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Roles of Phospholipase Cβ4 in Synapse Elimination and Plasticity in Developing and Mature Cerebellum

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Abstract

The β isoforms of phospholipase C (PLCβs) are thought to mediate signals from metabotropic glutamate receptor subtype 1 (mGluR1) that is crucial for the modulation of synaptic transmission and plasticity. Among four PLCβ isoforms, PLCβ4 is one of the two major isoforms expressed in cerebellar Purkinje cells. The authors have studied the roles of PLCβ4 by analyzing PLCβ4 knockout mice, which are viable, but exhibit locomotor ataxia. Their cerebellar histology, parallel fiber synapse formation, and basic electrophysiology appear normal. However, developmental elimination of multiple climbing fiber innervation is clearly impaired in the rostral portion of the cerebellar vermis, where PLCβ4 mRNA is predominantly expressed in the wild-type mice. In the adult, long-term depression is deficient at parallel fiber to Purkinje cell synapses in the rostral cerebellum of the PLCβ4 knockout mice. The impairment of climbing fiber synapse elimination and the loss of long-term depression are similar to those seen in mice defective in mGluR1, Gαq, or protein kinase C. Thus, the authors' results strongly suggest that PLCβ4 is part of a signaling pathway, including the mGluR1, Gαq and protein kinase C, which is crucial for both climbing fiber synapse elimination in the developing cerebellum and long-term depression induction in the mature cerebellum.

Index Entries: Phospholipase Cβ; cerebellum; synapse elimination; plasticity; long-term depression.

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Introduction

Activation of phosphoinositide-specific phospholipase C (PLC) by heterotrimeric guanosine triphosphate-binding proteins (G proteins) is one of the major mechanisms whereby hormones, neurotransmitters, growth factors, and other biologically active molecules transduce signals *(1).* PLC catalizes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to produce two second messengers, diacylglycerol and inositol 1,4,5-trisphosphate (IP3). Diacylglycerol activates protein kinase C (PKC), together with Ca^{2+} *(2);* IP3 mobilizes Ca^{2+} from intracellular stores *(3).* Molecular cloning has identified three classes of PLC: PLCβ, PLCγ, and PLCδ. Among them, the β class of PLC, including four isoforms (PLCβ 1–4), has been shown to be activated by the Gq class of G proteins, including Gαq, Gα11, Gα14, Gα15, and Gα16 *(4).* The β4 isoform has been purified from bovine cerebellum *(5)* and retina *(6),* and is highly homologous to the NorpA protein that mediates the phototransduction cascade in *Drosophila* photoreceptors *(7).* PLCβ4 is localized in the photoreceptors, bipolar cells, horizontal cells, and ganglion cells in the bovine retina *(8).* A study on the PLCβ4 knockout mouse suggests that PLCβ4 plays a role in visual processing that occurs after the initial phototransduction cascade in rod outer segment *(9).*

Besides abundant expression in the retina, the mRNA for PLCβ4 has been reported to be localized in several limited regions of the central nervous system, among which the cerebellar Purkinje cells (PCs) have strong reactions *(10–12).* PCs have been shown to express high levels of metabotropic glutamate receptor subtype 1 (mGluR1) *(13,14),* which has been shown to stimulate PLCβ through activation of heterotrimeric G proteins of the Gαq/11 family *(15,16).* PCs are also rich in the γ isoform of PKC (PKCγ) *(17–19)* and IP3 receptors *(20),* both of which are activated after hydrolysis of PIP2 by PLCβs. Gαq and Gα11 are shown to be co-localized with mGluR1 in the PC dendritic spines *(21).* Therefore, signal transduction cascade from mGluR1 to PKCγ, and IP3 receptor activation, via Gαq and PLCβ4 very likely plays important roles for PC functions.

PCs receive two distinct types of excitatory inputs from parallel fibers and climbing fibers *(22).* Parallel fibers consist of the bifurcated axons of granule cells, and form synapses onto the dendritic spines of PCs. Each parallel fiber synapse is weak, but one PC is thought to receive synaptic inputs from approx 10^5 parallel fibers in rodents. In contrast, climbing fibers originate from the inferior olive, and form strong excitatory synapses on proximal dendrites of PCs. In an adult mouse, the majority of PCs are innervated by single climbing fibers. These two distinct types of excitatory synapses are generated and modified dynamically during postnatal (PN) development. During early PN rodent development, PCs are multiply-innervated by climbing fibers *(23).* At PN d 5 (PN5), 3–4 climbing fibers innervate single PCs. Thereafter, parallel fiber to PC synapses are continuously generated, concurrent with the migration of granule cells from the external granular layer to the internal granular layer. Massive elimination of supernumerary climbing fibers occurs during PN wk 2–3, until a one-to-one relation is attained at approx PN20. This relation is then maintained through adult life. The climbing fiber synapse elimination proceeds in multiple steps during PN cerebellar development. The final phase of climbing fiber synapse elimination appears to require mGluR1, Gαq, PLCβ4, and PKCγ. The authors have previously shown that the four strains of mutant mice lacking these respective genes have persistent multiple climbing fiber innervation into adulthood, because of a defect in climbing fiber synapse elimination during PN wk 3 *(24–28).*

In the mature cerebellum, parallel fiber to PC synapses exhibit long-term depression (LTD), when these synapses are activated repeatedly in conjunction with climbing fiber to PC synapses *(29,30).* LTD is thought to be a cellular substrate for motor learning in the cerebellum *(22,30,31).* Accumulated evidence suggests that

the induction of LTD requires activation of mGluR1 and PKC in PCs *(24,32–37).* Thus, the mGluR1 and the downstream signal transduction play key roles in both climbing fiber synapse elimination during development and LTD in the adult. This article reviews the authors' recent analyses on the cerebellum of PLCβ4 knockout mice *(27).* The data strongly suggest that PLCβ4 transduces signals within PCs from mGluR1, which are required for climbing fiber synapse elimination during PN wk 3 and induction of LTD in the adult.

Localization of PLCβ Isoforms in Cerebellum

The author examined regional differences in mRNA expression of the four PLCβ isoforms along rostrocaudal and mediolateral axes of the cerebellum *(12,27).* The PLCβ1 mRNA was detected at low levels in the PC somata, showing no apparent regional differences (Fig. 1A). Hybridization signals for PLCβ3 mRNA were restricted to the PC layer (Fig. 1B,E), exhibiting stronger signals in the caudal cerebellum than in the rostral cerebellum (Fig. 1B). The PLCβ4 mRNA was expressed in PCs and granule cells (Fig. 1C,F). In contrast to the PLCβ3 mRNA expression pattern, levels of PLCβ4 mRNA in PCs were stronger in the rostral than in the caudal cerebellum (Fig. 1C). The boundary for regions with PCs expressing high levels of PLCβ3 or PLCβ4 mRNA was in the middle of lobule 6 (Fig. 1B,C). The boundary does not coincide with the classical anatomical border, the primary fissure, but rather corresponds to the newly found cerebellar boundary in some mutant mice *(38),* or that determined by the staining patterns of biochemical markers, such as zebrins *(39).* The expression of PLCβ2 mRNA was much lower than mRNAs of other three isoforms. With longer exposure to X-ray film, weak signal of PLCβ2 mRNA was detected in the white matter *(12).*

Examination of coronal sections revealed a reciprocal expression pattern of PLCβ3 and PLCβ4 mRNA expression in the mediolateral cerebellar axis. In sections through the rostral cerebellum, the majority of PCs expressing high levels of PLCβ4 and low levels of PLCβ3 mRNAs formed thick parasagittal zones, which were interspersed periodically by 1–2 PCs with low PLCβ4 and high PLCβ3 mRNAs (Fig. 1E,F). These results indicate that PLCβ3 and PLCβ4 are the major isoforms in PCs. Each individual PC appears to strongly express either PLCβ4 or PLCβ3 mRNA, and, in addition, to weakly express PLCβ1 mRNA.

Behavior and Cerebellar Morphology of PLCβ4 Knockout Mouse

To examine the roles of PLCβ4 in cerebellar synapse formation and plasticity, the authors analyzed PLCβ4 knockout mice *(27,40).* The PLCβ4 mutant mice were viable and fertile, but exhibited various signs of ataxia, which became obvious 2–3 wk after birth *(27,41,42),* including general uncoordinated movements, body swaying while moving, ataxic gait, and intention tremors. These abnormalities are not caused by muscle weakness, because the mutant mice showed normal grasping responses. The duration of balancing on the rotating rod of the PLCβ4 mutant was significantly shorter than that of wild-type mice *(41).*

The cerebellum of the PLCβ4 mutant mice showed size and foliation similar to that of wild-type mice at 2 mo of age *(41).* No appreciable differences were found in the thickness of cortical layers and in granule cell density. In both strains of mice, cell bodies of PCs were aligned in a monolayer at similar intervals, and their dendritic structures were indistinguishable *(41).* The formation of parallel fiber to PC synapses appeared normal when examined by electron microscopy *(41).* The synapses were surrounded by cell processes of the Bergmann astroglia. The density of parallel fiber to PC synapses (the profile number 100 mm^2) was 21.3 ± 1.3 for the wild-type and 20.1 ± 1.7 for the mutant mice *(27,41).*

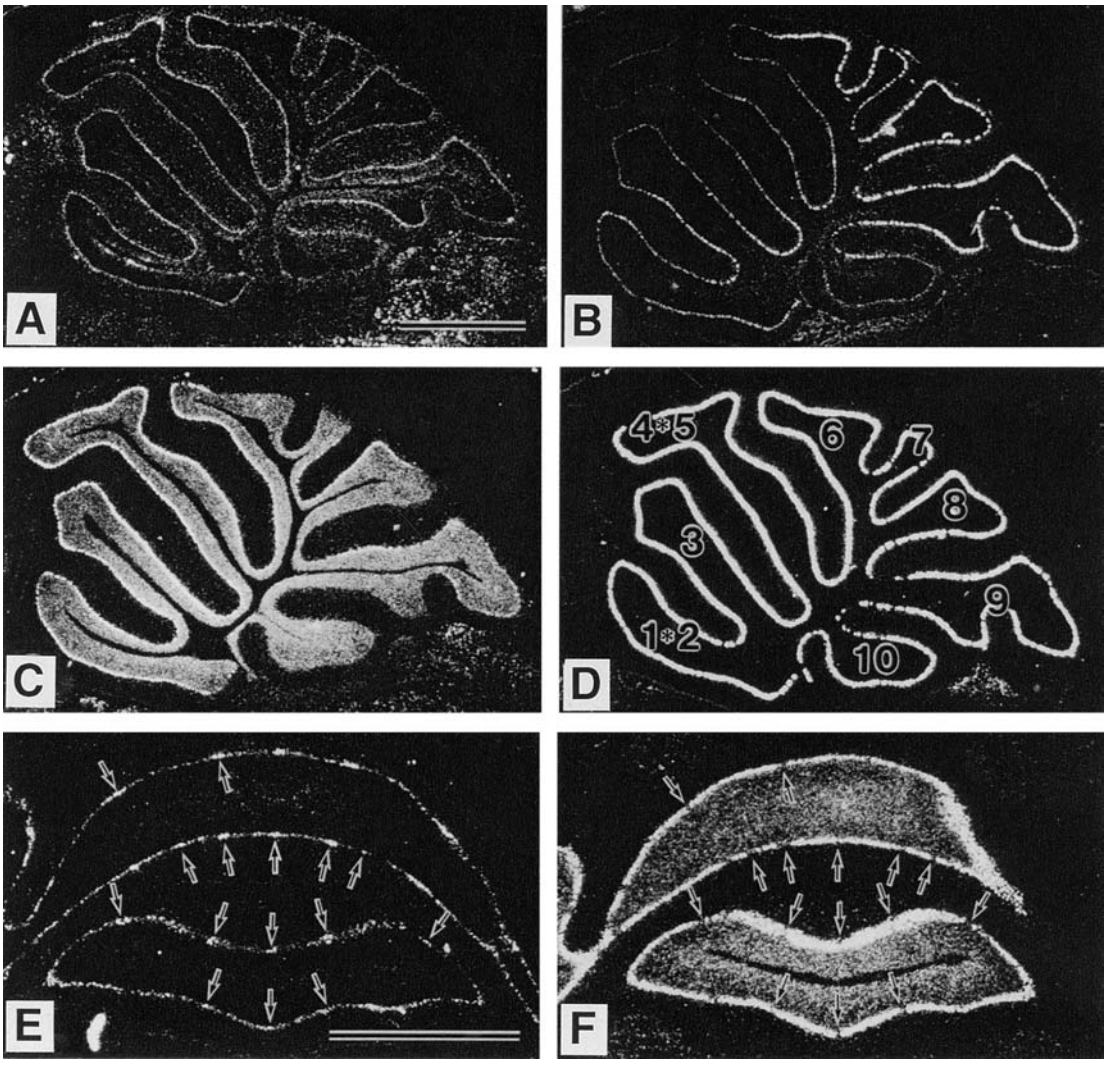

Fig. 1. Localization of PLCβ isoforms in the cerebellum. *In situ* hybridization showing expressions of the PLCβ1 **(A),** PLCβ3 **(B and E),** and PLCβ4 **(C and F)** mRNAs in the mature wild-type mouse cerebellum. **(A–D)** Dark-field micrographs taken from adjacent sagittal cerebellar sections near the midline. As a reference, cell bodies of PCs were visualized by detecting calbindin mRNAs in **D.** The numbers in **D** represent the cerebellar lobules in the vermis. **(E and F)** Dark-field micrographs taken from adjacent coronal sections through the rostral cerebellum. Note that locations of PCs with high PLCβ3 mRNA expression (arrows in **E**) correspond to those with low PLCβ4 mRNA expression in the adjacent section (arrows in **F**). Scale bar in **A** and **E:** 1 mm. (Modified from ref. *27.*)

PLCβ4 Is Required for Developmental Climbing Fiber Synapse Elimination

The authors made whole-cell recordings from visually identified PCs in parasagittal cerebellar slices *(43)* prepared from PN22–109 wild-type mice, or age-matched PLCβ4 mutant animals. Excitatory postsynaptic currents (EPSCs) were recorded, elicited by stimulating climbing fibers in the granule cell

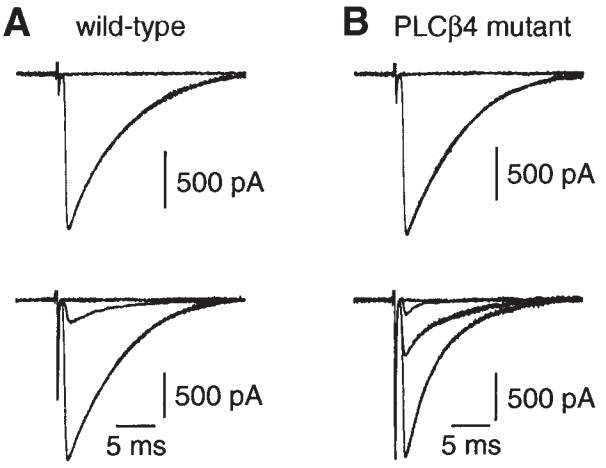

Fig. 2. Excitatory postsynaptic currents (EPSCs) elicited by stimulation of climbing fibers in wild-type **(A)** and PLCβ4 mutant **(B)** PCs. Records were taken from mice at P50 (**A,** upper), P80 (**A,** lower), P46 (**B,** upper) and P46 (**B,** lower). Stimuli were applied at 0.2 Hz. One or two traces are superimposed at each threshold intensity. Holding potential was –10mV. (Modified from ref. *27.*)

layer. The stimulation electrode was moved systematically in the granule cell layer. To search all climbing fibers innervating the recorded PC, the stimulus intensity was gradually increased at each stimulation site (pulse width 0.1 ms, strength 0–100V). When a climbing fiber was stimulated, a clearly discernible EPSC was elicited in an all-or-none fashion (e.g., Fig. 2A, upper traces). In some PCs, more than two discrete climbing fiber-induced EPSCs (CF-EPSCs) were elicited at one stimulus site, with different stimulus thresholds (e.g., Fig. 2A, lower traces). The number of climbing fibers innervating the recorded PC was estimated by the number of discrete CF-EPSC steps elicited in that PC *(25,26).* In wild-type PCs, 82.4 and 17.6% were innervated by one (e.g., Fig. 2A upper trace) and two climbing fibers (e.g., Fig. 2A lower trace), respectively. On the other hand, in PLCβ4 mutant PCs, 54.7% had single CF-EPSC steps (e.g., Fig. 2B, upper trace); 27.9, 15.1, and 2.3% had two, three, and four discrete steps, respectively (e.g., Fig. 2B, lower trace). In total, significantly higher percentages of PCs remain multiply-innervated in PLCβ4 mutant mice than in wild-type mice ($p < 0.01$, χ^2 test).

Because PCs expressing PLCβ4 were localized in the rostral cerebellum (Fig. 1), the authors looked for possible interlobular differences in climbing fiber innervation by plotting the recording sites of PCs on the standard sagittal plane. In wild-type mice, PCs with mono-innervation and those with multiple innervation were evenly distributed (Fig. 3A). By marked contrast, in PLCβ4 mutant mice, multiply-innervated PCs were much more numerous in the rostral half (lobule 1 to the rostral half of lobule 6) than in the caudal half (the caudal half of lobule 6 to lobule 10) of the cerebellum (Fig. 3B). The frequency distribution histogram, constructed from data in the rostral cerebellum (Fig. 3C), clearly shows that the difference between the wild-type mice and PLCβ4 mutant mice was highly significant ($p < 0.0001$, χ^2-test for independent samples). By marked contrast, in the caudal cerebellum, the frequency distribution of mono-innervated PLCβ4 mutant PCs was similar to that of wild-type PCs (Fig. 3D). These results indicate that PLCβ4 mutant mice have a defect in climbing fiber synapse elimination in the rostral half of the cerebellum, which exactly corresponds to the region where PLCβ4 is predominantly expressed in the wild-type mice (Fig. 1C).

The authors then followed the developmental course of climbing fiber innervation in the rostral and caudal cerebellum during the first three PN weeks. During the PN wk 1 (PN1–7), the majority of PCs in the rostral cerebellum were multiply-innervated by more than four climbing fibers in both wild-type and PLCβ4 mutant mice (Fig. 4A). The majority of PCs in the caudal cerebellum were also multiply-innervated, but by a generally smaller number of climbing fibers than those in the rostral cerebellum (Fig. 4B). This may reflect the gen-

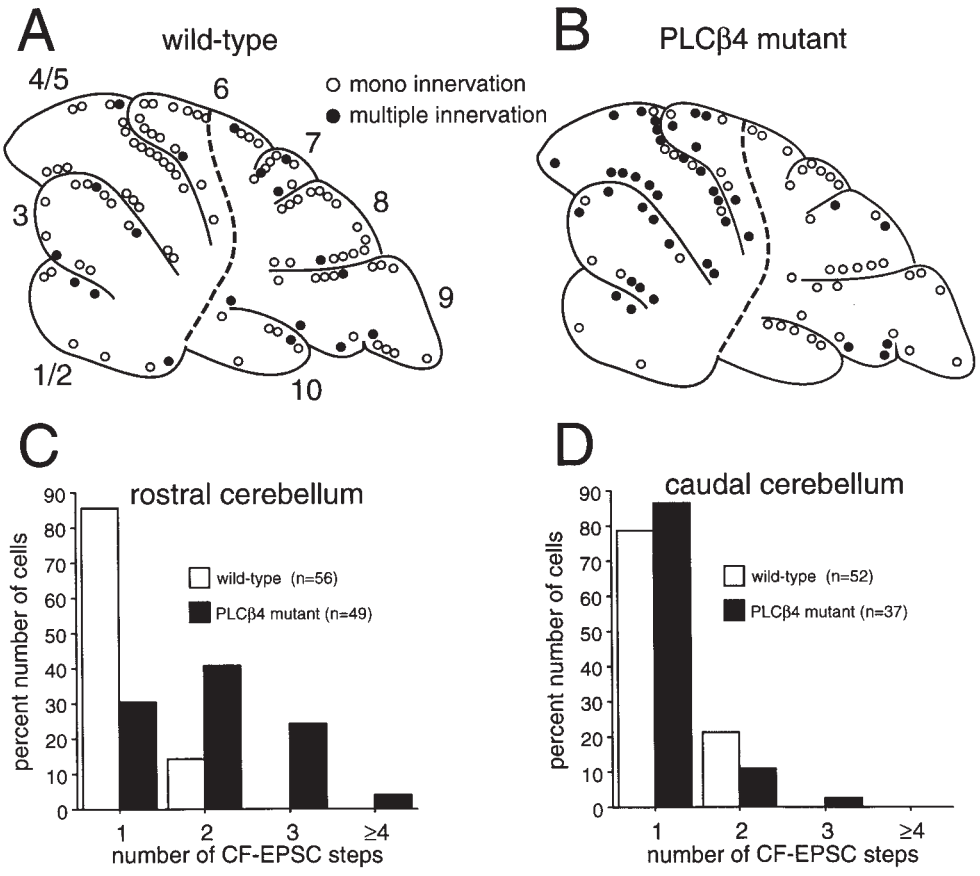

Fig. 3. Persistent multiple climbing fiber innervation of PCs in mature PLCβ4 mutant cerebellum. (**A** and **B**) Spatial distribution of mono-innervated (open circles) and multiply-innervated (filled circles) PCs of the wild-type **(C)** and PLCβ4 mutant **(D)** cerebella. Recording sites, obtained from 6 wild-type (n = 108 cells) and 6 PLCβ4 mutant (n = 86 cells) cerebella, were plotted on the standard midsagittal plane of the vermis. The broken line indicates the demarcation of the border of the rostral and caudal cerebellum, which corresponds to the border of the two cerebellar regions in terms of PLCβ4 and PLCβ3 mRNA expression patterns (*see* Fig. 1). (**C** and **D**)Summary histograms showing the number of discrete steps of CF-EPSCs of the wild-type (open columns) and PLCβ4 mutant (closed columns) PCs sampled in the rostral **(C)** and caudal **(D)** cerebellum. Data obtained from mice at PN35–55. The wild-type and mutant PCs were almost all analyzed before knowledge of the mouse genotype was revealed. (Modified from ref. *27*.)

eral tendency of the PN cerebellar development for the caudal lobules to mature earlier than the rostral lobules. During PN wk 2 (PN8–14), the percentage of multiply-innervated PCs decreased markedly in both wild-type and PLCβ4 mutant mice in the rostral (Fig. 4C) and caudal (Fig. 4D) cerebellum. During PN wk 1 and 2, the frequency distributions of the number of CF-EPSC steps per PC were not significantly different between the wild-type and the PLCβ4 mutant mice, in both rostral and caudal cerebellum ($p > 0.05$, χ^2 test). During PN wk 3 (PN15–21), clear differences between the rostral and caudal cerebellum became obvious. In the rostral cerebellum, a significantly higher percentage

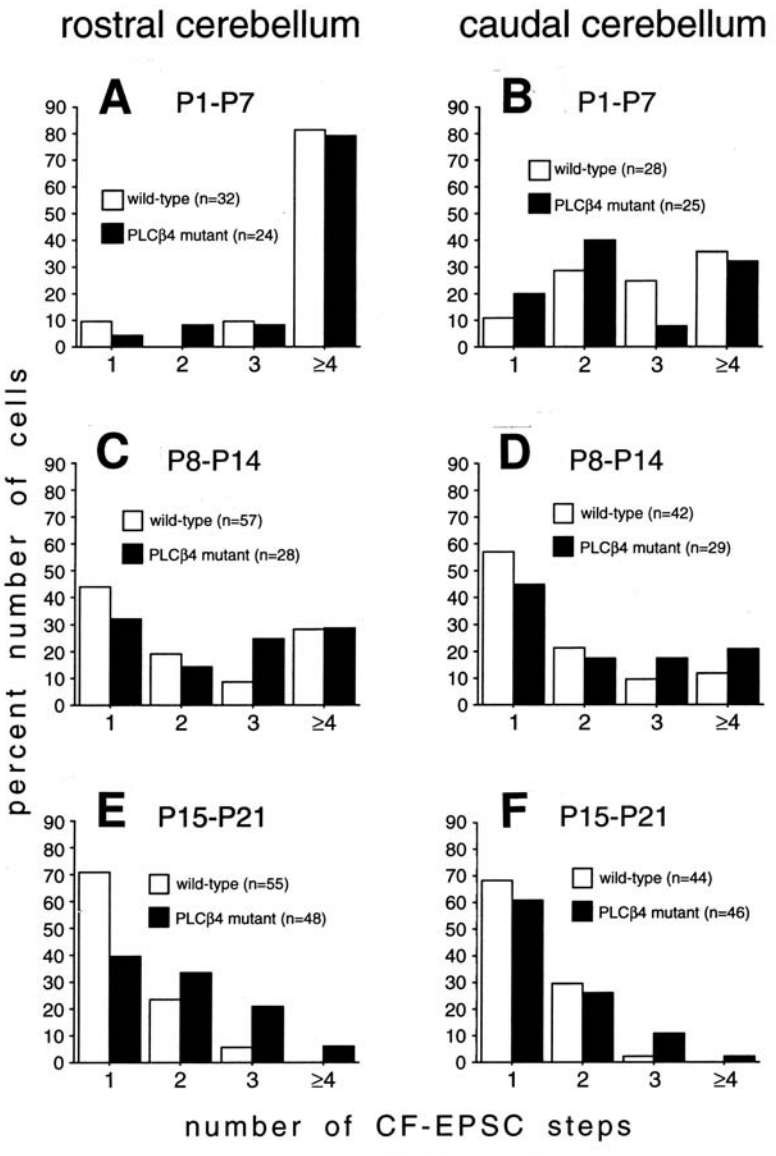

Fig. 4. Early PN development of climbing fiber innervation. (**A** and **B**) Frequency distribution histogram for PCs in the rostral **(A)** and caudal **(B)** cerebellum from four wild-type and three PLCβ4 mutant mice at PN1–7. (**C** and **D**) Similar to **A** and **B,** but for data from six wild-type and four PLCβ4 mutant mice at PN8–14. (**E** and **F**) Similar to **A** and **B,** but for data from six wild-type and seven PLCβ4 mutant mice at PN15–21. Most PCs in **A** and **B,** and all cells in **C–F,** were studied blind to the mouse genotype. (Modified from ref. *27*.)

of PCs remain multiply-innervated by climbing fibers in PLCβ4 mutant mice than in wild-type mice ($p < 0.01$, χ^2 test; Fig. 4E). By contrast, in the caudal cerebellum, the frequency distributions were not significantly different between the two strains of mice (Fig. 4F). These results indicate that the synapse elimination process that occurs during PN wk 3 is specifically impaired in the rostral cerebellum of the PLCβ4 mutant mice.

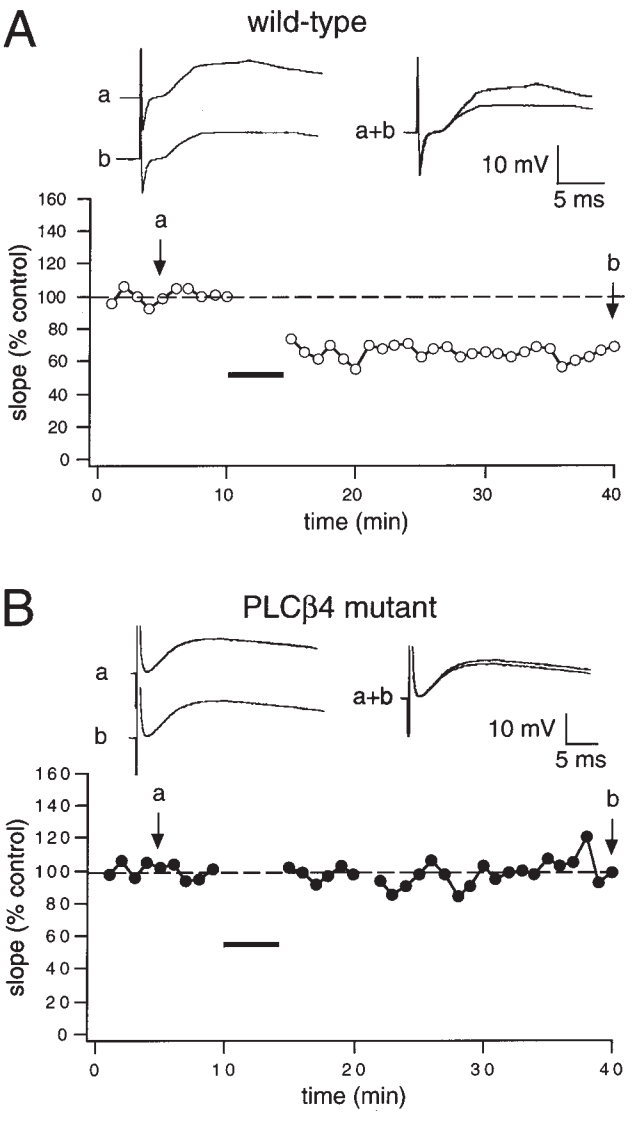

Fig. 5. LTD is absent in the rostral cerebellum of PLCβ4 mutant mice. **(A)** Conjunctive parallel fiber and climbing fiber stimulation (horizontal bar) resulted in LTD of the PF-EPSP initial slopes of a wild-type PC. Traces of PF-EPSPs were obtained before (a) and 30 minutes after the initiation of the conjunctive stimulation (b). These traces are superimposed on the upper right (a+b). Reduction in the latter EPSP is caused by LTD. **(B)** Traces of PF-EPSPs and time-course of changes in PF-EPSP initial slopes of a PLCβ4 mutant PC, showing an absence of LTD.

PLCβ4 Is Required for LTD Induction in Mature Cerebellum

The authors next asked whether PLCβ4 is involved in the induction of LTD at parallel fiber to PC synapses of mature cerebellum. For monitoring LTD, intracellular recordings were made from proximal dendrites of PCs in parasagittal slices, using a sharp glass microelectrode *(44,45).* PCs were sampled in the rostral cerebellum of the wild-type and PLCβ4 mutant mice. In the rostral portion of the wild-type cerebellum, LTD was readily induced by simultaneous activation (1 Hz for 5 min) of parallel fiber and climbing fiber synapses (Fig. 5A), a protocol that is optimal for inducing LTD *(44,45).* 30 min after the onset of this stimulation, the mean slope of excitatory postsynaptic potentials, elicited by parallel fiber stimulation, was depressed to 60.9 ± 5.6% (mean ± SEM; $n = 9$) of the control value measured prior to conjunctive stimulation. In the rostral portion of the PLCβ4 mutant cerebellum, the same conjunctive parallel fiber and climbing fiber stimulation paradigm did not induce LTD (Fig. 5B). In these experiments, special care was taken to sample PCs with full-sized complex spikes in response to climbing fiber stimulation, because ~60% of PCs in the rostral cerebellum of mature PLCβ4 mutant mice were multiply-innervated by climbing fibers (Fig. 3B,C). The mean value of excitatory postsynaptic potentials slope was not depressed (116.1 ± 12.0%; $n = 6$) when measured 30 min after paired synaptic stimulation. Furthermore, in preliminary experiments, LTD was readily induced in PLCβ4 mutant mice, when PCs were recorded in the caudal portion of the cerebellum. These results strongly suggest that PLCβ4 is essential for LTD induction in the rostral cerebellum, where the majority of PCs express PLCβ4 in the wild-type mice (Fig. 1C).

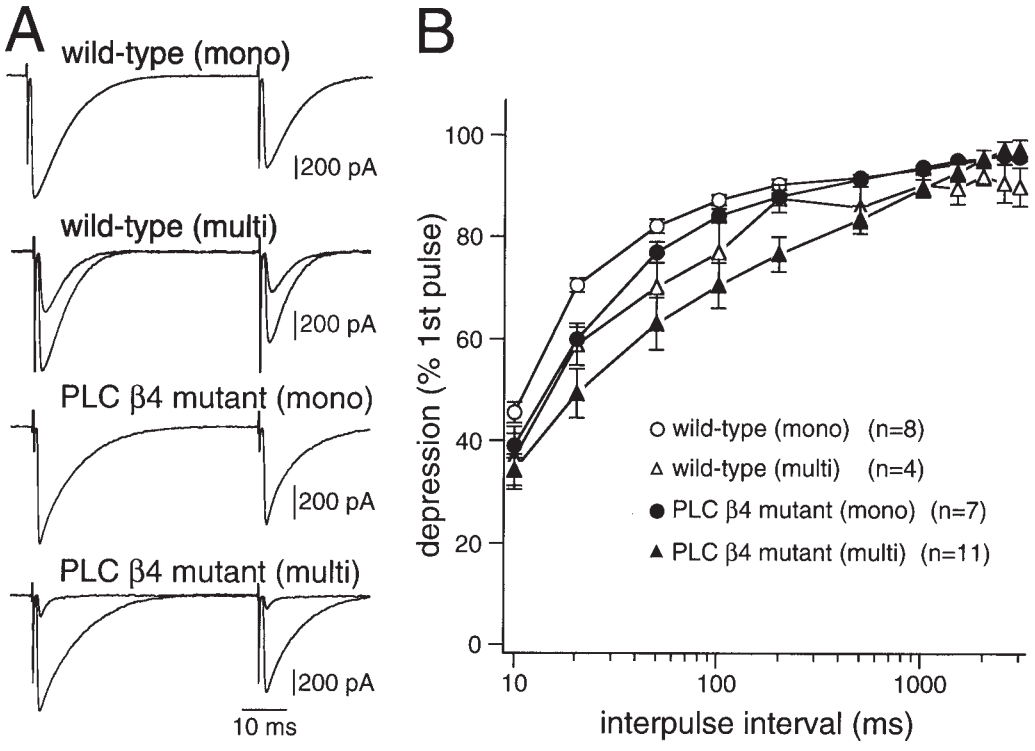

Fig. 6. Paired-pulse depression of CF-EPSCs in the rostral cerebellum. **(A)** Examples of CF-EPSCs in response to pairs of stimuli separated by 50 ms, recorded from PCs sampled in the rostral cerebellum. Average of three consecutive traces. Stimulus pairs were applied at 0.2 Hz. Holding potential was –10mV for all records. **(B)** Summary graphs showing paired-pulse depression of CF-EPSCs in the wild-type mono-innervated (open circles), wild-type multiply-innervated (open triangles), mutant mono-innervated (filled circles), and mutant multiply-innervated (filled triangles) PCs which were sampled in the rostral cerebellum from mice at PN35–55. The second response is expressed as percentage of response to the first pulse, and is plotted as a function of interpulse intervals. Each point represents mean ± SEM. Stimulus pairs were applied at 0.2 Hz. (Modified from ref. *27*.)

Basic Properties of Excitatory Synaptic Transmission of PLCβ4 Mutant Cerebellum

The authors found no significant differences in the rostral and caudal cerebellum, in basic electrophysiological properties of CF-EPSCs in the wild-type and PLCβ4 mutant mice. The 10–90% rise time or the decay time-constants were similar in the wild-type and PLCβ4 mutant mice. CF-EPSCs showed prominent paired-pulse depression in both mono-innervated and multiply-innervated PCs in the rostral cerebella of wild-type and PLCβ4 mutant mice (Fig. 6A). At varying interpulse intervals, the magnitudes of paired-pulse depression were mostly identical between the wild-type mono-innervated and PLCβ4 mutant mono-innervated PCs

(Fig. 6B). In both strains of mice, the magnitudes of paired-pulse depression of multiply-innervated PCs tend to be slightly larger than those of mono-innervated PCs (Fig. 6B). There was no significant difference between wild-type and PLCβ4 mutant mice in the caudal cerebellum (data not shown). These results suggest that climbing fiber presynaptic terminals of PLCβ4 mutant mice have properties mostly similar to those of the wild-type mice. In both the rostral and caudal cerebellum, the current-voltage relations were linear in both mono-innervated and multiply-innervated PCs derived from the two strains of mice (data not shown). CF-EPSCs in the wild-type and PLCβ4 mutant mice were not affected by AP5 (100 μ*M*), but were totally suppressed by CNQX (10 μ*M*) (data not shown). These results suggest that, in both the rostral and caudal cerebellum, climbing fiber to PC synapses in the PLCβ4 mutant mice are functional, and their electrophysiological properties are mostly similar to the wild-type mice.

The nature of EPSCs elicited by stimulation of parallel fibers was examined in mice at PN27–50. There was no significant difference between wild-type and PLCβ4 mutant mice in kinetics of parallel fiber-induced EPSCs (PF-EPSCs). Furthermore, in both wild-type and PLCβ4 mutant PCs, PF-EPSCs were little affected by AP5 (100 μ*M*), but were totally suppressed by CNQX (10 μ*M*) (data not shown). These results indicate that PF-EPSCs are mediated exclusively by non-N-methyl-D-aspartate receptors (NMDAR). Moreover, in both strains of mice, PF-EPSCs displayed similar paired-pulse facilitation at varying interpulse intervals from 10 to 300 ms. Taken together, these results suggest that the PLCβ4 deficiency had no detectable effect on the pre- or postsynaptic properties of the parallel fiber synapse. Therefore, the authors conclude that impairment in climbing fiber synapse elimination and loss of LTD in PLCβ4 mutant mice are caused by impaired PLCβ4-mediated signal transduction, rather than any change in basic synaptic properties.

Connection to mGluR1 Cascade

Results have demonstrated that PLCβ4 is required for climbing fiber synapse elimination in the rostral cerebellum during PN wk 3 *(27,41).* PLCβ4 is the major PLCβ isoform in the majority of PCs in the rostral cerebellum *(12,27).* PLCβ4 is thought to be activated at the downstream from mGluR1 in PCs. A number of knockout mice with defects in mGluR1, or in its downstream signal transduction pathway, also exhibit defects in elimination of climbing fiber synapses. Similar to the PLCβ4 mutant mice, adult mice defective in mGluR1 *(26,46),* Gαq *(28),* or PKCγ *(25)* all exhibit persistent multiple climbing fiber innervation, despite the normal formation and function of the parallel fiber–PC synapse. The author's recent results clearly indicate that mGluR1 and its downstream cascade must work in PCs, because the defects in mGluR1 knockout mice are restored by introducing mGluR1α transgene into their PCs under the control of a PC-specific promoter *(24).* Electrophysiological evidence indicates that mGluR1 is activated at parallel fiber–PC synapses *(47,48).* Rabbacchi et al. *(49)* found that the blockade of NMDARs in the rat cerebellum during the PN period impairs climbing fiber synapse elimination. The authors have also reported, in mice, that climbing fiber elimination process during PN wk 3 requires activation of NMDARs *(50).* NMDARs have no contribution to either PF- or CF-EPSCs in PCs, but play significant roles in mossy fiber-granule cell excitatory transmission *(50).* These lines of experimental evidence suggest that neural activity along the mossy fiber – granule cell – parallel fiber pathway activates mGluR1 and its downstream cascade in PCs, which is required for the climbing fiber synapse elimination during PN wk 3.

Climbing fiber synapse elimination proceeds in multiple steps that may involve distinct molecular mechanisms. The critical period for X-irradiation to cause persistent multiple climbing fiber innervation in rat is reported to be from PN4 to PN7 *(51,52).* The X-irradiation during this period suppresses the synapse elimination after PN8 in the rat *(53).* Kakizawa et al. *(50)* showed that an NMDAR antagonist, MK-801, had no effect on regression of multiple climbing fiber innervation, when applied from PN7 to PN14. In contrast, MK-801 caused persistent multiple innervation into adulthood, when applied from PN15 to PN16 *(50).* These lines of evidence suggest that the elimination of climbing fiber synapses occurs in at least three distinct phases: an early phase during PN wk 1 (in the rat), which does not depends on granule cell genesis; a second phase during PN wk 2 which is independent of NMDAR-mediated neural activity but dependent on granule cell genesis; and a third phase during PN15–16, which depends on NMDAR-mediated neural activity. The signaling cascades, mediating the first and the second phases, remain to be elucidated in future studies.

In the caudal half of the cerebellar vermis, the developmental climbing fiber elimination normally occurs in PLCβ4 mutant mice. Here, the expression of PLCβ4 mRNA is low, and that of PLCβ3 mRNA is high. Biochemical data indicate that PLCβ3 and PLCβ4 are indistinguishably activated by Gαq and Gα11 *(2,54),* although PLCβ3, but not PLCβ4, is activated by βγ subunits *(55,56).* Conceivably, therefore, PLCβ3, presumably together with PLCβ1, compensates for the lack of PLCβ4 function in the caudal cerebellum.

In the adult cerebellum, PLCβ4 is involved in the induction of LTD at parallel fiber to PC synapses. The signaling pathway involves mGluR1 and Gαq, because LTD is deficient in adult mice defective in mGluR1 *(32,33)* or Gαq (Miyata et al., unpublished). LTD is present in PKCγ knockout mice *(57),* presumably because of some compensatory mechanisms, but clearly, LTD requires activation of PKC, because pharmacological and genetic inactivation of PKC blocks LTD induction *(34,36).* In addition, several groups report that IP3-mediated Ca^{2+} release is required for LTD induction *(58,59).* The authors recent results suggest that IP3 must release Ca^{2+} locally, within the dendritic spines of parallel fiber – PC synapses, to induce LTD *(44).* The mGluR1 and its downstream cascade must work in PCs, because the loss of LTD in mGluR1 knockout mice was restored by introducing mGluR1α transgene into their PCs *(24).* Thus, the same signaling pathway involving PLCβ4 plays key roles in two different aspects of synaptic events in the cerebellum: climbing fiber synapse elimination during PN development and LTD in the adult.

Climbing fiber to PC synapse reportedly undergoes LTD after repetitive activation of climbing fibers (5 Hz for 30 s) in young rats aged 14–26 d *(60).* Activation of mGluR1, as well as elevation of intracellular Ca^{2+}, is required for the climbing fiber LTD *(60).* It is, therefore, possible that PLCβ4 may play a role in the rostral cerebellum for the induction of climbing fiber LTD. It is also known that climbing fibers are capable of remarkable structural plasticity in the adult *(61),* e.g., climbing fibers retract when their target PCs are deleted or their activities are suppressed by tetrodotoxin. Conversely, entire terminal arbors of climbing fibers grow when extra postsynaptic neurons are available. It is of interest to examine whether the mGluR1 cascade involving PLCβs plays any role in the structural plasticity of climbing fiber synapses.

Note added in proof: the original paper showing the dependency of cerebellar LTD in $PLC_{\beta 4}$ mutant mice has been published *(62).*

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