Molecular Neurobiology

ISSN0893-7648/06/33(1): 001–015/$30.00

Cerebellar Regulation Mechanisms Learned From Studies on GluRδ2

Tomoo Hirano*

Department of Biophysics, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan and CREST, Japan Science and Technology Corporation, Kawaguchi 332-0012, Japan

Abstract

The amino acid sequence suggests that glutamate receptor δ2 (GluRδ2) belongs to an ionotropic GluR (iGluR) subunit family. However, neither the direct binding to glutamate nor the incorporation into any native iGluRs has been demonstrated. One prominent feature of GluRδ2 is its predominant expression at parallel fiber–Purkinje cell synapses in the cerebellum. Knockdown or knockout of GluRδ2 impairs synaptic plasticity, stabilization, elimination, motor control, and learning. Therefore, GluRδ2 plays a crucial role in the cerebellar function. Several ataxic spontaneous mutant mice have defects in the *GluRδ2* gene. Numerous proteins interacting with GluRδ2 have been identified. Recent in vivo studies on GluRδ2 knockout mice shed light on the mechanism by which GluRδ2 deficiency causes ataxia and unveiled some secondary influence of the GluRδ2 deficiency on the function of the central nervous system. Studies on GluRδ2 might provide unique clues regarding not only the molecular mechanism of synaptic regulations but also the functioning mechanism of the entire cerebellar system.

Index Entries: Glutamate receptor; cerebellum; Purkinje cell; synaptic plasticity; synapse formation; motor control; learning; memory.

Introduction

At most excitatory synapses in the central nervous system (CNS), glutamate is used as a neurotransmitter. Glutamate receptors are classified into ionotropic and metabotropic receptors *(1).* The metabotropic receptors possess seven-transmembrane segments and bind to heterotrimeric G proteins. They regulate intracellular signaling cascades through G proteins and affect neuronal activities relatively slowly *(2).* The ionotropic glutamate receptor (iGluR) is a heterotetramer that possesses an ion channel pore in the center, in which the opening is

Received April 14, 2005; Accepted June 2, 2005.
*Author to whom correspondence and reprint requests should be addressed. E-mail: thirano@neurosci.biophys.kyoto-u.ac.jp

gated by glutamate binding. iGluRs have been classified into the following four subfamilies based on the sequence similarity, selective agonists, and antagonists: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainite, and δ-receptors. NMDA, AMPA, and kainate are selective agonists for each receptor family, although glutamate is regarded as a natural ligand. On the other hand, no agonist has been identified for δ-receptors. Eighteen complementary DNA (cDNA) for iGluR subunits have been cloned. Four are subunits for AMPA receptors, five are for kainate receptors, seven are for NMDA, and two (δ1 and δ2) are for δ-receptors.

Although glutamate receptor δ2 (GluRδ2) has been regarded as a subunit for iGluR, neither direct binding to glutamate nor incorporation into any native iGluRs has been demonstrated *(3,4).* The characteristic and intriguing feature of GluRδ2 is its selective expression in cerebellar Purkinje cells and developmentally related cells of the dorsal cochlear nucleus *(5–7).* However, GluRδ1 messenger RNA is expressed in several regions of the developing CNS *(4).* The first clue of GluRδ2 function was obtained by the knockdown study in culture.

The antisense oligonucleotide treatment of cultured Purkinje cells suppresses the induction of long-term depression (LTD; refs. *8* and *9*), a type of synaptic plasticity implicated in motor learning. Soon after, the GluRδ2 knockout mouse was generated *(10).* The mutant mice deficient in GluRδ2 show several interesting phenotypes, the impairment of LTD, destabilization of parallel fiber–Purkinje cell synapses, failure to eliminate redundant climbing fiber inputs, enhanced inhibitory synaptic transmission, motor discoordination, motor learning failure, and deficit in retention of cued fear memory *(10–15).* Numerous spontaneous mutant mouse lines with defects in the *GluRδ2* gene have been identified. They also show motor discoordination, indicating the critical role of GluRδ2 in motor control. Several molecules binding to GluRδ2 at the cytoplasmic C-terminus have been identified. The intermolecular interaction between GluRδ2 and these molecules in the postsynaptic density could be critical for the GluRδ2 function.

This article summarizes information about the sequence, structure, localization, and biochemical and physiological properties of GluRδ2. The article then describes and in vivo analyses on the GluRδ2 mutant mice. Several articles that are focused more on the structural and molecular level findings on GluRδ2 have been published elsewhere *(16,17).*

Sequence, Structure, Gene, and Expression

The rat and mouse GluRδ2 polypeptides predicted from cDNA sequences are about 1000 residues in length *(3,4).* The overall amino acid sequence homology between GluRδ2 and non-NMDA (-AMPA or -kainate) or NMDA receptors range from 22 to 28% and 7 to 20%, respectively. Therefore, GluRδ2 is almost equidistant from other iGluR subunit families. Based on the sequence similarity with other iGluR subunits and localization of hydrophobic regions, GluRδ2 has been suggested to possess a long extracellular N-terminus, three transmembrane segments (TM1, TM3, and TM4), a re-entrant loop segment (TM2) forming a wall of ion channel pore, and an intracellular C-terminus (Fig. 1).

The extracellular region of GluRδ 2 contains a leucine–isoleucine–valine-binding protein (LIVBP)-like domain and a lysine–arginine–ornitine-binding protein (LAOBP)-like domain *(16).* The LIVBP-like domain is formed by the distal region of N-terminus, and the LAOBP-like domain is composed of S1 formed by the membrane-proximal region of N-terminus and S2 formed by the extracellular region between TM3 and TM4 (Fig. 1). Both the LIVBP-like and the LAOBP-like domains are also found in other iGluR subunits. The LAOBP-like domain is responsible for glutamate binding. Mutagenesis studies on NMDA receptors and structural analysis on crystallized S1 and S2 domains in AMPA receptor

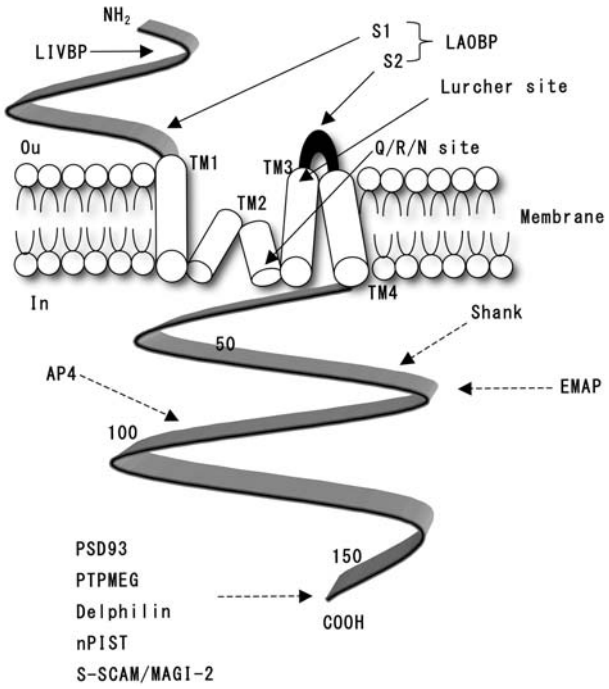

Fig. 1. Structure of GluRδ2 and molecules binding to the intracellular C-terminus. The leucine–isoleucine–valine-binding protein-like domain, lysine–arginine–ornitine-binding protein-like domain, Q/R/N site in TM2, *lurcher* mutation site in TM3 (arrows), and binding sites of proteins in the C-terminus (broken arrows) are indicated. Numbers indicate approximate positions of amino acid residues in the C-terminus.

subunits have identified critical residues for glutamate binding *(18–20).* These critical residues are conserved in GluRδ2. However, binding studies performed to date have failed to show glutamate-related ligands binding to GluRδ2 *(3,4,21).*

The asparagine (N) residue in TM2 of NMDA receptor subunits, or the glutamine (Q) or arginine (R) residue in AMPA or kainate receptor subunits corresponding to the N site, plays a critical role in Ca^{++} permeation *(22).* A negatively charged amino acid four residues downstream of Q/R/N sites is implicated in the channel block by intracellular polyamines *(23).* Therefore, TM2 has been suggested to constitute the wall of the ion channel pore. GluRδ2 also has Q and a glutamate (E) at corresponding sites.

Other data suggesting contribution of GluRδ2 to the channel formation has been obtained in *lurcher* mutant mice. The *lurcher* mouse, in which all Purkinje cells die during development, has a point mutation in the *GluRδ2* gene *(24).* In *lurcher,* the highly conserved alanine (A) residue in TM3 of GluRδ2 is replaced by threonine (T) (Fig. 1). The *lurcher* GluRδ2 forms a cationic channel that is open in the basal condition without binding to ligands *(24–28).* The ion flux through the mutated GluRδ2 channels might induce Purkinje cell death. Alternatively, the direct interaction of GluRδ2 C-terminus with the molecule implicated in autophagic pathway might play a critical role in cell death *(29).* The relatively long intracellular C-terminus is known to bind to several kinds of proteins (refs. *29–37;* Fig. 1). The motif that binds to class I PSD-95/Dlg/Zo-1 domain is located at the extreme C-terminus *(38).*

The *GluRδ2* gene, known as *Grid2,* is about 1.4 *M* basepairs long and contains 16 exons *(16).* This size is quite large compared with other iGluR subunit genes, which are around 200 K basepairs. GluRδ2 protein is highly expressed on the postsynaptic membrane at parallel fiber–Purkinje cell synapses (Fig. 2). Interestingly, GluRδ2 is not found at climbing fiber–Purkinje cell synapses in adult animals. Therefore, GluRδ2 expression is not only Purkinje cell-specific but is also restricted for parallel fiber–Purkinje cell synapse *(39–41).* Immunocytological studies have also shown that the proportion of cytoplasmic to membrane-bound GluRδ2 is relatively small among iGluR subunits, suggesting the efficient transport of GluRδ2 protein to the plasma membrane, in which the C-terminal juxtamembrane segment plays a role *(42,43).*

Long-Term Depression

LTD is the long-lasting decrease in synaptic transmission efficacy. At the parallel fiber–Purkinje cell synapse, stimulation of the presynaptic

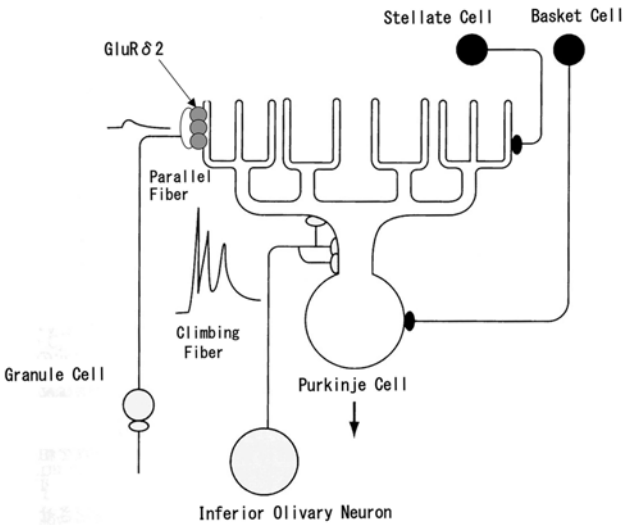

Fig. 2. Synapses on a cerebellar Purkinje cell. More than 100,000 parallel fibers and a climbing fiber form glutamatergic excitatory synapses. The latter input is much stronger than each of the former. The climbing fiber activation induces the characteristic complex spike, and activation of a parallel fiber induces a small excitatory postsynaptic potential. GluRδ2 is selectively expressed in the postsynaptic densities of parallel fiber–Purkinje cell synapses. Stellate and basket cells form γ-aminobutyric acidergic inhibitory synapses.

parallel fiber coupled with the climbing fiber activation induces LTD *(44–46).* This parallel fiber–Purkinje cell LTD has been considered as a cellular basis of motor learning *(47–49).* The climbing fiber stimulation can be replaced by direct postsynaptic depolarization of the Purkinje cell, and the parallel fiber stimulation can be replaced by direct application of transmitter glutamate to the Purkinje cell for the LTD induction *(50,51).* The necessity of an increase in intracellular Ca^{++} and activation of AMPA-type iGluR and metabotropic GluR (mGluR1) for LTD induction is known *(49,52,53).* mGluR1 activates phospholipase C and produces inositoltrisphosphate (IP)-3 and diacylglycerol through the Gq protein. IP-3 also works to increase the intracellular Ca^{++} concentration by inducing Ca^{++} release from the intracellular stores, and diacylglycerol works to activate protein kinase C (PKC) in concert with Ca^{++}. Phosphorylation of GluR2 (an AMPA-type iGluR subunit) by PKC facilitates unbinding of GluR2 from glutamate receptor interacting proteins and binding to proteins interacting with C-kinase-1, which induces the internalization of GluR2 containing iGluR, resulting in the induction of LTD *(54,55).* This is a prevailing scheme for the LTD expression.

Knockdown of GluRδ2 expression by an antisense oligonuceleotide in cultured Purkinje cells suppresses the induction of LTD *(8,9).* These results have been confirmed in the Purkinje cells prepared from GluRδ2 knockout mice *(10,56).* Hirai et al. have also reported the induction of a LTD-like phenomenon by treatment of the wild-type cultured Purkinje cells with an antibody that recognizes LAOBP-like extracellular domain of GluRδ2 *(57).* Application of the antibody induces the AMPA receptor endocytosis, attenuates parallel fiber–Purkinje cell synaptic transmission, and abrogates the LTD. These results might be interpreted to suggest that the antibody somehow affects the GluRδ2 activity by binding to the LAOBP-like domain, resulting in alteration of AMPA receptor trafficking implicated in the LTD.

A recent study in our laboratory demonstrated that exogenous expression of GluRδ2 in the knockout Purkinje cells by micro-injection of cDNA into the nucleus rescued the LTD induction, suggesting that GluRδ2 is directly implicated in the LTD (not through the secondary developmental alteration of Purkinje cells, unpublished observation). Presently, however, the mechanism by which GluRδ2 is involved in the induction of LTD is unresolved.

Rescue of LTD by GluRδ2 transgenic expression in the knockout mouse has also been reported *(58).* This study showed that the mutant GluRδ2 molecule with a mutation in the putative ligand-binding motif within the LAOBP-like domain also rescues the induction of LTD, suggesting that binding of a glutamate analog to GluRδ2 may be dispensable for the LTD. Implication of numbers of molecules not mentioned earlier (such as nitric oxide, PKG, mitogen-activated protein kinase, tyrosine kinase, phosphatase, corticotropin-releasing factor, and so on) in the LTD has also

Table 1
Mutant Mice With Defects in the Cerebellar Cortex

	LTD	CF	PF	Motor control	Motor learning	PC-specific
Knockout mice						
GluRδ2	X	X	X	X	X	○
mGluR1	X	X	○	X	X	X
PKCγ	△	X	○	△	○	X
Gαq	X	X		X		X
PLCβ4	X	X	○	X	X	X
IP-3R1	X	○		X		X
nNOS	X			○	X	X
PKG1	X	○	○	○	X	○
GFAP	X	○	○	○	X	X
Transgenic mice						
GluRδ2 rescue	○	○	○	○	○	○
mGluR1α rescue	○	○	○	○	○	○
PKC inhibitor	X	X	○	○	△	○

LTD, long-term depression; CF, climbing fiber innervation pattern; PF, parallel fiber–Purkinje cell synapses; GluRδ2, glutamate receptor δ2; mGluR1, metabotropic GluR1; PKC, protein kinase C; PLC, phospholipase C; IP, inositoltrisphosphate; nNOS, neuronal nitric oxide synthase; PKG, protein kinase G; GFAP, glial fibrillary acidic protein. X indicates defects such as LTD failure, multiple climbing fiber innervations, decrease in the number of synapses, motor discoordination, or motor learning deficits. ○ indicates no defect; △ indicates a slight alteration detected.

been reported *(49,59–64)*. Although there have been great advances in the study on molecular mechanism of cerebellar LTD, the precise role of each molecule and their respective mutual interactions have not been fully elucidated.

Synapse Formation and Stabilization at Parallel Fiber Synapses

The number of parallel fiber–Purkinje cell synapses is reduced to about one-half in GluRδ2 knockout mice. Electron microscopic observation has demonstrated that the length of postsynaptic density does not match that of the presynaptic active zone at some parallel fiber–Purkinje cell synapses in the GluRδ2 knockout mouse *(65–67)*. Another characteristic feature in a GluRδ2 knockout Purkinje cell is that there are many spines not contacted by presynaptic terminals. These results indicate that GluRδ2 somehow regulates synapse formation or stabilization and might suggest that GluRδ2 is implicated in the communication between pre- and postsynaptic elements. However, there are synapses that appear normal. Therefore, the precise role of GluRδ2 in synapse formation or stabilization is unclear.

Formation of Abnormal Climbing Fiber Synapses

In the adult wild-type cerebellum, proximal dendrites of a Purkinje neuron are innervated by a single climbing fiber. At an early stage of normal development, several climbing fibers form synapses on a Purkinje cell, but all climbing fibers—with the exception of one that forms the strongest connection—lose synaptic connections during development *(68)*. The adult GluRδ2 knockout mouse, as well as mGluR1, Gαq, PKC, or phospholipase Cβ knockout mice, shows multiple innervations on a Purkinje cell by climbing fibers (Table 1; refs. *10* and *69–75*). Therefore, in these mutant mice, the

synaptic elimination process of climbing fibers appears to be somewhat disturbed.

Notably, the pattern of multiple innervations in the GluRδ2 knockout mouse is slightly different from those in mGluR1 knockout mice *(69).* In GluRδ2 knockout mice, climbing fibers form synapses not only on proximal dendrites but also on distal dendrites, where only parallel fibers form synapses in the wild-type mice. Conversely, climbing fibers do not form synapses on distal dendrites in mGluR1 knockout mice. The climbing fiber innervations to distal dendrites in GluRδ2 knockout mice have been correlated with the scarcity of parallel fiber synapses *(70).* The authors reason that the climbing fibers invade into distal dendritic regions, because there are vacant spaces for synapse formation on distal dendrites of Purkinje cells in GluRδ2 knockout mice. Blocking neuronal activity with tetrodotoxin in the wild-type rat induces formation of new spines in the proximal dendrites, which are mainly innervated by parallel fibers. In this condition, all synapses, including those innervated by climbing fibers, bear GluRδ2 *(76).* Restoration of neuronal activity induces reinnervation of climbing fibers to proximal dendrites expressing GluRδ2 *(77).* These results suggest that GluRδ2 are expressed at the climbing fiber–Purkinje cell synapses at early stages and are then downregulated by the climbing fiber activity. Therefore, GluRδ2 might favor parallel fibers regarding synapse formation or stabilization. The Ca^{2+}-dependent regulation of GluRδ2 number on the plasma membrane by endocytosis has been reported *(78).*

Molecular Mechanism of Function

As discussed earlier, previous studies have failed to show glutamate binding to the GluRδ2 protein *(3,4,21).* However, the effect of an antibody recognizing the LAOBP-like domain of GluRδ2 on the synaptic function suggests that GluRδ2 could be regulated through ligand binding *(57).* Therefore, two questions arise: what is the ligand and what function is regulated by the ligand?

The GluRδ2 with *lurcher* mutation forms an ion channel, and the GluRδ2 amino acid sequence is homologous to other iGluR subunits *(24–28).* The heterologous expression study in cultured cells shows that GluRδ2 can form a heteromultimer with other iGluR subunits *(79),* suggesting that GluRδ2 protein functions as a component of ion channel. However, as mentioned earlier, no direct experimental data exist to support the contribution of GluRδ2 to the native iGluR channel formation in neurons.

The existence of unmatched synapses in the GluRδ2 knockout mouse *(67)* suggest that GluRδ2 is implicated in the cell–cell communication-like adhesion molecules such as cadherin, neuroligin or integrin or receptor-like molecules such as receptor tyrosine kinases or Notch *(80–86).* Therefore, the ligand for GluRδ2 could be not only a diffusible molecule but also a substrate-bound molecule. A molecule located on the presynaptic active zone could be a candidate. In such cases, GluRδ2 might function not as an iGluR component but as a structural component stabilizing synaptic structure or as a membrane-bound signal transducer regulating the postsynaptic intracellular molecular signaling. Notably, AMPA-type iGluR functions not only as a ligand-gated ion channel but also as a signaling molecule that influences protein tyrosine kinase activity *(86),* and the extracellular domain of GluR2 subunit is implicated in regulation of dendritic spine morphology *(87).*

Identification of molecules interacting with GluRδ2 could provide clues to clarify the functioning mechanism of GluRδ2. GluRδ2 binds to nPIST, PSD93, PTPMEG, Delphilin, and S-SCAM/MAGI-2 at the extreme C-terminus through PSD-95/Dlg/Zo-1 domains of these molecules (Fig. 1; refs. *29, 31–33,* and *37*). It also binds to Spectrin, Shank, AP4, and a microtubule-associated protein (EMAP) at intracellular C-terminal segments *(30,34–36).* These molecular interactions may be implicated in trafficking of GluRδ2 (AP4, S-SCAM/MAGI-2), its selective localization at the postsynaptic densities (PSD93, Delphilin), interaction with cytoskeletons (Spectrin, EMAP), and/or the

intracellular signal transduction (PTPMEG, nPIST). Colocalization of GluRδ2 with the monocarboxylate transporter MCT2 has also been demonstrated *(88).*

Motor Discoordination

GluRδ2 knockout mice show motor discoordination *(10,13).* Surprisingly, motor control ability of the knockout mouse is worse than the *lurcher* mouse, in which all Purkinje cells (the sole output neurons in the cerebellar cortex) are lost *(13).* Therefore, the dysfunction of the cerebellar system caused by GluRδ2 deficiency disturbs motor coordination more severely than the complete loss of cerebellar cortical outputs. Presumably, aberrant Purkinje cell activities in the knockout mouse disturb motor control. Involuntary spontaneous movement, which is caused by the enhanced aberrant climbing fiber influence on Purkinje cell activity in GluRδ2 knockout mice, has been suggested to be a cause of the severe motor discoordination *(13).*

The authors noticed the continuous spontaneous eye movement with a 10-Hz rhythm and found that the movement correlated with climbing fiber inputs on Purkinje cells in the flocculus, a cerebellar region implicated in the control of reflex eye movement such as vestibulo-ocular reflex (VOR) and optokinetic response (OKR; refs. *89* and *90*). The inferior olivary neurons sending climbing fibers have approx 10 Hz of intrinsic subthreshold oscillation of membrane potential *(91,92).*

In the GluRδ2 knockout mouse, multiple climbing fibers innervate a Purkinje cell, and the number of parallel fibers–Purkinje cell synapses is reduced *(10).* Yoshida et al. reasoned that these abnormalities in the synaptic organization should have increased the impact of climbing fiber outputs on the Purkinje cell activity *(13)*. They also noted that the LTD failure in the GluRδ2 mutant mouse was likely to affect motor learning. The learning deficit might have enhanced climbing fiber activity transmitting error signals *(49).* Therefore, they suggest that the enhanced rhythmic climbing fiber activity in GluRδ2 knockout mice induces the abnormal Purkinje cell activity with a 10-Hz rhythm, resulting in the involuntary surplus movement that disturbs the motor control. The same group also reported aberrant dynamic properties of reflex eye movements in a later study *(93).* Abnormally large gain in the VOR and large phase lag in the OKR were demonstrated. These properties should also disturb motor control, because such dynamics in the reflex eye movements should result in poor performance in fixating the visual image during head motion.

Motor Learning

Motor learning is impaired in GluRδ2 knockout mice. In studies, the mice have shown defects in the vestibular compensation, classical eye-blink conditioning, and adaptive modification of VOR and OKR *(11,12,93).* Vestibular compensation is the behavioral recovery from the symptoms caused by the unilateral destruction of vestibular organ. The destruction induces tilt of the body and head toward the damaged side, the tendency to rotate to that direction, and the decrease in VOR gain *(94).* GluRδ2 mutant mice show difficulty to stop rotation as well as slower recovery of VOR gain after the destruction *(11,95).* The downregulation of GluRδ2 messenger RNA expression in the vestibulo-cerebellum after unilateral labyrinthectomy also has been reported *(96).*

In the eye-blink conditioning, a conditioning stimulus (CS) such as sound is coupled with an unconditioned stimulus (US) such as air puff to the eye, which in itself induces eye blinking. After several couplings, mice begin to respond to the CS *(97).* There are two paradigms: delay and trace conditioning. In the former, an US starts before the end of a CS, and in the latter, there is an interval between the end of a CS and the start of an US. Therefore, trace conditioning is more difficult than delay conditioning. Unexpectedly, GluRδ2 knockout mice

show the defect in delay conditioning but not in trace conditioning. One explanation may be that the cerebellum plays a critical role in the delay paradigm, but in the trace paradigm, other CNS regions (such as the hippocampus) are essential *(12,98).*

The VOR and the OKR are compensatory reflex eye movements that prevent the external images from slipping across the retina, and both show adaptive modification *(89,90, 99–101).* The VOR is driven by head movement sensed by the vestibular organ. The signal is transmitted to the neurons in vestibular nuclei and then to the motor neurons of extra-occular muscles. The vestibular signals also go to the cerebellum, and the cerebellar outputs from Purkinje cells converge on neurons in the vestibular nuclei. Therefore, the cerebellar outputs regulate the amplitude and timing of the VOR. The dynamic properties of the VOR need to be continuously fine-tuned to prevent the retinal slip during head motion. This actually occurs in the wild-type animal. For example, if the head rotation is continuously coupled with the rotation of surrounding screen to the opposite direction, eye movement becomes larger to prevent the retinal slip. This phenomenon is called VOR adaptation and has been studied as a model of motor learning. This VOR adaptation does not occur in the GluRδ2 knockout mice *(93).*

In wild-type mice, OKR adaptation is reported *(102).* The motion of large visual fields drives the OKR. In the basal condition, eye movement is smaller than the movement of surrounding visual scene. Therefore, retinal slip exists. The continuous movement of the screen surrounding the wild-type mouse gradually increases the eye movement so that the retinal slip is reduced. This OKR adaptation does not occur in the GluRδ2 knockout mice *(93).*

The motor learning failures described earlier are consistent with the idea that the LTD is implicated in motor learning. However, other abnormalities in GluRδ2 knockout mice, such as the reduction in the number of parallel fiber–Purkinje cell synapses and multiple climbing fiber innervations to a Purkinje cell, could be responsible.

Direct and Indirect Effects of GluRδ2 Deficiency

Selective localization of GluRδ2 protein on the postsynaptic membrane at the parallel fiber–Purkinje cell synapse suggests that the major role of GluRδ2 is related to regulation of that synapse. Therefore, LTD regulation and stabilization of structure at the parallel fiber–Purkinje cell synapse may be directly controlled by GluRδ2. On the other hand, multiple climbing fiber innervations on a Purkinje cell appear to be caused indirectly. The reduction in the number of parallel fiber–Purkinje cell synapses on the distal dendrites could be a cause *(70).*

Additional change in the CNS caused indirectly by GluRδ2 deficiency has been reported. Ohtsuki et al. have demonstrated that inhibitory synaptic transmissions on Purkinje cells are enhanced in GluRδ2 knockout mice *(14).* It has been suggested that the influence of enhanced climbing fibers on a Purkinje cell in GluRδ2 knockout mice saturates rebound potentiation (RP)—the long-lasting increase in transmission efficacy (a form of synaptic plasticity) induced by postsynaptic depolarization at the inhibitory interneuron–Purkinje cell synapses *(103,104).* Therefore, in the cerebellar slices prepared from GluRδ2 knockout mice, the amplitudes of inhibitory postsynaptic currents (IPSCs) in Purkinje cells are large, and the RP cannot be induced. The cultured Purkinje cells prepared from the mutant mice show RP, suggesting that GluRδ2 is not directly implicated in the RP induction *(14).* It has also been suggested that during development, some indirect changes occur in the brain stem neuronal circuit controlling the VOR and the OKR in GluRδ2 knockout mice, because the dynamic properties of the VOR and the OKR after destruction of the flocculus (a cerebellar region regulating the VOR and the OKR) in the wild-type mice are different from those in GluRδ2 knockout mice *(93).*

Spontaneous Mutant With Defects in *Grid2*

There are numerous spontaneous mutant mice with defects in *Grid2,* the GluRδ2 gene. The *lurcher* mouse is one example. Other mutants are called *hotfoot* mice. Seventeen *hotfoot* mutant mice lines have been reported *(16,105).* Some *hotfoot* mice (*ho-Nancy, ho-5J, ho-tpr*) have null mutation similar to GluRδ2 knockout mice, and others have limited mutations. All these mice show motor discoordination. *ho-4J, ho-7J,* and *ho-11* have in-frame deletions in the extracellular N-terminus, which suppresses the export of the GluRδ2 protein from the endoplasmic reticulum *(106,107).*

Comparison to Other Cerebellar Mutant Mice

There are several mutant mice lines showing the LTD failure, multiple climbing fiber innervations on a Purkinje cell, motor discoordination, and/or motor learning deficits (Table 1), such as mGluR1 knockout mice, which show all of these *(108,109).* However, no morphological abnormality of parallel fiber–Purkinje cell synapses has been noted. Selective transgenic expression of mGluR1α in cerebellar Purkinje cells rescues these anomalies in mGluR1 knockout mice *(72),* suggesting that the phenotypes result from the absence of mGluR1 in Purkinje cells. Mice deficient in Gαq, PLCβ4, or IP-3 receptor, which are molecules located downstream of mGluR1 in the intracellular signaling cascade in a Purkinje cell, also show similar phenotypes *(110–114).*

Neuronal nitric oxide synthase, glial fibrillary acidic protein, and protein kinase G knockout mice demonstrate motor learning deficits and LTD failure but have normal motor control *(115–117).* Therefore, there is good correlation between the LTD failure and motor learning deficits, suggesting implication of LTD in motor learning. However, the transgenic mouse expressing a PKC inhibitor in Purkinje cells that lack the LTD shows slowly developing motor learning *(118,119),* suggesting that there are some LTD-independent motor learning processes. Table 1 also shows a relatively good correlation between the multiple climbing fiber innervations and motor discoordination or learning. However, the PKC inhibitor transgenic mouse shows normal motor control with multiple climbing fiber innervations, and the PKCγ knockout mouse with the multiple innervations shows apparently normal motor learning. A specific phenotype of the GluRδ2 knockout mouse is a reduction in the number of parallel fiber–Purkinje cell synapses.

Although certain correlations are noted in Table 1, caution should be exercised when drawing a general conclusion from such a summary in the following respects. First, most knockout studies are not cell-specific. Therefore, deficiency of a particular molecule in cells outside the cerebellum may be responsible for some phenotypes. Second, any molecules can have several functions and multiple roles, which might not be examined or noticed. Third, conditions or tests to examine each phenotype often are not identical in different studies. Therefore, a certain mutant mouse may show deficits in certain learning tests but not in others. Furthermore, the degree of abnormality may be quantitatively different among mutant mice, despite the fact that the phenotype is classified into a same category in Table 1. For example, the number of climbing fibers innervating a Purkinje cell in a PKC inhibitor transgenic mouse may be smaller than that in the GluRδ2 knockout mouse or the mGluR1 knockout mouse. Finally, some anomalies can be induced secondarily, or original anomalies may be masked by some compensatory mechanisms during development or even for short periods. Careful examination to correlate phenotypes and analyses to clarify causal relations appear to be required.

GluRδ2 in Animals Other Than Mice and Rats

GluRδ2 has also been studied in humans and zebrafish. In humans, the GluRδ2 gene is

mapped to chromosome 4q22 *(120).* In zebrafish, the GluRδ2 protein is localized not only in Purkinje cells but also at the apical dendrites of crests cells of the medial octavolateral nucleus and of type I neurons of the optic tectum *(121).* These neurons also receive large numbers of parallel-fiber-like inputs at the apical dendrites.

Conclusion

GluRδ2 is a glutamate-receptor-related molecule selectively localized at parallel fiber–Purkinje cell synapses, where it plays a critical role for their normal function and also indirectly affects regulation of information processing in the cerebellar circuit. Recent studies on GluRδ2 have provided intriguing information that contributes to the understanding of not only synaptic regulation mechanisms but also functioning mechanisms of the whole cerebellar system.

Acknowledgments

I would like to thank K. Funabiki, Y. Tagawa, S. Kawaguchi, S. Yawata, T. Yoshida, G. Ohtsuki, and K. Tsumura for comments on the manuscript and A. Itahashi for preparing the figures and a table.

References

1. Hollmann M. and Heinemann S. (1994) Cloned glutamate receptors. *Annu. Rev. Neurosci.* **17,** 31–108.
2. Conn P. J. and Pin J. P. (1997) Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol.* **37,** 205–237.
3. Araki K., Meguro H., Kushiya E., Takayama C., Inoue Y., and Mishina M. (1993) Selective expression of the glutamate receptor channel δ2 subunit in cerebellar Purkinje cells. *Biochem. Biophys. Res. Commun.* **197,** 1267–1276.
4. Lomeli H., Sprengel R., Laurie D. J., et al. (1993) The rat delta-1 and delta-2 subunits extend the excitatory amino acid receptor family. *FEBS Lett.* **315,** 318–322.
5. Rossi F. and Borsello T. (1993) Ectopic Purkinje cells in the adult rat: olivary innervation and different capabilities of migration and development after grafting. *J. Comp. Neurol.* **337,** 70–82.
6. Takayama C., Nakagawa S., Watanabe M., Mishina M., and Inoue Y. (1995) Light- and electron-microscopic localization of the glutamate receptor channel δ2 subunit in the mouse Purkinje cell. *Neurosci. Lett.* **188,** 89–92.
7. Petralia R. S., Sans N., Wang Y. X., et al. (2004) Loss of GluR2 alpha-amino-3-hydroxy- 5-methyl-4-isoxazoleproprionic acid receptor subunit differentially affects remaining synaptic glutamate receptors in cerebellum and cochlear nuclei. *Eur. J. Neurosci.* **19,** 2017–2029.
8. Hirano T., Kasono K., Araki K., Shinozuka K., and Mishina M. (1994) Involvement of the glutamate receptor δ2 subunit in the long-term depression of glutamate responsiveness in cultured rat Purkinje cells. *Neurosci. Lett.* **182,** 172–176.
9. Jeromin A., Huganir R. L., and Linden D. J. (1996) Suppression of the glutamate receptor δ2 subunit produces a specific impairment in cerebellar long-term depression. *J. Neurophysiol.* **76,** 3578–3583.
10. Kashiwabuchi N., Ikeda K., Araki K., et al. (1995) Impairment of motor coordination, Purkinje cell synapse formation, and cerebellar long-term depression in GluR δ2 mutant mice. *Cell* **81,** 245–252.
11. Funabiki K., Mishina M., and Hirano T, (1995) Retarded vestibular compensation in mutant mice deficient in δ2 glutamate receptor subunit. *Neuroreport* 7, 189–192.
12. Kishimoto Y., Kawahara S., Suzuki M., Mori H., Mishina M., and Kirino Y. (2001) Classical eyeblink conditioning in glutamate receptor subunit δ2 mutant mice is impaired in the delay paradigm but not in the trace paradigm. *Eur. J. Neurosci.* **13,** 1249–1253.
13. Yoshida T., Katoh A., Ohtsuki G., Mishina M., and Hirano T. (2004) Oscillating Purkinje neuron activity causing involuntary eye movement in a mutant mouse deficient in the glutamate receptor δ2 subunit. *J. Neurosci.* **24,** 2440–2448.
14. Ohtsuki G., Kawaguchi S., Mishina M., and Hirano T. (2004) Enhanced inhibitory synaptic transmission in the cerebellar molecular layer

of the GluRδ2 knock-out mouse. *J. Neurosci.* **24,** 10,900–10,907.
15. Sacchetti B., Scelfo B., Tempia F., and Starata P. (2004) Long-term synaptic changes induced in the cerebellar cortex by fear conditioning. *Neuron* **42,** 973–982.
16. Yuzaki M. (2003) The δ2 glutamate receptor: 10 years later. *Neurosci. Res.* **46,** 11–22.
17. Yuzaki M. (2004) The δ2 glutamate receptor: a key molecule controlling synaptic plasticity and structure in Purkinje cells. *Cerebellum* **3,** 89–93.
18. Hirai H., Kirsch J., Laube B., Betz H., and Kuhse J. (1996) The glycine binding site of the N-methyl-D-aspartate receptor subunit NR1: identification of novel determinants of co-agonist potentiation in the extracellular M3-M4 loop region. *Proc. Natl. Acad. Sci. USA* **93,** 6031–6036.
19. Laube B., Hirai H., Sturgess M., Betz H., and Kuhse J. (1997) Molecular determinants of agonist discrimination by NMDA receptor subunits: analysis of the glutamate binding site on the NR2B subunit. *Neuron* **18,** 493–503.
20. Armstrong N., Sun Y., Chen G. Q., and Gouaux E. (1998) Structure of a glutamate-receptor ligand-binding core in complex with kainate. *Nature* **395,** 913–917.
21. Mayat E., Petralia R. S., Wang Y. X., and Wenthold R. J. (1995) Immunoprecipitation, immunoblotting, and immunocytochemstry studies suggest that glutamate receptor δ subunits form novel postsynaptic receptor complexes. *J. Neurosci.* **15,** 2533–2546.
22. Burnashev N. (1996) Calcium permeability of glutamate-gated channels in the central nervous system. *Curr. Opin. Neurobiol.* **6,** 311–317.
23. Panchenko V. A., Glasser C. R., Partin K. M., and Mayer M. L. (1999) Amino acid substitutions in the pore of rat glutamate receptors at sites influencing block by polyamines. *J. Physiol.* **520,** 337–357.
24. Zuo J., De Jager, P. L., Takahashi K. A., Jiang W., Linden D. J., and Heintz N. (1997) Neurodegeneration in Lurcher mice caused by mutation in δ2 glutamate receptor gene. *Nature* **388,** 769–773.
25. Kohda K., Wang Y., and Yuzaki M. (2000) Mutation of a glutamate receptor motif reveals its role in gating and δ2 receptor channel properties. *Nat. Neurosci.* **3,** 315–322.
26. Taverna F., Xiong Z. G., Brandes L., Roder J. C., Slater M. W., and MacDonald J. F. (2000) The lurcher mutation of an alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunit enhances potency of glutamate and converts an antagonist to an agonist. *J. Biol. Chem.* **275,** 8475–8479.
27. Wollmuth L. P., Kuner T., Jatzke C., Seeburg P. H., Heinz N., and Zuo J. (2000) The lurcher mutation identifies δ2 as an AMPA/kainite receptor-like channel that is potentiated by Ca^{2+}. *J. Neurosci.* **20,** 5973–5980.
28. Ikeno K., Yamakura T., Yamazaki M., and Sakimura K. (2001) The lurcher mutation reveals Ca^{2+} permeability and PKC modification of the GluRδ channels. *Neurosci. Res.* **41,** 193–200.
29. Yue Z., Horton A., Bravin M., Dejager P. L., Selimi F., and Heinz N. (2002) A novel protein complex linking the δ2 glutamate receptor and autophagy: implications for neurodegeneration in *lurcher* mice. *Neuron* **35,** 921–933.
30. Hirai H. and Matsuda S. (1999) Interaction of the C-terminal domain of δ glutamate receptor with spectrin in the dendritic spines of cultured Purkinje cells. *Neurosci. Res.* **34,** 281–287.
31. Roche K. W., Ly C. D., Petralia R. S., et al. (1999) Postsynaptic density-93 interacts with the δ2 glutamate receptor subunit at parallel fiber synapses. *J. Neurosci.* **19,** 3926–3934.
32. Hironaka K., Umemori H., Tezuka T., Mishina M., and Yamamoto T. (2000) The protein-tyrosine phosphatase PTPMEG interacts with glutamate receptor δ2 and ε subunits. *J. Biol. Chem.* **275,** 16,167–16,173.
33. Miyagi Y., Yamashita T., Fukaya M., et al. (2002). Delphilin: a novel PDZ and forming homology domain-containing protein that synaptically colocalizes and interacts with glutamate receptor δ2 subunit. *J. Neurosci.* **22,** 803–814.
34. Uemura T., Mori H., and Mishina M. (2004) Direct interaction of GluRδ2 with Shank scaffold proteins in cerebellar Purkinje cells. *Mol. Cell. Neurosci.* **26,** 330–341.
35. Yap C. C., Murate M., Kishigami S., et al. (2003) Adaptor protein complex-4 (AP-4) is expressed in the central nervous system neurons and interacts with glutamate receptor δ2. *Mol. Cell. Neurosci.* **24,** 283–295.
36. Ly C. D., Roche K. W., Lee H., and Wenthold R. J. (2002) Identification of rat EMAP, a δ-glutamate receptor binding protein. *Biochem. Biophys. Res. Commun.* **291,** 85–90.
37. Yap C. C., Muto Y., Kishida H., Hashikawa T., and Yano R. (2003) PKC regulates the δ2 glutamate receptor interaction with S-SCAM/

MAGI-2 protein. *Biochem. Biophys. Res. Commun.* **301,** 1122–1128.
38. Hung A. Y. and Sheng M. (2002) PDZ domains: structural modules for protein complex assembly. *J. Biol. Chem.* **277,** 5699–5702.
39. Takayama C., Nakagawa S., Watanabe M., Mishina M., and Inoue Y. (1996) Developmental changes in expression and distribution of the glutamate receptor channel δ2 subunit according to the Purkinje cell maturation. *Brain Res. Dev. Brain Res.* **92,** 147–155.
40. Landsend A. S., Amiry-Moghaddam M., Matsubara A., et al. (1997) Differential localization of δ glutamate receptors in the rat cerebellum: coexpression with AMPA receptors in parallel fiber-spine synapses and absence from climbing fiber synapses. *J. Neurosci.* **17,** 834–842.
41. Zhao H. M., Wenthold R. J., Wang Y. X., and Petralia R.S. (1997) δ-glutamate receptors are differentially distributed at parallel and climbing fiber synapses on Purkinje cells. *J. Neurochem.* **68,** 1041–1052.
42. Matsuda I. and Mishina M. (2000) Identification of a juxtamembrane segment of the glutamate receptor δ2 subunit required for the plasma membrane localization. *Biochem. Biophys. Res. Commun.* **275,** 565–571.
43. Matsuda S., Hannen R., Matsuda K., Yamada N., Tubbs T., and Yuzaki M. (2004) The C-terminal juxtamembrane region of the δ2 glutamate receptor controls its export from the endoplasmic reticulum. *Eur. J. Neurosci.* **19,** 1683–1690.
44. Ito M., Sakurai M., and Tongroach P. (1982) Climbing fibre induced depression of both mossy fibre responsiveness and glutamate sensitivity of cerebellar Purkinje cells. *J. Physiol.* **324,** 113–134.
45. Sakurai M. (1987) Synaptic modification of parallel fibre-Purkinje cell transmission in *in vitro* guinea-pig cerebellar slices. *J. Physiol.* **394,** 463–480.
46. Hirano T. (1990) Depression and potentiation of the synaptic transmission between a granule cell and a Purkinje cell in rat cerebellar culture. *Neurosci. Lett.* **119,** 141–144.
47. Ito M. (1989) Long-term depression. *Annu. Rev. Neurosci.* **12,** 85–102.
48. Hansel C., Linden D. J., and D'Angelo E. (2001) Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. *Nat. Neurosci.* **4,** 467–475.
49. Ito M. (2001) Cerebellar long-term depression: characterization, signal transduction, and functional roles. *Physiol. Rev.* **81,** 1143–1195.
50. Hirano, T. (1990) Effects of postsynaptic depolarization in the induction of synaptic depression between a granule cell and a Purkinje cell in rat cerebellar culture. *Neurosci. Lett.* **119,** 145–147.
51. Linden D. J., Dickinson M. H., Smeyne M., and Connor J. A. (1991) A long-term depression of AMPA currents in cultured cerebellar Purkinje neurons. *Neuron* **7,** 81–89.
52. Linden D. J. (1995) Long-term synaptic depression. *Annu. Rev. Neurosci.* **18,** 319–357.
53. Shigemoto R., Abe T., Nomura S., Nakanishi S., and Hirano T. (1994) Antibodies inactivating mGluR1 metabotropic glutamate receptor block long-term depression in cultured Purkinje cells. *Neuron* **12,** 1245–1255.
54. Matsuda S., Launey T., Mikawa S., and Hirai H. (2000) Disruption of AMPA receptor GluR2 clusters following long-term depression induction in cerebellar Purkinje neurons. *EMBO J.* **19,** 2765–2774.
55. Xia J., Chung H. J., Wihler C., Huganir R. L., and Linden D. J. (2000) Cerebellar long-term depression requires PKC-regulated interactions between GluR2/3 and PDZ domain-containing proteins. *Neuron* **28,** 499–510.
56. Hirano T., Kasono K., Araki K., and Mishina M. (1995) Suppression of LTD in cultured Purkinje cells deficient in the glutamate receptor δ2 subunit. *NeuroReport* **6,** 524–526.
57. Hirai H., Launey T., Mikawa S., et al. (2003) New role of δ2-glutamate receptors in AMPA receptor trafficking and cerebellar function. *Nat. Neurosci.* **6,** 869–876.
58. Hirai, H., Miyazaki T., Kakegawa W., et al. (2005) Rescue of abnormal phentypes of the δ2 glutamate receptor-null mice by mutant δ2 transgenes. *EMBO Rep.* **6,** 90–95.
59. Crepel F. (1998) Nitric oxide and long-term depression in the cerebellum. *Trends. Neurosci.* **21,** 63,64.
60. Feil R., Hartmann J., Luo C., et al. (2003) Impairment of LTD and cerebellar learning by Purkinje cell-specific ablation of cGMP-dependent protein kinase I. *J. Cell Biol.* **163,** 295–302.
61. Kawasaki H., Fujii H., Gotoh Y., et al. (1999) Requirement for mitogen-activated protein kinase in cerebellar long term depression. *J. Biol. Chem.* **274,** 13,498–13,502.

62. Boxall A. R., Lancaster B., and Garthwaite J. (1996) Tyrosine kinase is required for long-term depression in the cerebellum. *Neuron* **16,** 805–813.
63. Launey T., Endo S., Sakai R., Harano J., and Ito M. (2004) Protein phosphatase 2A inhibition induces cerebellar long-term depression and declustering of synaptic AMPA receptor. *Proc. Natl. Acad. Sci. USA* **13,** 676–681.
64. Miyata M., Okada D., Hashimoto K., Kano M., and Ito M. (1999) Corticotropin-releasing factor plays a permissive role in cerebellar long-term depression. *Neuron* **22,** 763–765.
65. Kurihara H., Hashimoto K., Kano M., et al. (1997) Impaired parallel fiber->Purkinje cell synapse stabilization during cerebellar development of mutant mice lacking the glutamate receptor δ2 subunit. *J. Neurosci.* **17,** 9613–9623.
66. Lalouette A., Lohof A., Sotelo C., Guenet J., and Mariani J. (2001) Neurobiological effects of a null mutation depend on genetic context: comparison between two hotfoot alleles of the delta-2 ionotropic glutamate receptor. *Neuroscience* **105,** 443–455.
67. Takeuchi T., Miyazaki T., Watanabe M., Mori H., Sakimura K., and Mishina M. (2005) Control of synaptic connection by glutamate receptor δ2 in the adult cerebellum. *J. Neurosci.* **25,** 2146–2156.
68. Hashimoto K. and Kano M. (2003) Functional differentiation of multiple climbing fiber inputs during synapse elimination in the developing cerebellum. *Neuron* **38,** 785–796.
69. Hashimoto K., Ichikawa R., Takechi H., et al. (2001) Roles of glutamate receptor δ2 subunit (GluRδ2) and metabotropic glutamate receptor subtype 1 (mGluR1) in climbing fiber synapse elimination during postnatal cerebellar development. *J. Neurosci.* **21,** 9701–9712.
70. Ichikawa R., Miyazaki,T., Kano M., et al. (2002) Distal extension of climbing fiber territory and multiple innervation caused by aberrant wiring to adjacent spiny branchlets in cerebellar Purkinje cells lacking glutamate receptor δ2. *J. Neurosci.* **22,** 8487–8503.
71. Kano M., Hashimoto K., Kurihara H., et al. (1997) Persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking mGluR1. *Neuron* **18,** 71–79.
72. Ichise T., Kano M., Hashimoto K., et al. (2000) mGluR1 in cerebellar Purkinje cells essential for long-term depression, synapse elimination, and motor coordination. *Science* **288,** 1832–1835.
73. Offermans S., Hashimoto K., Watanabe M., et al. (1997) Impaired motor coordination and persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking Gαq. *Proc. Natl. Acad. Sci. USA* **94,** 14,089–14,094.
74. Kano M., Hashimoto K., Watanabe M., et al. (1998) Phospholipase Cβ4 is specifically involved in climbing fiber synapse elimination in the developing cerebellum. *Proc. Natl. Acad. Sci. USA* **95,** 15,724–15,729.
75. Kano M., Hashimoto K., Chen C., et al. (1995) Impaired synapse elimination during cerebellar development in PKCγ mutant mice. *Cell* **83,** 1223–1231.
76. Morando L., Cesa R., Rasetti R., Harvey R., and Strata P. (2001) Role of glutamate δ2 receptors in activity-dependent competition between heterologous afferent fibers. *Proc. Natl. Acad. Sci. USA* **98,** 9954–9959.
77. Cesa R., Morando L., and Strata P. (2003) Glutamate receptor δ2 subunit in activity-dependent heterologous synaptic competition. *J. Neurosci.* **23,** 2363–2370.
78. Hirai H. (2001) Ca^{2+}-dependent regulation of synaptic δ2 glutamate receptor density in cultured rat Purkinje neurons. *Eur. J. Neurosci.* **14,** 73–82.
79. Kohda K., Kamiya Y., Matsuda S., Kato K., Umemori H., and Yuzaki M. (2003) Heteromer formation of δ2 glutamate receptors with AMPA or kainate receptors. *Brain Res. Mol. Brain Res.* **110,** 27–37.
80. Takai Y., Shimizu K., and Ohtsuka T. (2003) The roles of cadherins and nectins in interneuronal synapse formation. *Curr. Opin. Neurobiol.* **13,** 520–526.
81. Yamagata M., Sanes J. R., and Weiner J. A. (2003) Synaptic adhesion molecules. *Curr. Opin. Cell Biol.* **15,** 621–632.
82. Washbourne P., Dityatev A., Scheiffele P., et al. (2004) Cell adhesion molecules in synapse formation. *J. Neurosci.* **24,** 9244–9249.
83. Clegg D. O., Wingerd K. L., Hikita S. T., and Tolhurst E. C. (2003) Integrins in the development, function and dysfunction of the nervous system. *Front. Biosci.* **8,** 723–750.
84. Huang E. J. and Reichardt L. F. (2003) Trk receptors: roles in neuronal signal transduction. *Annu. Rev. Biochem.* **72,** 609–642.

85. Gaiano N. and Fishell G. (2002) The role of notch in promoting glial and neural stem cell fates. *Annu. Rev. Neurosci.* **25,** 471–490.
86. Hayashi T., Umemori H., Mishina M., and Yamamoto T. (1999) The AMPA receptor interacts with and signals through the protein tyrosine kinase Lyn. *Nature* **397,** 72–76.
87. Passafaro M., Nakagawa T., Sala C., and Sheng M. (2003) Induction of dendritic spines by an extracellular domain of AMPA receptor subunit GluR2. *Nature* **424,** 677–681.
88. Bergersen L., Waerhaug O., Helm J., et al. (2001) A novel postsynaptic density protein: the monocarboxylate transporter MCT2 is co-localized with δ-glutamate receptors in postsynaptic densities of parallel fiber-Purkinje cell synapses. *Exp. Brain Res.* **136,** 523–534.
89. Robinson D. A. (1981) Control of eye movement. In: Brooks V., ed., Handbook of Physiology, Sect. 1, Bethesda, MD: Williams and Wilkins, pp. 1275–1320..
90. Raymond J. L., Lisberger S. G., and Mauk M. D. (1996) The cerebellum: a neuronal learning machine? *Science* **272,** 1126–1131.
91. Llinas R. and Volkind R. A. (1973) The olivocerebellar system: functional properties as revealed by harmaline-induced tremor. *Exp. Brain Res.* **18,** 69–87.
92. Llinas R. and Yarom Y. (1986) Oscillatory properties of guinea-pig inferior olivary neurones and their pharmacological modulation: an in vitro study. *J. Physiol.* **376,** 163–182.
93. Katoh A., Yoshida T., Himeshima Y., Mishina M., and Hirano T. (2005) Defective control and adaptation of reflex eye movements in mutant mice deficient in either the glutamate receptor δ2 subunit or Purkinje cells. *Eur. J. Neurosci.* **21,** 1315–1326.
94. Darlington C. L., Dutia M. B., and Smith P. F. (2002) The contribution of the intrinsic excitability of vestibular nucleus neurons to recovery from vestibular damage. *Eur. J. Neurosci.* **15,** 1719–1727.
95. Murai N., Tsuji J., Ito J., Mishina M., and Hirano T. (2004) Vestibular compensation in glutamate receptor δ-2 subunit knockout mice: dynamic property of vestibulo-ocular reflex. *Eur. Arch. Otorhinolaryngol.* **261,** 82–86.
96. Kitahara T., Takeda N., Uno A., Kubo T., Mishina M., and Kiyama H. (1998) Unilateral labyrinthectomy downregulates glutamate receptor δ-2 expression in the rat vestibulocerebellum. *Mol. Brain Res.* **61,** 170–178.
97. Thompson R. F. (2005) In search of memory traces. *Annu. Rev. Psychol.* **56,** 1–23.
98. Takatsuki K., Kawahara S., Kotani S., et al. (2003) The hippocampus plays an important role in eyeblink conditioning with a short trace interval in glutamate receptor subunit δ2 mutant mice. *J. Neurosci.* **23,** 17–22.
99. Ito M. (1982) Cerebellar control of the vestibulo-ocular reflex—around the flocculus hypothesis. *Annu. Rev. Neurosci.* **5,** 275–296.
100. du Lac S., Raymond J. L., Sejnowski T. J., and Lisberger S. G. (1995) Learning and memory in the vestibulo-ocular reflex. *Annu. Rev. Neurosci,* **18,** 409–441.
101. Boyden E. S., Katoh A., and Raymond J. L. (2004) Cerebellum-dependent learning: the role of multiple plasticity mechanisms. *Annu. Rev. Neurosci.* **27,** 581–609.
102. Katoh A., Kitazawa H., Itohara S., and Nagao S. (2000) Inhibition of nitric oxide synthesis and gene knockout of neuronal nitric oxide synthase impaired adaptation of mouse optokinetic response eye movements. *Learn. Mem.* **7,** 220–226.
103. Kano M., Rexhausen U., Dreessen J., and Konnerth A. (1992) Synaptic excitation produces a long-lasting rebound potentiation of inhibitory synaptic signals in cerebellar Purkinje cells. *Nature* **356,** 601–604.
104. Kawaguchi S. and Hirano T. (2000) Suppression of inhibitory synaptic potentiation by presynaptic activity through postsynaptic $GABA_B$ receptors in a Purkinje neuron. *Neuron* **27,** 339–347.
105. Lalouette A., Guenet J. L., and Vriz S. (1998) Hotfoot mouse mutations affect the δ2 glutamate receptor gene and are allelic to lurcher. *Genomics* **15,** 9–13.
106. Wang Y., Matsuda S., Drews V., Torashima T., Meisler M. H., and Yuzaki M. (2003) A hot spot for *hotfoot* mutations in the gene encoding the δ2 glutamate receptor. *Eur. J. Neurosci.* **17,** 1581–1590.
107. Matsuda S. and Yuzaki M. (2002) Mutation in *hotfoot-4J* mice results in retention of δ2 glutamate receptors in ER. *Eur. J. Neurosci.* **16,** 1507–1516.
108. Aiba A., Kano M., Chen C., et al. (1994) Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. *Cell* **7,** 377–388.
109. Conquet F., Bashir Z. I., Davies C. H., et al. (1994) Motor deficit and impairment of synap-

tic plasticity in mice lacking mGluR1. *Nature* **372,** 237–243.
110. Hashimoto K., Watanabe M., Kurihara H., et al. (2000) Climbing fiber synapse elimination during postnatal cerebellar development requires signal transduction involving Gαq and phospholipase Cβ4. *Prog. Brain Res.* **124,** 31–48.
111. Hartmann J., Blum R., Kovalchuk Y., et al. (2004) Distinct roles of Gαq and Gα11 for Purkinje cell signaling and motor behavior. *J. Neurosci.* **24,** 5119–5130.
112. Miyata M., Kim H., Hashimoto K., et al. (2001) Deficient long-term synaptic depression in the rostral cerebellum correlated with impaired motor learning in phospholipase Cβ4 mutant mice. *Eur. J. Neurosci.* **13,** 1945–1954.
113. Inoue T., Kato K., Kohda K., and Mikoshiba K. (1998) Type 1 inositol 1,4,5-trisphosphate receptor is required for long-term depression in cerebellar Purkinje neurons. *J. Neurosci.* **18,** 5366–5373.
114. Matsumoto M. and Nagata E. (1999) Type 1 inositol 1,4,5-trisphosphate receptor knock-out mice: their phenotypes and their meaning in neuroscience and clinical practice. *J. Mol. Med.* **77,** 406–411.
115. Lev-Ram V., Nebyelul Z., Ellisman M. H., Huang P. L., and Tsien R. Y. (1997) Absence of cerebellar long-term depression in mice lacking neuronal nitric oxide synthase. *Learn. Mem.* **4,** 169–177.
116. Feil R., Hartmann J., Luo C., et al. (2003) Impairment of LTD and cerebellar learning by Purkinje-cell specific ablation of cGMP-dependent protein kinase I. *J. Cell Biol.* **163,** 295–302.
117. Shibuki K., Gomi H., Chen L., et al. (1996) Deficient cerebellar long-term depression, impaired eyeblink conditioning, and normal motor coordination in GFAP mutant mice. *Neuron* **16,** 587–599.
118. De Zeeuw C. I., Hansel C., Bian F., et al. (1998) Expression of a protein kinase C inhibitor in Purkinje cells blocks cerebellar LTD and adaptation of the vestibulo-ocular reflex. *Neuron* **20,** 495–508.
119. van Alphen A. M. and De Zeeuw C. I. (2002) Cerebellar LTD facilitates but is not essential for long-term adaptation of the vestibulo-ocular reflex. *Eur. J. Neurosci.* **16,** 486–490.
120. Hu W., Zuo J., De Jager P. L., and Heintz N. (1998) The human glutamate receptor δ2 gene (GRID2) maps to chromosome 4q22. *Genomics* **47,** 143–145.
121. Mikami Y., Yoshida T., Matsuda N., and Mishina M. (2004) Expression of zebrafish glutamate receptor δ2 in neurons with cerebellum-like wiring. *Biochem. Biophys. Res. Commun.* **322,** 168–176.