release of transmitter.

A concern from a computational point of view is that PF beams distribute information conveyed by MFs to more than one microzone. Information of MFs may spread in the cerebellar cortex via branches of MF terminals and further via PFs across boundaries of microzones. However, how a microcomplex acquires its functional specificity out of such broad input connections can be speculated in the following way along with the microcomplex concept (Fig. 8). Suppose that when signals in a set of MFs activate PCs in a number of microcomplexes through the divergent MF-GA-PC connections, PC inhibi-
tion dominates in them. However, if one of these microcomplexes is involved in execution of a motor control, the consequently arising CF error signals will induce LTD in this microcomplex (b in Fig. 8). In the cerebellar cortex of this microcomplex, the excitatory action of GA-PC synapses will then be reduced, but the excitatory action of GAs on basket and stellate cells remains and may surplus GA-PC excitation so that PCs will be inhibited. Thus nuclear neurons are released from PC inhibition and add to the output of the microcomplex responding to the MF input. This process could continue until an adequate number of nuclear neurons are released from PC inhibition to generate an activity required for the optimal operation of the microcomplex in the given motor control. Microcomplexes initially kept inhibited through the preexisting divergent MF-GA-PC connections will thus individually acquire a specific functional involvement through CF error-driven LTD-based learning.

C. Other Types of Synaptic Plasticity

A third complication in the microcomplex structure is the presence of several other types of synaptic plasticity than conjunctive LTD.

1. Homosynaptic LTD in GA-PC synapses

The homosynaptic LTD occurring after relatively strong stimulation of GAs alone (188) could represent a normalizing process against overstimulation of GA-PC synapses (113). Alternatively, it could be involved in a type of learning in which there is no specialized input lines for error signals. Any MF input that evokes GA signals sufficiently strong to cause Ca$^{2+}$ entry to a PC could serve as error signals and depress other GA inputs conjunctively imposed to the PC. This type of learning without a specialized error signal system occurs in the part of a fish cerebellum devoid of CFs (44, 45). In the cerebellar cortex equipped with CFs, both types of learning appear to be implemented in superposition. However, as pointed out in section V B1, homosynaptic LTD could be a major learning mechanism in the peripheral branchlets of PCs, where CF terminals do not reach (424) and CF-induced Ca$^{2+}$ spikes do not reliably spread (378).

2. Heterosynaptic LTD in GA-PC synapses

Recent observations that LTD spreads to neighboring synapses up to 100 μm from those involved in GA/CF conjunction (189, 445, 539) make the input specificity for LTD ambiguous. Wang et al. (539) guess that conjunction involving one PF causes LTD in 600 PFS. However, Reynolds and Hartell (445) recognized that LTD did not spread to the neighboring synapses when these were left unstimulated at all for 20 min. LTD developed only after their stimulation at 0.2 Hz was resumed for monitoring LTD. Therefore, heterosynaptic LTD occurs only in those neighboring synapses that are active even at a low rate of 0.2 Hz during the postconjunction period for 20 min. In other words, LTD is induced not only in those synapses directly coactivated with CF signals, but also in the neighboring synapses activated in a loose time relationship with CF signals. If this is the case, the input specificity of LTD still holds in a wider sense.

Heterosynaptic LTD may reduce the memory capacity of a Perceptron-like neuronal network, but spreading memory may bring a certain advantage such as has been discussed for spread of LTP in hippocampus neurons.
LTP induced in a neuron by pairing 1-Hz presynaptic stimulation and membrane depolarization was shown to spread to neighboring neurons, which displayed LTP in those synapses activated by the 1-Hz presynaptic stimulation under no membrane depolarization (470). These synapses in neighboring cells were not imposed with membrane depolarization, and therefore they should not have displayed LTP unless an intercellular influence reached them from the cell undergoing LTP. Diffusible substances such as NO, CO, and arachidonic acid may be considered as its mediator. In the hippocampus, a small number of synapses involved in generation of LTP influence many other synapses in the vicinity, which have recently been active. Here, learning is thought to occur not at levels of individual synapses, but rather at levels of small local volumes (volume learning, see Ref. 386).

Exact meaning of heterosynaptic LTD in the operation of cerebellar network needs to be explored further. One likely situation to be considered is that, in natural conditions, MF signals from a source may reach synapses in a segment of a PC dendrite after being temporally dispersed across successive relays. Conjunctive activation with a time-limited occurrence of CF error signals during behavior may take place only with some of these synapses. Nevertheless, LTD could spread to all other synapses located nearby and receiving the dispersed MF signals. This model assumes local volumes in the cerebellar cortex as mapping MF inputs. To test the validity of this model, one needs to investigate how MF signals arising from a source are represented in PC dendrites.

3. Presynaptic LTP in GA-PC synapses

Brief stimulation of GAs at 2–8 Hz induces LTP in PCs (104, 207, 459), which is due to an increase in the release of transmitters from GA terminals (see sect. vC5). The observation that LTD is induced postsynaptically and LTP presynaptically in PCs is in contrast to that in pyramidal cells, where tetanus-induced LTD is converted to LTP depending on the postsynaptic membrane potential which determines $[\text{Ca}^{2+}]_{i}$ (25, 566). A seeming conversion from LTD to LTP was reported to occur in PCs during intracellular injection of a Ca2+ chelator (485), but the possibility is not excluded that presynaptic LTP was simply unmasked by the disappearance of LTD. In cultured hippocampal neurons, repetitive postsynaptic spiking within a time window of 20 ms after presynaptic activation has been reported to result in LTP, whereas postsynaptic spiking within a window of 20 ms before the repetitive presynaptic activation led to LTD (52). However, no such conversion between postsynaptic LTD and presynaptic LTP has been observed in PCs by changing the timing of CF and GA stimulation.

4. Homosynaptic LTD in CF-PC synapses

Homosynaptic LTD occurs also in CF-PC transmission during stimulation of CFs at 5 Hz for 30 s (184). Signal transduction for this CF-LTD overlaps that for GA/CF conjunctive-induced LTD. The functional meaning of the modest depression (by 20–30%) of large all-or-none CF-evoked EPSCs is unclear for the present, but since such depression of CF responses does not occur during CF stimulation at 1 Hz (265), it may imply a possibility that the effects of CFs to induce conjunctive LTD or rebound facilitation (see below) decline after excessive activation of CFs.

5. Rebound potentiation of inhibitory synapses on PCs

CF activation in PCs is followed by a prolonged potentiation of GABA$_{A}$ receptor-mediated IPSPs (rebound potentiation), which is due to a Ca$^{2+}$-dependent upregulation of postsynaptic GABA$_{A}$ receptor function (262) and involves activation of CaMKII (261). Correspondingly, photolytic release of IP$_{3}$ in PCs combined with membrane depolarization resulted in facilitation of inhibitory postsynaptic currents, while it induced LTD in GA-evoked EPSCs (280). Rebound facilitation may have a synergistic action with LTD in releasing a microcomplex from PC inhibition (Fig. 8).

6. LTP in MF-GA synapses

LTP is induced at MF-GA synapses after a high-frequency stimulation of MFs paired with membrane depolarization. This LTP induction involves NMDA receptors, mGluRs, Ca$^{2+}$, and PKC (107) and is accompanied by changes of intrinsic excitability of granule cells (24). In the three-layered Simple Perceptron model of the cerebellum, connections of MFs to granules cells at the second layer perform certain transformation of MF inputs and provide the decision hyperspace where PCs select connections for their correct responses (15). In this model, if MF-GA LTP increases functionally the divergence number from a mossy fiber to granule cells, it is expected that the dimension of the hyperspace expands so that the computational capacity of the model is enhanced.

7. Excitability of nuclear neurons

The presence of synaptic plasticity at the major excitatory input to nuclear neurons has been suggested (332). There is a report of such LTP in vivo (440), but a recent observation in rat cerebellar slices revealed no presynaptic tetanus-induced increase of EPSPs in cerebellar nuclear neurons except for some broadening of the EPSP duration (9). Instead, Aizenman and Linden (9) found in nuclear neurons a marked increase of the membrane excitability as represented by an increase of the
number of spikes evoked by a given depolarizing pulse. The membrane excitability change depends on a Ca\(^{2+}\) load imposed by activation of NMDA receptors or direct current injection. In in vitro whole brains of guinea pig, excitatory input from the vestibular nerve to vestibular nuclear neurons was paired with the inhibitory input from flocculus PCs and revealed no traces of synaptic plasticity in the vestibular nucleus neurons, whereas paired stimulation of the vestibular nerve and inferior olive effectively induced LTD in flocculus PCs (31). Hence, there is no evidence for input-specific synaptic plasticity in the major excitatory synaptic input to the nuclear neurons.

8. LTD in PC-to-nuclear neuron synapses

Tetanic stimulation of inhibitory synapses on cerebellar nuclear neurons, presumably supplied by PC axons, results in long-lasting depression (389). This depression is due to a reduced postsynaptic GABA sensitivity caused by increases in [Ca\(^{2+}\)]\(_i\) and activation of protein phosphatases in nuclear neurons but does not require activation of the GABA\(_A\) receptors. This synaptic plasticity explains how the increased discharge in PCs in the absence of CF activity (96) results in a decreased efficacy of PC inhibition of nuclear neurons (54, 239, 264). Tetanic induction of IPSPs in nuclear neurons also induced a prominent rebound excitation and associated Na\(^+\) spike burst upon release from hyperpolarization, which are regulated by [Ca\(^{2+}\)]\(_i\) (8). A long-lasting increase or decrease of the IPSPs, depending on the amount of rebound-induced spikes, followed the rebound excitation (10). The effects described above in sections \(\text{vC7}\) and \(\text{vC8}\) may add to use-dependent flexibility of the microcomplex structure. However, because these effects are input nonspecific, it is uncertain how far they afford the simple divergence-convergence connections in a cerebellar nucleus of a capacity to maintain elaborate memory information.

D. Accessory Circuits

1. Nucleocortical projection

Collaterals of axons that larger nuclear neurons send to the red nucleus, thalamus, and other brain stem structures pass back to the cerebellar cortex (368, 520, 521). These fibers terminate in the cerebellar cortex as mossy fibers. The nucleocortical projection is mainly reciprocally organized with corticonuclear projections by PCs (71, 123, 124, 522). Because the nuclear neurons supply excitatory synapses to brain stem structures, and because the major transmitter of the nucleocortical projection is glutamate, but not GABA (41, 296), the nucleocortical projection should have excitatory action upon granule cells as mossy fibers. Because the nucleocortical projection would activate PCs that in turn inhibit nuclear neurons, it may stably maintain the activity of nuclear neurons at a low level in the manner of negative feedback. If LTD occurs in the GA-PC synapses activated by the nucleocortical feedback, the negative feedback will be removed so that nuclear neurons may maintain a higher activity. If this is the case, the nucleocortical projection will add to the release mechanism of an error-driven microcomplex from dominating PC inhibition as postulated in Figure 8.

2. Nucleoolivary projection

Smaller GABA-containing neurons that occupy 31.7% of nuclear neurons (41) are the source of the nucleoolivary inhibitory projection (119). The function of this nucleolivary projection has been suggested to regulate the occurrence of LTD in PCs by providing negative feedback information to the olive. For example, when a learned response reaches a sufficient amplitude, the olive would be inhibited and further learning blocked (201, 281, 475). This suggestion has been supported in studies on classic conditioning (see sect. \(\text{vA2}\)). The nucleolivary projection may thus have an action of protecting a microcomplex, which has already learned, from further modification. A modeling study suggests that inclusion of the nucleolivary inhibition into cerebellar circuitry makes learning stable (495).

3. Dentatorubroolivary triangle

Cerebellar nuclei send excitatory synapses to neurons in the parvocellular part of the red nucleus, which also receive excitatory input from the parietal association cortex (417). Parvocellular red nucleus neurons in turn send axons to the inferior olive (72, 73). The rubroolivary projection supplies GABA-negative, excitatory synaptic terminals to inferior olive neurons (119, 120). Because inferior olive neurons supply excitatory synapses to cerebellar nuclear neurons via collaterals of CF afferents, the dentatorubroolivary triangle may form a reverberating circuit, which, however, would be depressed by the PC inhibition on the nuclear neurons.

A role of the dentatorubroolivary triangle in motor learning was suggested by an experiment in which motor disturbance produced by lesioning the rubrospinal tract that originates from the magnocellular part of the red nucleus was compensated well only when the parvocellular part of the red nucleus was intact (279). It is noted that collaterals of CFs supply excitatory synapses to not only the major nuclear neurons projecting to the brain stem, but also small GABA-containing nucleolivary neurons (121). Inferior olive neurons are thus equipped with both positive feedback via the dentatorubroolivary circuit and negative feedback through
E. As an Internal Model

Modern control system theories have been useful in accurately defining roles played by a microcomplex in motor control. In the usual design of a control system, precise control is secured by feedback (Fig. 9A). However, such feedback is not always available in living bodies, and an essential role of the cerebellum appears to secure precise control without feedback (225, 227, 276). In a typical feed-forward control (Fig. 9B), the controller converts instruction for a movement to command signals that act on the controlled object. The controlled object in turn converts the command signals to an actual movement. If the instruction/command conversion is inversely equivalent to the command/movement conversion by the controlled object, the actual movement becomes equivalent to the instruction (Fig. 9B). This inverse model principle was applied to the control of a robot’s arm (214) and has been considered as a major mechanism for bodily motor control. A unique two-degrees-of-freedom adaptive control system for voluntary movement proposed by Kawato and colleagues (171, 276) combines feedback control by the cerebral cortex with feed-forward control by the cerebellum (Fig. 10).

Another way of performing a precise control in a seemingly feed-forward manner is to utilize an internal loop through a model that simulates the command/movement conversion by the controlled object (forward model) and thereby predicts the movement to be produced by the controlled object (Fig. 11). This model was applied to interpret functional meanings of the cerebrocerebellar communication loop (223, 227). If the internal loop contains not only dynamic properties of the controlled object but also the delay time involved in the external feedback, exactly the same effect as the external feedback from the actual movement will be reproduced. This is what is done in an engineering model called Smith Predictor, and hence Smith Predictor has been proposed as a form of internal models in the cerebellum (372). The two modes of control in Figures 10 and 11 should contribute differently to learning of movement, and both may be executed in combination (171, 229, 276, 557, 558).

A forward model mimics the conversion of command of a movement in the coordinates of the body to an actual movement in the coordinates of the workspace, and an inverse model converts vice versa. When these conver-
sions are performed on the dynamic characteristics of the controlled object such as torque and muscle tension, the internal model represents forward or inverse dynamics of the controlled object. When the conversion is performed on the kinematic properties of the controlled object such as movement direction and target position, the internal model represents forward or inverse kinematics of the controlled object. These internal models may not necessarily be limited to the cerebellum. They may distribute to various parts of the central nervous system such as spinal cord, basal ganglia, and cerebral cortex, and the cerebellum may contribute to an important portion of them (473).

Computational potentialities of the cerebellum in forming such an internal model, either forward or inverse, within its elaborate neuronal networks by learning have extensively been explored in recent years. An equation representing dynamics or kinematics, or their inverse, for a multi-joint arm, for example, consists of many terms including mass, inertia, length of segments of the arm, and viscosity in joints so that analytical solution of the equation requires an enormous amount of computation. A surprising capability of the Simple Perceptron-like network of the cerebellum has been found to be such that it quickly forms an internal model for a multi-joint arm without specific information about individual terms of the equation, simply by the error-driven LTD-based learning (275, 472, 473, 558).

VI. EXPERIMENTAL EVIDENCE FOR FUNCTIONAL ROLES OF LONG-TERM DEPRESSION

The above-presented hypothesis that the microcomplex makes precise feed-forward control possible by forming an internal model through error-driven LTD-based learning is examined below in the light of available experimental data. These data were collected from the three particular viewpoints: 1) error representation by CFs, 2) involvement of LTD in motor learning, and 3) behaviors of PCs, in various forms of motor control.

A. Error Representation by CFs

The ways of detecting errors vary, and the errors represented by CF signals are of varied nature, as reviewed here for various forms of motor learning. In simple situations, CF error signals are derived from sensory systems detecting a harmful consequence of a wrongly executed movement (sect. vi, A1 and A2) or monitoring a deviation of a realized movement from a desired one (sect. vi, A3 and A4). However, in more complex situations, errors appear to be sensed even before the actual movement is executed, by comparing the instruction with a consequence predicted within the central nervous system (sect. vi, A4 and A5). The notion of error representation by CFs is expanded to include not only those errors detected externally through sensory systems but also those errors derived internally through a neural mechanism for feed-forward control.

1. Errors in flexion reflex and startling

Complex spikes elicited in the paravermal zone of the cerebellum in response to nociceptive stimuli from the skin of a finger (140) would inform PCs about the occurrence of errors in the flexion reflex that withdraws the finger from the noxious stimulus. In awake monkeys startled by sudden loud sounds, complex spike discharges were evoked in PCs of lobule VI, paramedian lobule, and dorsal paraflocculus (391). These complex spikes may reflect errors of the startle response in minimizing the impact of the sudden loud sounds.

2. Unconditioned stimulus in conditioning

Classical conditioning of the rabbit nictitating membrane/eyelid response (eye blink) can be established by combining an innocuous stimuli such as a tone as conditioned stimulus (CS) and a noxious stimulus such as an air puff to or electrical stimulation around the eye as unconditioned stimulus (US) (282, 366, 518, 567, 568). Complex spikes are elicited by corneal stimulation as US and represent errors in timely eye closure to avoid corneal stimuli. The air puff-induced responses in the dorsal accessory inferior olive neurons of rabbits were initially large, but they diminished during acquisition of the eyeblink conditioning (477). Disrupting inhibition of the inferior olive prevented this “blocking” (281). In decerebrate ferrets, electrical skin stimulation of the forelimb as CS was paired with periorcular skin stimulation as US to form eye-blink conditioning (200). The CS did not inhibit the occurrence of US-induced CF responses in PCs at the beginning of training, but it did so in some animals when conditioned responses became large. The negative feedback via the inhibitory nuceloivillary projection is likely to mediate the behavioral phenomenon of blocking (sect. viD2).

3. Retinal slip in ocular movement

PCs in the flocculus of rabbits and cats exhibit complex spike discharges in response retinal slip (128, 159, 176, 301). Continuous rotation of the visual field upregulated CRF and its mRNAs in the inferior olive, apparently due to an enhanced retinal slip input (36, 37). PCs in the monkey’s cerebellar area, which was originally taken as flocculus, but later redefined as the ventral paraflocculus (169, 498), exhibited an increased complex spike discharge during smooth pursuit eye movement, representing retinal slip resulting from inadequate tracking (498).
In the ocular following movement elicited by slow whole visual field movement, PCs in the monkey’s ventral paraflocculus showed a modulation pattern of complex spike discharges correlated with retinal slip (290).

4. Perturbed posture and locomotion

During roll oscillation of frogs, PCs exhibited complex spike discharges, which were larger in the absence of appropriate compensatory limb movements, suggesting an error-signaling role of CFs (18). Inferior olive neurons in the awake cats frequently responded to passive displacement of a limb (168). These responses can be interpreted as signaling errors in a postural adjustment of the limb (491).

In experiments on decerebrate ferrets, where a bar that extended into the trajectory of the right forelimb at a specific phase of the step cycle perturbed locomotion, a significant increase occurred in the discharges of complex spikes from PCs in lobule V or VI, at times immediately after the perturbation (345). This complex spike response could represent errors in avoiding perturbation. During stable treadmill locomotion of decerebrate cats, complex spike responses of lobule V PCs were only slightly modulated with a weak increment at the swing phase of the ipsilateral forelimb, but when the contralateral forelimb alone was suddenly imposed with a faster belt velocity, the occurrence of the climbing fiber discharges was significantly enhanced during the late swing phase of the ipsilateral forelimb (565). These climbing fiber responses appear to represent errors in interlimb coordination during locomotion.

CF responses evoked in the C1/C3 zones of lobules V and VI by electrical stimulation of the superficial radial nerve of the ipsilateral forelimb displayed modulation of their sensitivity related to locomotion steps (20). The largest CF responses occurred overwhelmingly during the E1 step phase when the limb is extended forward and down to establish footfall, whereas the smallest responses were seen during the stance phase. This may suggest that the spinolivary afferent system is gated to be most sensitive when the foot is touching down, that is, when errors tend to happen. In experiments with cats walking on a horizontal ladder, perturbation by an unexpected 2-cm descent of a stepped-on rung induced complex spike discharges from lobule V PCs, closely time-locked to the onset of rung movement but not its cessation, which they often preceded (19). Therefore, the complex spikes appear to compare commands from higher motor centers via descending connections, with the activity that these commands evoke in spinal motor circuits, that is, internal feedback, but not with the peripheral input resulting from the commanded movement. Because the descending pathways from the motor cortex and midbrain and the ascending pathways from the spinal cord do not converge onto the same inferior olivary neurons (120), the comparison between the descending and ascending signals may not directly be made in the inferior olive as suggested earlier (375, 422).

5. Three types of errors in hand/arm movement

When a monkey was holding a lever driven by torque motor force against flexion and extension, a shift of the load at some unpredictable time induced complex spikes in the intermediate cortex of lobules III through V (170). These complex spikes occurred just after the load switch (at 50–150 ms) and apparently represented errors caused by a sudden change of the load. Gilbert and Thach (170) observed that the simple spike discharge decreased reciprocally to the complex spike discharge and remained decreased after the complex spike discharge had returned to normal, the early evidence supporting Albus’ (15) hypothesis (sect. I4).

A significant increase in complex spike discharges was also observed when a monkey, manipulating a stick to shift a cursor on the screen from a starting box to a target box, was required to modify the ongoing movement of placing the cursor within a repositioned target box (540). This result allows an interpretation that CF signals occur when the motor state changes and/or during errors in motor performance. In a paradigm in which a monkey learned to adapt to a change of the relationship between the cursor and the hand, complex spike discharges occurred in those trials in which the velocity was inappropriate for the cursor-hand relationship that the animal is required to learn, suggesting that complex spikes are associated with velocity-related error signals (416). The complex spike discharges observed in a multiple-joint arm-reaching task of monkeys were found to encode distance and/or direction, suggesting that they are spatially tuned and related to movement kinematics of the arm (155). This observation is consistent with the hypothesis of error representation by complex spikes, because in this experiment the monkey controls kinematic properties of a multiple joint arm.

However, complex spike discharges were found to occur in various phases of hand/arm movements, often unrelated to feedback of consequences from the performed movement (415). In a visually triggered tracking movement of the wrist by monkeys, the firing rate of complex spikes increased immediately following electromyography activity in prime mover muscles, before the beginning of the actual movement (358). The timing of complex spike discharges was analyzed in detail in monkeys performing short-lasting reaching hand/arm movements (286). The monkeys saw the hand and the target before and after the movements, but the reaching movements were performed without visual feedback. When monkeys moved the hand to touch a visual target that
appeared at a random location on a screen, complex spikes were shown to occur in three phases (first, second, and third responses) (Fig. 12). The third response falling after the end of the reaching movements apparently represents visually perceived deviations between the target and the reached finger position, but the first and second responses appear too early to be interpreted similarly. Kawato et al. (276) postulated, as the most plausible possibility, that errors are derived from the output of the cerebral cortex, which also generates the motor commands to the hand/arm system (feedback-error learning) (Figs. 10 and 11). In this design, the discrepancy between the instructed target position and the hand position predicted by an internal forward model in the cerebellum is fed to the inferior olive via the cerebral cortex (Fig. 11). This may explain the second complex spike response in the reaching movement (286).

The earliest component of complex spike discharges can also be explained by the feedback-error learning scheme. When the monkey initiates the reaching in dark, the cerebral cortex is driven by the instruction of movement toward the target position, and this instruction is fed to the inferior olive via the cerebral cortex (Figs. 10 and 11). If the instruction represents the discrepancy between the initial set position of the hand and the visually instructed target position, it implies in a sense an error. The initial set error, as it may be called, might be referred to if the monkey learned to shift the initial set position of the hand closer to the target zones before initiating the reach, but this was prevented in the experiment. Or, the initial set error might represent the discrepancy between the target position actually viewed and that which the monkey anticipated from preceding experiences. Such an error might be referred to if the monkey learned to reach a target repeatedly appearing at the same position, but this learning was also prevented by randomizing the target position. Therefore, the earliest complex spike discharges would play no role in the given situation where learning is prevented. A computer simulation of learning reaching movement adopting the feedback-error learning reproduced complex spike discharges in two phases: the early response locked to movement onset, which was always present, and the later response, which disappeared after learning (475). Because the learning was stable and maintained, the early CF response is considered as inevitable, but playing no significant roles in performing the given motor task.

B. Involvement of LTD in Motor Learning

There are numerous data of lesion experiments using surgical ablation or application of a toxic dose of amino acids to the cerebellum. However, this section mainly focuses on recent data obtained by pharmacological or genetic manipulation of LTD, which has been made possible due to recent advances in our knowledge of signal transduction in LTD (sects. III and IV).

1. Adaptation in ocular movement

VOR, producing eye movement compensatory for head movement, is adaptively enhanced or depressed under persistent vestibular-visual mismatching conditions (172, 242, 243, 448). The flocculus hypothesis (226) that the VOR adaptation occurs due to LTD-based learning in the flocculus is supported by the following observations.

1) Injection of an inhibitor of NOS into the goldfish cerebellum also inhibited the adaptive increase of VOR gain (318). 2) Transgenic mice that selectively express the pseudosubstrate PKC inhibitor, PKC-
[19—31], in PCs lacked the VOR adaptation consistent with the loss of LTD induction in cerebellar slices obtained from these mice (118). In testing VOR adaptation, a caution is needed to avoid the mouse strain 129/sv because of its abnormally low VOR gain, while C57BL/6 has a normal VOR gain (273).

Whether the memory trace for VOR adaptation is eventually formed in the cerebellar cortex or the nucleus, or even in the brain stem is still controversial (444). Application of lidocaine to the goldfish cerebellum through a microdialysis probe abolished the VOR adaptation attained by a few hour stimulation, indicating that the memory for this adaptation, at least for the initial few hours, is located in the cerebellar cortex (369).

Continued rotation of the visual field around a stationary rabbit induced an increase in the optokinetic eye movement response (OKR) in 1 h (396, 397). 3-AP-induced depletion of CFs abolished OKR adaptation in mice (272), and nNOS-deficient mice lacked OKR adaptation (273). Fyn is a member of the src subfamily of the genes encoding nonreceptor PTKs (357), which have been implicated in LTD induction (sect.IV C3), but Fyn-deficient mice exhibited normal adaptability of OKR (287). Probably, some other member(s) of the src subfamily is involved in LTD.

When a monkey making a smooth pursuit eye movement is repeatedly faced with a sudden increase in the velocity of the moving target, it adapted to start the pursuit with an increased velocity. Subdural applications of NO scavenger or NOS inhibitor to the paraflocculus-flocculus scarcely affected the smooth pursuit, but markedly depressed its adaptation, suggesting that cerebellar LTD underlies the adaptation of smooth pursuit (400).

2. Eye-blink conditioning

The microcomplex involved in eye-blink conditioning contains HVI lobule cortex (C1 and C3 zones), an anterior part of the interpositus nucleus and the medial parts of rostral dorsal accessory olivary, since lesions in these regions disrupt conditioned responses (306, 307, 567–569). Reversible inactivation of the anterior part of the interpositus nucleus by local injection of muscimol prevented the acquisition of conditioned responses (300) as well as its extinction (441). Eye-blink conditioning is significantly impaired in Purkinje cell degeneration (pcd) mutant mice (89).

In rabbit cerebellar area HVI, an increase of membrane-bound PKC was detected after eye-blink conditioning (150), which could be related to LTD (sect. vC1). Those rabbits given a NOS inhibitor, which blocks LTD induction (sect. mA2), exhibited learning deficits in the conditioned eye-blink response (85). GFAP-deficient mutant mice showed loss of LTD (sect. vD3) and also impaired eye-blink conditioning (482). Injection of an IGF-I antisense oligonucleotide in the inferior olivary, which reduces IGF-I levels in the cerebellum (sect. mA5), blocked conditioned eye-blink learning in freely moving rats (83).

The fact that some conditioned responses remain after cortical lesions or PC degeneration raises the possibility that a portion of memory trace is retained in cerebellar nuclei or brain stem (444). However, when the eye-blinking evoked from the conditioning stimulation of the arm skin was reproduced by direct stimulation of the cerebellar peduncle, the latency of the conditioned responses suggested signal transfer via the cerebellar cortex, but not via the nucleus (202). Furthermore, a unique memory role of the cerebellar cortex is indicated by the finding that cerebellar cortical lesions specifically disrupted the timing of conditioned eyelid responses acquired by learning, and left responses that peaked inappropriately at very short latencies (366, 430). LTD-based learning in the cerebellar cortex may account for the well-timed conditioned responses, while the input non-specific enhancement of excitability in nuclear neurons (see sect. vC7) may be responsible for the untimely conditioned responses that appear after cortical lesioning.

3. Adaptation in posture and locomotion

Compensation of the impaired righting response by unilateral lesion of the vestibular organ has been studied as a type of learning in the cerebellum. The compensation was retarded in mutant mice deficient in δ2-protein (158), consistent with the loss of LTD in this mutant (269). The behavioral recovery from unilateral labyrinthectomy in rats was accompanied by asymmetric expression of PKC-α, -γ, and -δ isoforms in the flocculonodular lobe with a regionally selective increase in the number of PKC-immunopositive PCs contralateral to the lesion (175). This asymmetry occurred within 6 h after the labyrinthectomy and was resolved to the control, symmetric pattern within 24 h. The compensation was retarded in the rats after intracerebroventricular application of PKC inhibitors (33), consistent with the requirement of PKC in LTD induction (sect. vC1).

When a decerebrate cat walking on a treadmill experienced sudden increase in the speed of the running belt only under the left forelimb, regular stable locomotion was restored in 50–100 steps. Injection of a NOS inhibitor into the lobule V vermis blocked this adaptation, consistent with the hypothesis that LTD induction is a major mechanism for cerebellar adaptation (564). mGluR1-deficient mice walking on a treadmill exhibited an abnormally dispersed locomotor cycle of two limbs, which were sharply distributed around 180° in wild-type mice, and did not adapt to an increase of the belt velocity, whereas wild-type mice progressively decreased step cycle duration (220). These signs of impaired interlimb coordination in locomotion diminished when the mGluR1 deficiency was rescued in the cerebellum.
4. Motor coordination

Coordination of complex movements has been regarded as a major function of the cerebellum (516). In experiments on mice, motor coordination is tested by observation of locomotor ataxia, rope climbing, running on an elevated runway with low obstacles, and evaluated by measuring the time during which an animal can remain on a horizontally placed rotating rod. Significant shortening of the staying time has been reported in a majority of the 10 types of gene-manipulated mice (Table 1). It is to be noted that nNOS-deficient mice show no obvious motor discoordination during daytime, but they exhibit peculiar motor discoordination during night (299).

Six of the eight types of gene-manipulated mice showing motor discoordination were tested for LTD induction in cerebellar slices obtained from them. Four types, namely, mGluR1- (6, 100), GluRδ2 (209, 269), Gαq- (381) and nNOS-deficient (316) mice lacked LTD; however, the other two, namely, PKC-γ (86) and mGluR4-deficient (428) mice retained LTD. Hence, lack of LTD is not the sole cause for motor discoordination. PKC-γ-deficient mice (258) exhibited persistent multiple innervation of PCs by climbing fibers (CFs); which, even though LTD is retained, is expected to impair the function of cerebellar neuronal circuit due to loss of the microzone specificity of CF innervation (sect. V, A2 and B3, and Fig. 8). Multiple CF innervation was also found in the three mutants lacking LTD [mGluR1 (259, 311), GluRδ2 (269), Gαq (414)], and hence which of LTD or multiple CF innervation is the major cause for motor discoordination is unclear. The abnormal transmission in GA-PC synapses found in the mGluR4-deficient mice (sect. uBI) could explain dysfunction of the cerebellar neuronal circuit and consequently motor discoordination in this mutant despite the presence of LTD. LTD induction in PLC-β4-deficient mice has not been tested, but the occurrence of multiple CF innervation (260) in them explains motor discoordination. NR2A/NR2C-deficient mice (252) are likely to develop multiple CF innervation of PCs by climbing fibers (CFs); hence, they impair the function of cerebellar neuronal circuit due to loss of the microzone specificity of CF innervation (516).

Because genes are manipulated all over the body in these mutants, specific relationships of the observed motor discoordination to the cerebellum may be questioned; it might arise from dysfunction of the cerebral cortex, basal ganglia, or spinal cord. This question was addressed in the experiment in which mGluR1 deficiency was rescued in the cerebellum (220). It was remarkable that the rescue-induced regression of multiple CF innervation and recovery of LTD paralleled improvement of motor coordination (220).

However, GFAP-deficient mice were reported to exhibit motor coordination indistinguishable from a wild type of mice despite the lack of LTD (482). PKC-inhibitor-transfected mice also were reported to exhibit seemingly normal motor coordination in the absence of LTD and in the presence of multiple CF innervation (118). These observations are difficult to reconcile with the hypothesis of cerebellar learning, but the author points out the fact that, compared with ocular adaptation, eye-blink conditioning, adaptive locomotion or hand/arm movement, motor coordination has been investigated only poorly in terms of responsible neuronal circuits as well as quantitative evaluation methods of the motor deficiency. Impairment of eye-blink conditioning in GFAP-deficient mice (482) and loss of VOR adaptation in PKC-inhibitor-transfected mice (118) appears to be a more reliable index for cerebellar dysfunction (see also sect. VI D6).

5. Adaptation in hand/arm movement

To throw balls of clay at a visual target while wearing wedge prism spectacles, normal subjects initially threw in
the direction of prism-bent gaze, but with 10–30 repeated throws adapted to hit the target. Patients with focal olivo-cerebellar lesions had impaired or absent prism adaptation, suggesting that climbing fibers play a role for this adaptation (361). Monkeys learn to reach and touch a target either with or without wearing the “learned” prism spectacles, thus acquiring and storing two gaze-reach calibrations (360). Martin et al. (360) found that, when the posterior cerebellar cortex was inactivated by lidocaine injections, the monkey failed to perform short-term adaptation to novel prisms and lost the learned prism adjustment. Motor memory may be located in the cerebellar cortex, or it may be accessed through the cerebellar cortex. The quick switching between the two gaze-reach calibrations raises an interesting possibility that a neural mechanism exists, probably outside of the cerebellum, for selecting an appropriate one out of a number of microzones specialized for the same movement but with different parameters (see sect. IV).

C. Functional Representation of PC Activities

The assumption that each microcomplex plays a unique functional role in neural control system functions is tested here based on the data obtained by recording simple spikes that are the major output signals of PCs.

1. HVI area in eye-blink conditioning

After training for eye-blink conditioning in rabbits, some PCs, mostly sampled from HVI lobule, exhibited a transient decrease of their simple spike discharges in response to the conditioning stimuli (50, 200, 518). This decrease occurred at about the time when anterior interpositus neurons discharged to induce conditioned responses. This observation can be explained based on the microcomplex model if there is a delay line mechanism converting the temporal pattern of the CS-induced activation in a set of MFs into a spatial pattern of GAs each of which discharges at varied time during the conditioned stimulation (387). Although these GAs converge onto a PC, LTD would depress only those GA-PC synapses conjunctively activated with the CF signals, and hence PCs would be deactivated around the time of unconditional stimulation. The possibility that such temporospatial conversion occurs in the brain stem is unlikely because single pulse stimuli directly given to MFs stimuli can reproduce eye-blink conditioning acquired by paired forelimb and periocular stimulation (202). A likely possibility is that the temporospatial conversion occurs in the MF-GA circuit including Golgi cells (156).

2. Flocculus in VOR adaptation

During sinusoidal whole body rotation of a rabbit or monkey in the horizontal plane, PCs in the microzone connected to the horizontal VOR (H zone) exhibited a significant modulation of simple spike discharges in relationship with the head velocity on the horizontal plane (166, 544). The pattern of simple spike discharges from rabbit flocculus PCs changes in parallel with adaptation of VOR in the manner that suggests a causal relationship between the former and the latter (128, 397, 544). Changes correlated with OKR adaptation were also found in PCs of the rabbit flocculus (396).

3. Ventral paraflocculus in ocular following

When a monkey moves its eyes following a movement of the whole visual field (ocular following), simple spike discharges in PCs of the ventral paraflocculus were modulated with a pattern, which matches well with the inverse dynamics of the eyeball (486). The inverse dynamics of the eyeball are represented by an equation that contains terms of eye acceleration, eye velocity, and eye position. This observation can be explained based on the control system design of Figure 9B. It was also found that the simple spike modulation was a mirror image of the pattern of complex spike discharges (290). Apparently, the inverse dynamics pattern of simple spike modulation is formed through LTD-based learning. The retinal slip caused by deviations between the movement of visual surroundings and that of the eyes contains information about the dynamics of the eyeball, which is conveyed by CFs to shape the behavior of PCs by LTD.

4. HV/HVI areas in arm movement

When a monkey makes a visuomotor arm tracking in a two-dimensional workspace, simple spike discharges in PCs sampled from intermediate and hemispheric cortex of V and VI (HV and HVI) were modulated in relation with direction, distance, or target location (154), most strongly at specific combinations of direction and speed, suggesting that they had a preferred velocity (97). These observations match the kinematics representation found with complex spikes (155) and are consistent with the hypothesis that the cerebellum forms a kinematics model of the arm (sect. vE).

5. Internal model for a new tool

A recent functional magnetic resonance imaging (fMRI) study on a human subject revealed a persistent change of local blood circulation in the cerebellum, which is presumed to represent an internal model postulated in Figures 10 and 11 (221). While a subject manipulated a computer mouse to follow a moving small square target with a small cross-hair cursor on a screen, the position of the cursor was rotated 120° around the center of the screen to provide a novel mouse condition. In the first session, large regions of the cerebellum were significantly
activated, but the extent of activation decreased during repeated test trials in parallel with a reduction in the tracking errors. Eventually, certain subregions (near the posterior superior fissure) continued to be activated. This remaining restricted activity may represent an internal model formed during the repeated test trials, which implies a novel relationship between the cursor movement and the mouse movement. Because the subject can switch between the old and novel mice quickly, this also suggests a neural mechanism for selecting an appropriate one out of many internal models for various mouse situations (see sects. vD3 and vB3).

When we consider how the activity detected by fMRI in the cerebellum is related to LTD, it should be realized that diverse chemical reactions underlie the induction of LTD, including the release of NO (sect. vA2), which has a well-known action of relaxing blood capillaries (231). Actually, the cerebral blood flow measured directly on the surface of the cerebellum, using laser-Doppler flowmetry, increased in relation with electric stimulation of PFs and was attenuated by application of a NOS inhibitor (14). Because picrotoxin did not affect the cerebral blood flow, inhibitory synaptic activity did not appear to contribute to the observed cerebral blood flow changes. Therefore, an increase of cerebral blood flow in the cerebellar cortex is likely to reflect an increased activity in GAs.

One may suppose that, at the beginning of motor learning, the cerebral cortex sends MF signals via pontine nuclei widely to the cerebellum so that GAs are activated in a wide area of the cerebellum, as occurs in the early phase of learning (221). In this early phase, there could be little output from cerebellar nuclei because of dominant PC inhibition. When a microcomplex receives error signals conveyed by CFs during repeated trials of the movement, LTD will develop in the microzone, and consequently its nuclear output will be released from PC inhibition. If the released nuclear output contributes to improve the movement, the cerebral cortex would reduce exploratory signals to the cerebellum, as represented by the subsidence of activation in the cerebellum (221). Then, how does GA activity grow and is maintained only in that particular microcomplex, as seen by Imanizu et al. (221)? The author pays a special attention to the neocortical projection (Fig. 6), which may act to maintain the high activity of GAs in the microzone connected to the nuclear neurons released from PC inhibition during learning (sect. vB2).

D. Consistency of LTD With Learning

While evidence accumulates in favor of the hypothesis that LTD is the core process of learning mechanisms in the cerebellum, as reviewed in section vi, A–C, a number of criticisms against the hypothesis and other different views have been presented. Even though it may require additional data before we clarify the entire situation for the microcomplex hypothesis on experimental bases, current major objections can be debated on the basis of already available data.

1. Timing between CF and GA signals

In actual motor control situations, it may happen that CF error signals arrive at PCs well after activation of GA-PC synapses so that no CF/GA conjunction could occur. However, the GA-CF temporal relationship for LTD induction is broadly tuned (88, 265), and LTD occurs even if CF signals reach PCs after GA signals (468, 538) (sect. vA4). A basis for explaining the “credit assignment” has been derived from knowledge of often long-lasting signal transduction processes underlying LTD (sect. vF). It has also become evident that CF error signals are derived not only from the final consequence of the operation of a neural system involving the microzone, but also from a prediction made within the central nervous system before completion of the operation (sect. vE).

2. Spontaneous GA and CF activities

Provided that each granule cell discharges spontaneously at 10–50 Hz, there will be a frequent chance of conjunctive activation of GAs with spontaneous CF impulses so that all GA synapses in a PC could be depressed in a relatively short time (336). However, LTD induction has a prominent frequency dependence (sect. vA4) so that it would not happen with slow, irregular background discharges of CFs.

3. Reciprocal modulation

Simple spikes and complex spikes are not always modulated reciprocally to each other, even though this is expected to occur after LTD-based learning (491, 492). Reciprocal modulation has been observed typically in monkey ventral paraflocculus during ocular following (290) and also in rabbit flocculus during head rotation in the light (122). However, part (11 of 43) of flocculus PCs tested by rotation in dark showed parallel modulation of simple and complex spikes instead of reciprocal modulation (122). It is not surprising because the simple spike modulation of flocculus PCs reflects a summed effect of excitation mediated by GAs and inhibition mediated by basket and stellate cells (379). Even if LTD depresses GA-PC synapses, the inhibition-dependent component of modulation will remain unchanged because LTD does not influence the MF-induced inhibition in PCs (241). Another seeming contradiction was that when PCs discharged complex spikes at the onset of monkey’s wrist tacking movements, a slightly more than one-half of the PCs also exhibited an increase of simple spike discharges (358).
Since, however, this experiment was made after the monkeys well learned the movement, the observed simple spike discharge might be a residue remaining after LTD had already shaped it from the original, probably larger response. It might not undergo a further depression in the postlearning phase where the complex spikes were driven by wrist tracking at a very low rate.

4. Natural stimulus-evoked CFs

Conjunction of natural stimulus-evoked simple spikes with spontaneously occurring complex spikes in cat cerebellum did not induce LTD (58). When a small flexion of a forepaw wrist was applied to evoke simple spike responses (either increase or decrease of the discharge rates) in PCs 20–70 ms after spontaneously arising complex spikes, the simple spikes exhibited a short-term facilitation rather than a long-lasting depression (131). However, slow, irregular spontaneous discharges of complex spikes and also the occurrence of inhibition in PCs after the forepaw flexion may account for the failure of LTD induction. It was also reported that when the forepaw flexion evoked complex spikes in combination with simples spikes, the simple spike responses were accentuated compared with those trials where no complex spikes were evoked (132). However, the effects of complex spikes upon simple spike responses were estimated by constructing poststimulus histograms during 39–53 trials, which do not seem to be sufficiently large in number for inducing LTD. The short-term facilitation observed in the simple spike responses combined with complex spikes was attributed to a change of responsiveness of PCs to MF inputs, but a possibility might remain that it was due to a change of transmission efficacy in the pathways relaying cutaneous and proprioceptive signals to PCs.

5. Opposing process to LTD

LTD may be incomplete as a mechanism for learning because of the lack of a known opposing process (336). Without such a process, all GA-PC synapses could eventually be depressed. The major assumption adopted in neuronal network models of the microcomplex is that those GA-PC synapses escaping conjunctive activation with CFs are potentiated (156), or that the total effect of GA-derived synapses in each PC is kept constant by non-specific occurrence of LTP (475). In reality, presynaptic LTP occurs in GA synapses activated without conjunction with CFs (sect. vC3). It counteracts LTD in terms of synaptic efficacy so that the combination of LTD and LTP provides a complete learning mechanism from computational viewpoints. Whether there is any mechanism that counteracts LTD at molecular levels is still an open question.

6. Discrepancy in motor discoordination

Behavioral disturbances observed in various types of gene-manipulated mice are not always correlated to LTD (336). Although lack of LTD is well associated with impairment of ocular movement adaptation (sect. viB) (230) and eye-blink conditioning (sect. viB2) (282, 366), LTD is less correlated with motor discoordination. LTD is present in two of the six types of gene-manipulated mice exhibiting motor discoordination and tested for LTD induction (Table 1). This may be because motor discoordination can be impaired by various causes, not only lack of LTD but also multiple CF innervation or abnormal MF-GA-PC transmission. However, the two cases in which motor coordination was indistinguishable from wild-type mice despite the lack of LTD (Table 1) are difficult to reconcile, and further investigation is required to find the reason for this discrepancy. It is necessary to identify neuronal circuits responsible for motor coordination and to introduce sensitive methods for evaluating motor disturbances under various test conditions. It is to be recalled that nNOS-deficient mice show no obvious motor discoordination during daytime, but they exhibit peculiar motor discoordination during the night (299).

7. Clock function of CFs

An alternative hypothetical role of the CFs is that they provide a clock for movement control (336, 341). This view is based on two lines of evidence that, under the influences of harmaline, complex spikes discharge rhythmically at a rate ~10 Hz (303, 340) and that, in slice conditions, inferior olive neurons exhibit a marked oscillation in membrane potentials (342). The clock hypothesis, however, is not consistent with the results of recording from PCs in awake behaving monkeys, which did not reveal clocklike discharge patterns of complex spikes (277, 278). A computer simulation study suggests an interesting possibility that electrical coupling between inferior olive neurons through gap junction acts to either synchronize or desynchronize coupled inferior olive cells depending on the coupling strength (474).

8. CF activity and licking

Licking is skilled tongue movements repeated in a form of a series of rhythmic trains. In a certain area of rat cerebellar cortex, PCs were found to discharge complex spikes rhythmically and time-locked to movement at about the time when the tongue was fully extended, some PCs firing in synchrony with each other (550). These complex spikes do not represent consequences of movement, since they are unaffected by deafferentation of the oral or perioral structures. Welsh et al. (550) suggest that the complex spike activities are transferred to cerebellar nuclear neurons via PC axons and eventually aid tongue
motoneurons that execute movement. Another possibility may remain, however, that complex spikes reflect discrepancies between instructions for licking movements and the internal feedback, as appears to be the cases in perturbed locomotion (sect. VI A4) and hand/arm movement (sect. VI A5).

9. Protective action of LTD

LTD may function for avoiding overexcitation of PCs (113) or Ca^{2+}-mediated excitotoxicity (336). These possibilities are, however, not exclusive to the memory and learning function considered in this review.

VII. CONCLUSION

Owing to the great efforts made during the past two decades, our understanding of the mechanisms of LTD and its functional roles has remarkably deepened. However, more data need to be accumulated for testing hypotheses, and hypotheses need to be more refined to match the actual data. Toward the final goal of understanding learning mechanisms of the cerebellum, the following questions are to be addressed.

How is LTD eventually converted to permanent memory? Efforts are required to extend the observation time for LTD further (sect. VI D) to reveal persistent changes in either molecular or cellular structures at the synaptic sites. Investigation of gene regulatory mechanisms for LTD will be of essential importance. A possibility exists that LTD as functional depression is consolidated as a structural change in synaptic contacts and spines. Even though evidence is available for the dependence of spine density in PC dendrites on afferent input activities (67), there is no evidence yet showing morphological changes correlated to LTD. The functional architecture of the microcomplex including accessory circuits also needs to be analyzed further to understand the operational principles of the entire cerebellar circuits.

Can the microcomplex concept be expanded to apply beyond motor learning to implicit learning in general? It has been applied to various physical functions including reflexes, compound movements (such as locomotion and saccade), animal behavior, and voluntary movement, and an obvious next target of cerebellar research is to determine the cerebellar contribution to certain mental functions such as cognition, language, and thought, as has been suggested based on an analogy with movement (229, 233). Studies on human subjects are becoming increasingly important, and requirements for new research technologies applicable to humans will greatly increase. A profound central problem in these studies is to find out, both experimentally and theoretically, how internal models for mental functions are formed and represented within the elaborate neuronal networks of the cerebellum after error-driven LTD-based learning.

The two research directions, one to extend LTD studies to reveal memory mechanisms of the cerebellum and the other to extend from motor learning to implicit learning in general, are mutually dependent and should be promoted together. Future studies of the cerebellum are thus expected to lead us to understanding of the entire mechanisms and functional roles of the cerebellum in implicit memory and learning in general, which in fact govern a large part of our life.

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