Mol Neurobiol (2008) 37:126–141
DOI 10.1007/s12035-008-8025-0

To Gate or not to Gate: Are the Delta Subunits in the Glutamate Receptor Family Functional Ion Channels?

Sabine M. Schmid · Michael Hollmann

Received: 31 March 2008 / Accepted: 9 May 2008 / Published online: 3 June 2008

Abstract The two delta receptor subunits remain the most puzzling enigma within the ionotropic glutamate receptor family. Despite the recent elucidation of the ligand-binding domain structure of delta2, many fundamental questions with regard to the subunits' mechanism of function still remain unanswered. Of necessity, the majority of studies on delta receptors focused on the metabotropic function of delta2, since electrophysiological approaches to date are limited to the characterization of spontaneous currents through the delta2-*lurcher* mutant. Indeed, accumulated evidence primarily from delta2-deficient transgenic mice suggest that major physiological roles of delta2 are mediated via metabotropic signaling by the subunit's C terminus. Why then would the subunits retain a conserved ion channel domain if they do not form functional ion channels? Any progress with regard to ionotropic function of the two delta subunits has been hampered by their largely unknown pharmacology. Even now that a pharmacological profile for delta2 is being established on the basis of the ligand-binding domain structure, wild-type delta2 channels in heterologous expression systems stay closed in the presence of molecules that have been demonstrated to bind to the receptor's ligand-binding domain. In this paper, we review the current knowledge of delta subunits focusing on the disputed ionotropic function.

Keywords Glutamate receptors · Delta receptors · Delta1 · Delta2 · D-serine · Cerebellar LTD · Lurcher · Spontaneous currents

S. M. Schmid
Department of Biochemistry I—Receptor Biochemistry,
Ruhr University Bochum,
Building NC Room 6/167, Universitätsstr. 150,
44780 Bochum, Germany

M. Hollmann (✉)
Department of Biochemistry I—Receptor Biochemistry,
Ruhr University Bochum,
Building NC Room 6/171, Universitätsstr. 150,
44780 Bochum, Germany
e-mail: Michael.Hollmann@rub.de

S. M. Schmid
International Graduate School of Neuroscience,
Ruhr University Bochum,
Universitätsstr. 150,
44780 Bochum, Germany

Introduction

In 1993, two new ionotropic glutamate receptor (iGluR) subunits were discovered by low stringency screening of mouse [1, 2] and rat [3] brain libraries using α-amino-3-hydroxy-5-methylisoxazole propionate (AMPA) receptor probes. They were tentatively designated as iGluRs on the basis of substantial sequence homology. However, the two subunits were more closely related to each other than to any previously described iGluR. Consequently, both subunits were taken to constitute a new, separate subfamily within the iGluR family. Following an alternative iGluR nomenclature introduced at the time for mouse subunits, both members of this new subtype were designated 'delta receptors' and numbered accordingly (delta1 and delta2).

Subsequent attempts to electrophysiologically characterize the two subunits in heterologous expression systems were unsuccessful [2, 3]. Both subunits were insensitive to common iGluR agonists, and radioligand-binding assays failed to identify a ligand [3, 4]. This prompted the designation 'orphan' receptors as an alternative name for this iGluR subtype, which emphasized that the agonist(s) required to activate the delta receptors remained unknown.

In the absence of a possibility to examine their electrophysiological properties, studies initially focused on the expression patterns of the two subunits, revealing a highly specialized expression pattern for both subunits, which is clearly different from the expression pattern of other iGluRs [3–11] (Table 1).

The next important step toward elucidating the function of delta subunits was the generation of delta2-deficient mice whose phenotype was first described in 1995 [12]. This delta2 knock-out confirmed what prior knock-down studies using antisense oligonucleotides in cell culture had already indicated [13–15]: delta2 deprivation resulted in impaired long-term depression (LTD), the major form of synaptic plasticity at parallel fiber synapses of cerebellar Purkinje cells. In the following years, the special phenotype of delta2-deficient mice was thoroughly investigated, revealing that not only LTD but also synaptogenesis, motor coordination, and motor learning were disturbed [16–23]. Whereas the delta2-deficient mice were described more than a decade ago, it took until last year to generate delta1-deficient mice [24].

A breakthrough with regard to the function of the delta2 subunit was the discovery that the phenotype of so-called *lurcher* mutant mice originated from a point mutation within the delta2 subunit [25]. This point mutation is an alanine-to-threonine conversion located in the most conserved sequence motif (the SYTANLAAF motif) of iGluRs. It yields delta2-*lurcher* channels that spontaneously gate in the absence of agonist. Investigators now had the means to examine ionic currents through the delta2-*lurcher* pore using electrophysiological techniques [26–28]. However, these currents could only be manipulated by substances that presumably did not bind to the ligand-binding domain (LBD) of the receptor. To date, it remains unclear whether the introduction of the corresponding mutation to delta1 yields spontaneously gating delta1-*lurcher* channels [28, 29]. Furthermore, the cell type-selective death of cerebellar Purkinje cells in *lurcher* mice was suggested to provide a good experimental system to address the mechanism of delayed, excitotoxic neuronal cell death after ischemia [25, 30]. For this reason, cell death pathways in the *lurcher* mouse cerebellum drew a lot of attention [31–38].

More recent progress stems from a transgenic rescue approach. This technique was used to elucidate which of the delta2 domains is critical for cerebellar LTD. The individual domains of delta2 were manipulated by mutagenesis, and the ability of the resulting mutants to rescue the impaired LTD in delta2-deficient mice was tested. The experiments showed that the C terminus of delta2 is necessary for LTD induction and suggested that the ion flux through the ion channel pore and ligand-binding capability are not [39–44].

In parallel to all these advancements, yeast two-hybrid screens identified numerous proteins that interact with the delta2 subunit's C terminus and link the receptor to well-known key molecules implicated in cerebellar LTD [45–52]. However, a precise pathway and mechanism of LTD induction that involves delta2 has not been described yet.

In 2007, the crystal structure of the delta2 LBD was reported [53]. This study finally started the establishment of a pharmacological profile for delta2. Unexpectedly, the isolated LBD of delta2 binds glycine and D-serine, like the NR1 and NR3 *N*-methyl-D-aspartate (NMDA) receptor subunits [53]. Disappointingly though, neither glycine nor D-serine evoked current responses at the wild-type delta2 subunit. Rather, both molecules reduced the spontaneous current through delta2-*lurcher* channels.

The wild-type delta subunits have persistently escaped electrophysiological characterization over the last 15 years, although they apparently belong to the glutamate receptors and thus are part of the most important excitatory neurotransmitter receptor family. Therefore, besides compiling the available data on the two subunits, the present review attempts to look at both delta receptors from an 'ion

Table 1 Expression pattern of delta subunits

delta subunit	Brain region	Expression level (and time)	Reference
delta1	Hippocampus: pyramidal and dentate granule cell layer	Weak but specific (adult)	Lomeli et al. [3]
	Caudate putamen	High (during development)	
	Cochlea: inner hair cells Spiral ganglia: neurons and satellite glia cells Vestibular hair cells Vestibular ganglion neurons	High and specific (adult)	Safieddine and Wenthold [5]
	Retina: bipolar cells		Jakobs et al. [11]
delta2	Cerebellum: Purkinje cells	High and specific (Adult)	Mayat et al. [4]; Takayama et al. [69]; Takayama et al. [6]; Zhao et al. [7]; Landsend et al. [8]; Petralia et al. [9];
	Within the Purkinje cells: Restricted to parallel fiber-Purkinje cell synapses		
	Pineal glia-like cells in culture	–	Yatsushiro et al. [10]

List of all reported brain regions in which delta subunits have been found to be expressed. The respective references are indicated as well as the level and time of expression, if that data was available.

channel point of view'. This review covers the literature up to and including February 2008.

Genes and Subunit Structure

Both delta receptor genes have been identified in mouse (delta1, NM008166; delta2, NM008167), rat (delta1, U08255; delta2, U08256), and human (delta1, NM017551; delta2, NM001510) [1–3, 54–59]. In addition, homologs of the genes exist in the genomes of *Xenopus tropicalis* (delta1, BC136207) and zebrafish (*Danio rerio*; delta2, NM001004123) [55, 60, 61]. The human delta1 gene (GRID1) is encoded by 16 exons on chromosome 10 (q23), and the delta2 gene (GRID2) is encoded by 16 exons on chromosome 4 (q22) [55, 62–64]. Comparable gene structure and sizes are found in rat and mouse [55]. Given the average iGluR gene size, the delta genes are unusually large. The delta2 subunit is encoded by the largest (1,400 kb) and the delta1 subunit by the second largest (770 kb) human iGluR gene [59, 64]. Sequence comparison places both delta receptors slightly closer to non-NMDA than to NMDA receptors (Fig. 1a). In addition, the intron–exon arrangement encoding the pore region of delta subunits is similar to that of AMPA receptors [64]. Neither delta1 nor delta2 undergo any known process of alternative splicing or RNA editing. The extensive sequence homology to other iGluRs strongly suggests that the delta receptors share the same topology and general structure as AMPA, kainate, and NMDA receptors (Fig. 1b).

Hence, functional receptors likely assemble from four single subunits as a dimer of dimers [65]. In principle, tetramers can be composed either of identical subunits (homomers) or of different subunits (heteromers). According to a general rule that applies to other iGluRs, heteromer formation is only observed among subunits of one pharmacological subtype [66]. However, whether this holds true for the delta subtype is presently unclear.

Ionotropic glutamate receptor (iGluR) subunits have a highly modular structure composed of four discrete domains: two large, globular extracellular domains referred to as N-terminal domain (NTD) and LBD, a membrane-resident region, and the intracellular C-terminal domain (CTD; Fig. 1b). The membrane-resident region of iGluRs comprises three α-helical transmembrane domains (TMDs A to C) and a membrane-resident hairpin loop (pore) between TMD A and TMD B [67]. The LBD folds from two discontinuous segments of amino acids, termed S1 and S2, with S1 located N-terminal of TMD A and S2 C-terminal of TMD B. This membrane topology places the NTD extracellularly before S1 and the CTD intracellularly, following TMD C.

Where are Delta Subunits Expressed and What do Mice Need Them for?

delta1 The delta1 subunit is the least abundant iGluR in the adult CNS but shows a pronounced developmental peak in late embryonic and early postnatal stages with high messenger RNA (mRNA) levels in the caudate putamen [3]. In the adult, weak but specific expression in the pyramidal and dentate granule cell layers of the hippocampus can be detected [3]. Furthermore, delta1 expression was detected in the inner ear in the organ of Corti and in the vestibular end organ [5]. In the ear, expression is restricted to the inner hair cells, whereas in the spiral ganglia, delta1 is expressed in neurons as well as their satellite glial cells. In the vestibular organ, expression was seen in both classes of vestibular hair cells and in vestibular ganglion neurons [5]. Recently, delta1 mRNA was detected in ganglion and bipolar cells of the retina [11].

Mice lacking the delta1 subunit show impaired hearing [24]. The cochlear threshold for frequencies higher than 16 kHz is shifted significantly and correlates with a substantial loss of type IV spiral ligament fibrocytes. Furthermore, the endolymphatic potential in high-frequency cochlear regions is reduced, and mice are more susceptible to acoustic injury. However, gross innervation patterns of inner hair cells are unaffected, and the morphology and function of hippocampus and vestibular system appear normal. This phenotype suggests a role for delta1 in high-frequency hearing and ionic homeostasis in the basal cochlea and corresponds well with the delta1 expression pattern [24].

delta2 The delta2 subunit is abundantly and specifically expressed in a major cell type of the cerebellum, the Purkinje cell. Purkinje cells receive two types of excitatory input: one from climbing fibers that originate from inferior olivary neurons in the brainstem and the other from parallel fibers that arise from granule cells in the granule cell layer of the cerebellum. Climbing fibers form synapses on the proximal dendrites of Purkinje cells, parallel fibers on the distal portion of the Purkinje cell's dendritic tree. While Purkinje cells are innervated by multiple climbing fibers in early postnatal stages, a strict one-to-one climbing fiber to Purkinje cell innervation pattern is established during maturation [68]. Delta2 mRNA can be detected as early as embryonic day 15 in Purkinje neuroblasts [4]. Upon maturation of the cells and their recruitment into cerebellar circuitry, the delta2 protein is redistributed to dendritic spines of excitatory synapses [6, 69]. While at postnatal day 10 delta2 is still present at both excitatory inputs, it is absent from climbing fiber synapses and restricted to parallel fiber synapses in the adult [7–9]. The selective targeting of delta2 subunits to parallel fiber synapses is

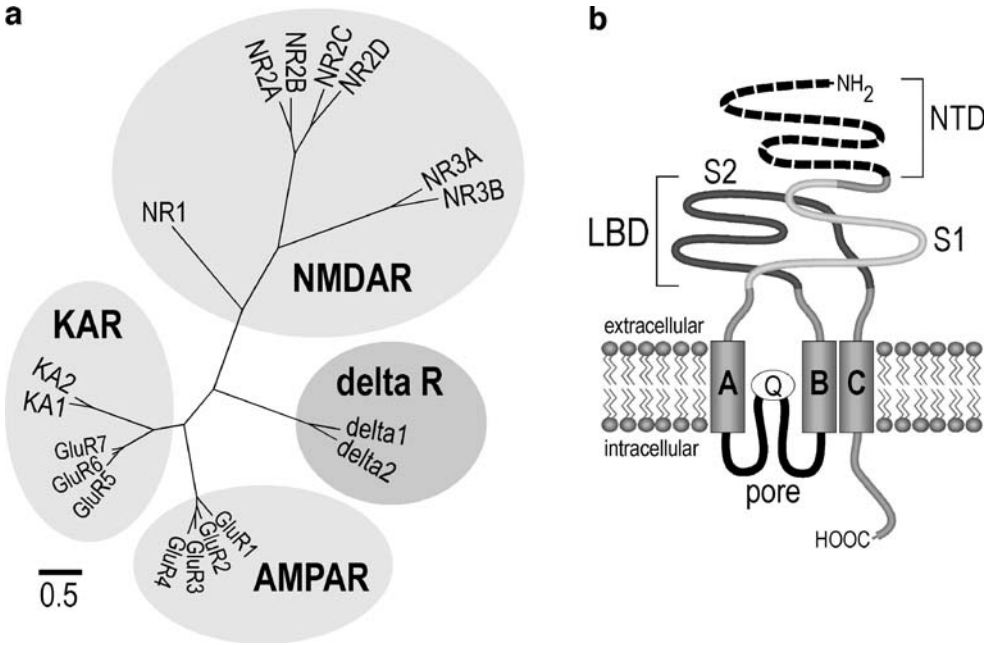

Fig. 1 Classification of mammalian ionotropic glutamate receptor proteins. **a** Unrooted tree of the 18 full-length mammalian iGluRs that are further classified according to sequence homology and pharmacological profiles into AMPA, kainate, NMDA, and delta receptor subunits. The alignment was calculated with MegAlign (DNASTAR). A distance analysis (minimum evolution) was performed using MrBayes 3.1, calculating 10^6 generations. Negative branch-length handling was set to zero. **b** Schematic membrane topology of an iGluR subunit. Color coding reflects the modular structure of the protein: NTD (*black*, *dashed*), S1 (*light gray*), S2 (*dark gray*), and ion pore (*black*). Receptor parts not related to other proteins are shown in *gray*, including the short stretch linking the NTD to S1, the three short stretches connecting the LBD (formed by S1 and S2) with TMDs A, B, and C, as well as TMD C and the CTD. The Q/R editing site in AMPA and kainate receptors is indicated. Both delta receptor subunits are believed to share a similar topology. They both carry a glutamine at the Q/R site

activity-dependent. Block of neuronal activity with tetrodotoxin (TTX) induces a redistribution of delta2 to all Purkinje cell spines and the formation of numerous new spines at proximal dendrites that become aberrantly innervated by parallel fibers [70]. After removal of TTX, climbing fibers reinnervate proximal dendrites and repress delta2 expression [71]. Interestingly, delta2 expression was also detected in a subpopulation of pineal glial-like cells in culture, suggesting a role of the receptor in the regulation of melatonin synthesis in the pineal gland [10].

Mice lacking the delta2 subunit display deficits in motor coordination and motor learning [12] which become apparent around postnatal day 12. In addition, the synaptogenesis in the cerebellum is abnormal, although gross cerebellar histoarchitecture appears normal [12]. The number of parallel fiber synapses on Purkinje cells is reduced to half, multiple innervation of Purkinje cells by climbing fibers persists in the adult, and numerous free spines emerge [17, 19, 20]. The reduced number of parallel fiber synapses causes climbing fiber innervation to extend to distal dendrites and to aberrantly jump to adjacent Purkinje cells [17, 19, 20]. The density of delta2 is directly related to the number of synaptic contacts with parallel fibers [72]. Upon gradual reduction of delta2 density, first the presynaptic active zone at parallel fiber synapses shrinks, then mismatches between the active zone and the Purkinje cell postsynaptic density (PSD) occur, and finally completely free spines emerge. These findings have led to the suggestion that the delta2 subunit might exert its role in promoting and stabilizing parallel fiber contacts via a direct interaction to the presynaptic terminal [73, 74]. The NTD of delta2 has been suggested to promote such contacts in a manner similar to GluR2 interaction with presynaptic N-cadherin [75].

The main form of synaptic plasticity at the parallel fiber synapse, LTD, is abrogated in mice lacking delta2 [12–15]. Concurrently, inhibitory synaptic transmission at interneuron synapses of Purkinje cells is enhanced to compensate for the non-depressed excitatory parallel fiber input [76]. Cerebellar LTD plays an essential role in the fine-tuning of motor coordination. Accordingly, mutant mice show deficits in a number of motor learning tasks such as eye blink conditioning [18, 21], vestibular compensation [16], and vestibulo-ocular reflex compensation [23]. Mutant mice fail to adapt oculomotor reflexes in response to training, eye movements are delayed and wiggly [23], and oscillatory eye movements arise spontaneously [22]. The delta2-deficient phenotype implies an important role of delta2 in synaptic plasticity and synaptogenesis in the cerebellum, which fits well with the specific expression of

delta2 in the sole cerebellar output neuron, the Purkinje cell.

The C Terminus of Delta Receptors Regulates Trafficking and Synapse Localization

Using the yeast two-hybrid system, a number of proteins have been identified that interact with the CTD of delta receptors (Table 2; for abbreviations not given in the text, please refer to the legend of Fig. 2).

The delta2 subunit interacts with two members of the family of MAGUKs (membrane-associated guanylate kinases), namely PSD-93 [45] and synaptic scaffolding molecule (S-SCAM) [50]. Both proteins bind to the distal four amino acids of the C terminus of delta2 via one of their conserved PDZ domains and cluster the receptor subunits at the PSD.

Several other proteins have been described that link delta2 to the cytoskeleton and mediate its trafficking to the plasma membrane: spectrin [77], echinoderm microtubule associated protein (EMAP) [47], AP-4 [49], and delphilin [48]. The expression pattern of EMAP suggests that in vivo it might be an interaction partner for delta1 that is involved in multiple pathways of yet unknown nature. AP-4 is a protein complex that traffics proteins from the Golgi network to the plasma membrane. Neither EMAP nor AP-4 interact with the distal four amino acids of the C terminus of delta receptors but bind to an internal motif in the CTD (Fig. 2, Table 2).

Spectrin is an actin-binding protein and anchors delta2 to the cytoskeleton in a calcium-dependent manner. Stimulation of ionotropic or metabotropic GluRs in cultured Purkinje cells induces a significant decrease in the density of synaptic delta2 receptors [78]. This effect is dependent on a rise in intracellular calcium and is blocked by glutamatergic antagonists or removal of extracellular calcium [78]. Morphological changes in the actin cytoskeleton are thought to regulate this decrease of delta2 in dendritic spines without affecting the number of synaptic contacts. The calcium-dependent dissociation of delta2 from spectrin might mediate the observed reduction of delta2 receptors at the PSD [78, 79]. Delphilin is a PSD protein at parallel fiber synapses that binds specifically to delta2, clusters the receptor subunits, and links them to the cytoskeleton as well as to the protein tyrosine kinase nSrc (neuronal sarcoma) [48].

A second link to downstream protein kinase and phosphatase pathways is the protein tyrosine phosphatase MEG that associates with the C terminus of delta2 via its PDZ domain [46].

A nine amino-acid deletion in the CTD four positions downstream of TMD C blocks the delivery of delta2 receptors to the plasma membrane [80]. This membrane-proximal region of the CTD was suggested to play a different role in surface expression, synapse location, and signal transduction than the distal four amino acids of the C terminus and their interaction with PDZ domain-containing proteins [81]. A stretch of 12 amino acids located in the central part of the delta2 CTD stabilizes the receptors at the synapse, rendering delta2 resistant to endocytosis [82]. By contrast, complete truncation of the CTD leaving only five amino acids protruding from TMD C was found not to interfere with location of delta2 at dendritic spines [52].

Metabotropic Signaling Links the Delta2 Subunit to Essential Players in LTD Induction

Calcium entry through voltage-gated calcium channels, the presence of delta2, mGluR1 activation, and AMPA receptor endocytosis are essential for LTD induction at the parallel fiber synapse of Purkinje cells [78, 83, 84]. The delta2 subunit is linked to LTD pathways in at least three ways. First, delta2 binds directly to the PDZ domain of Shank proteins via a stretch of amino acids in the CTD that folds into a β-turn [51] (Fig. 2). Shank proteins are linked to AMPA receptors via GRIP1 and to mGluR1 via Homer, thus placing delta2 in the immediate vicinity of two essential players in LTD induction. Secondly, 14 amino acids in the region of the CTD immediately following TMD C in delta2 were shown to interact with the BAR domain of PICK1, and deleting these amino acids hampered the induction of LTD [52]. Thirdly, upon induction of LTD, S945 of the receptor is phosphorylated by PKC, which in turn enhances the interaction with the MAGUK S-SCAM [50, 85].

Although many CTD interaction partners for delta2 have been identified, no clear picture for delta2's involvement in LTD has been established yet. It is worth mentioning that a number of interactions require the CTD of delta2 to adopt a distinct folding such as a β-turn and that delta2 density seems to be reduced upon calcium influx.

The Mouse Delta2 Gene is a Hot Spot for Spontaneous Mutation

In the absence of pharmacological means to examine the delta receptors, the majority of clues regarding their physiological roles originate from studies of knockout or transgenic mice. In addition to these intentional manipulations of delta genes, two spontaneous, naturally occurring mutations affect the delta2 gene in mice: *lurcher* and *hotfoot*. The hypermutability of delta2 in mice has been attributed to the unusually large size of the gene. However, no spontaneous mutations linked to the human delta2 gene

Table 2 Intracellular proteins interacting with the CTD of delta receptors

Interacting protein	Interaction site in delta subunit	Experimental system	Localization of interacting protein	Function	Reference
PSD93 PSD95 SAP97	delta1, delta2: PDZ-binding motif (GTSI)	Yeast two-hybrid, bait: complete CTD of delta1, delta2	Purkinje cell Weak expression in Purkinje cells	Clustering of delta2 at synapse	Roche et al. [45]
Spectrin (α and β)	delta2: CTD	Immunoprecipitation, Immunoblots with His-tagged delta2-CTD	High level expression in spines of Purkinje cells	Linkage to cytoskeleton, interaction inhibited by Ca^{2+} *in vitro*	Hirai and Matsuda [77]
PTP MEG	delta2: PDZ-binding motif (GTSI)	Yeast two-hybrid, bait: CTD delta2; Immunoprecipitation	Purkinje cells	Role of PTPase activity in downstream signaling	Hironaka et al. [46]
EMAP	delta1, delta2: aa 904–955, necessary and sufficient	Yeast two-hybrid, bait: complete CTD of delta1, delta2	Weak: in cortex, hippocampus and cerebellum; High: in superior and inferior colliculi and brainstem	Protein-protein interaction, link to cytoskeleton, *in vivo* interaction partner might be delta1	Ly et al. [47]
nPIST	delta1, delta2: PDZ-binding motif (GTSI)	Yeast two-hybrid, bait: complete CTD of delta2	Colocalization of delta2 and nPIST in HEK293 cells	nPIST interacts with Beclin1, link to autophagy and apoptosis pathways	Yue et al. [37]
Delphilin	delta2-specific: PDZ-binding motif (GTSI)	Yeast two-hybrid, bait: C-terminal part of delta2 CTD	Specifically localized to parallel fiber synapses of Purkinje cells	Link to actin cytoskeleton	Miyagi et al. [48]
				Delphilin interaction dependent on phosphorylation status of PDZ-binding motif by PKA	Sonoda et al. 2006
AP-4	delta2: aa 941–991	Yeast two-hybrid, bait: membrane-distal part of delta2 CTD	Dendritic shafts of Purkinje cells	Trafficking from Golgi network to plasma membrane	Yap et al. [49]
S-SCAM	delta2: PDZ-binding motif (GTSI)	Yeast two-hybrid, bait: delta2 CTD; immunoprecipitation	Purkinje cell	Sorting and clustering at PSD, interaction regulated by PKC phosphorylation of S945	Yap et al. [50]
Shank1 Shank2	delta2-specific: aa 908–936, necessary and sufficient	Yeast two-hybrid, bait: complete CTD of delta1, delta2	Purkinje cell, molecular layer Purkinje cell, mRNA present	Connection to mGluR1 and AMPAR via homer and GRIP	Uemura et al. [51]
PKC	delta2: S945			Phosphorylation	Kondo et al. [85]
PICK1	delta2: SKEDDKE motif	Yeast two-hybrid, bait: membrane-proximal part of delta2 CTD	Dendritic spines of Purkinje cells	BAR domain of PICK1 interacts with delta receptor, multimerization, connection to AMPAR	Yawata et al. [52]

List of all reported proteins that interact with the CTD of delta receptors. In addition, the respective interaction site on the delta subunits is given as well as the experimental system used to identify the interaction, information regarding the colocalization of the delta subunits with the respective interacting protein, the suggested functional implications of such an interaction, and the respective reference.

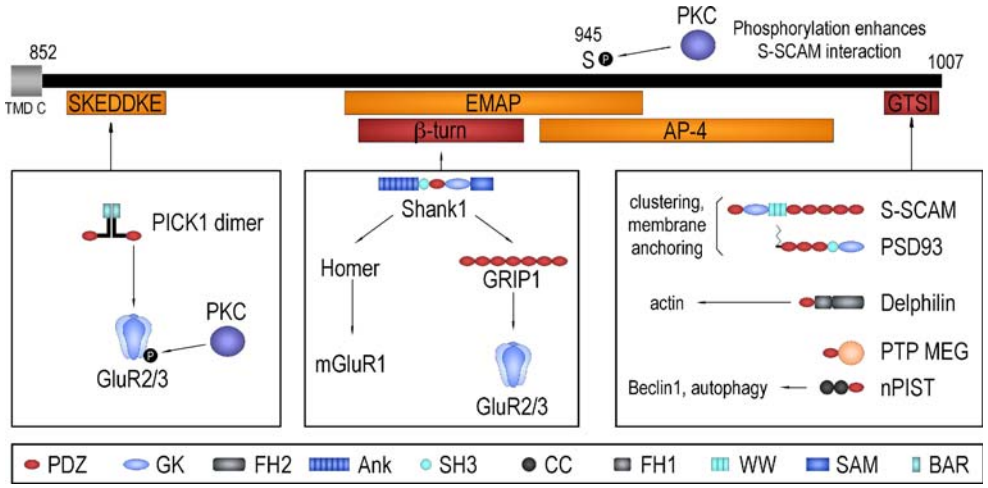

Fig. 2 Intracellular protein interactions with the CTD of delta2. Schematic representation of the delta2 CTD (155 amino acids) and its interactions with intracellular proteins. Indicated are all described interacting proteins with the exception of spectrin since its exact binding site has not been characterized yet. The respective interaction sites of all other proteins interacting with the CTD of delta2 are indicated: PDZ domain-mediated interactions with delta2 (*red*) and interactions via other domains (*orange*). Downstream signaling and domain composition of interacting proteins are indicated when relevant. Abbreviations of domains: *PDZ* PSD-95/Dig/ZO-1, *GK* guanylate kinase, *FH2* formin homology 2, *Ank* ankyrin repeats, *SH3* Src homology 3, *CC* coiled-coil, *FH1* formin homology 1, *WW* domain with two conserved Trp (*W*) residues, *SAM* sterile (α) motif, *BAR* Bin1/amphiphysin/RVS167. Abbreviations of proteins: *Shank* SH3 and ankyrin repeat-containing protein, *PSD-93* postsynaptic densitiy protein 93, *S-SCAM* synaptic scaffolding molecule, *GRIP1* glutamate-receptor-interacting protein 1, *PICK1* protein interacting with C-kinase 1, *PKC* protein kinase C, *Delphilin* delta2-philic-protein, *nPIST* neuronal protein interacting specifically with TC10, *PTP MEG* protein tyrosine phosphatase MEG, *AP-4* adaptor protein 4, *EMAP* echinoderm microtubule associated protein

have been described so far, although the gene size in humans is comparable to that in mouse.

Lurcher Mice The autosomal, dominant *lurcher* phenotype arose spontaneously and owes its name to the characteristic lurching gait of the mice [86]. Homozygous animals die within the first postnatal day due to the autonomous death of all large trigeminal motor neurons during embryonic development [87]. Although the patterning of the brain is regular and trigeminal neurons arise normally, the cells start dying at embryonic day 15, coinciding with the terminal differentiation of motor control circuits in the hindbrain. Heterozygous animals are viable and breed but display impaired motor coordination [88–90]. Symptoms manifest around postnatal day 12, coinciding with the onset of Purkinje cell degeneration. During the next 5 weeks, animals lose 100% of their Purkinje cells, 90% of granule cells, and 75% of inferior olivary neurons [91]. The observed neurodegeneration arises from a single amino acid substitution within the delta2 subunit: An alanine within the conserved SYTANLAAF motif of iGluRs at the C-terminal end of TMD B is replaced with a threonine (A654T) [25]. Purkinje cells of *lurcher* mice display a high membrane conductance and depolarized resting potentials [25]. In accordance with this, heterologous expression of the mutant receptors confirms that homomeric delta2-*lurcher* channels mediate the high membrane conductance, since the application of open channel blockers can block these spontaneous currents [26].

Active caspases and DNA fragmentation in dying Purkinje and granule cells point to an apoptotic mechanism [31, 34] that is aided by extracellular signaling cascades [36]. Blocking apoptosis can completely rescue granule cell death, while Purkinje cell and inferior olivary neuron degeneration is only delayed, indicating that an alternative cell death pathway is activated in these cells [33–35]. This alternative cell death program was suggested to be autophagy [37]. The C terminus of the delta2 subunit is linked to the PDZ domain of neuronal protein interacting specifically with TC10 (nPIST) and the nPIST-associated Beclin1 [37]. Dissociation of the nPIST/Beclin1 complex from delta2 receptors can lead to the induction of autophagy through the release of Beclin1 from nPIST. Interestingly, the onset of delta2-*lurcher*-induced membrane depolarization is not correlated with the degeneration of Purkinje cells [38].

Hotfoot Mice Hotfoot is a naturally occurring recessive mouse mutation causing ataxia [92, 93]. A total of nine different *hotfoot* alleles have been identified as deletions of one or more exons in the delta2 gene [64, 94, 95]. Six of them are in-frame mutations leading to deletions in the NTD of delta2, the other three are frameshift mutations that cause truncation of delta2. Although delta2 is abundantly expressed in *hotfoot*-4J mice cerebelli, the mutant receptors accumulate in the soma of Purkinje cells. Intersubunit interactions are weakened by the deletions, implying that

oligomerization of delta2 subunits is disturbed and unstable oligomers are retained in the ER. Homozygous mice behave very similar to delta2-deficient mice, while in heterozygous mice, mutant delta2 is selectively eliminated and not detectable, explaining the recessivity of the mutation. Besides the nine characterized alleles, yet another 11 *hotfoot* alleles have been reported [64, 95], making the delta2 gene a hot spot for spontaneous mutations.

The C Terminus of Delta2 is Decisive for the Subunit's Involvement in LTD

In recent transgenic rescue studies, delta2 receptors carrying a mutation that is predicted to disrupt ligand binding were still able to rescue aberrant synaptogenesis and LTD induction in delta2-deficient mice [39, 40]. Similarly, disrupting ion permeability or solely calcium permeability of delta2 had no effect on these mutant receptors' ability to rescue LTD [41, 42]. By contrast, C-terminally truncated delta2 did not rescue LTD in delta2-deficient mice, despite the fact that the truncated subunits were trafficked normally and restored abnormal synapse formation almost completely [43, 44]. These findings indicate that the role of delta2 in LTD is dependent on its C terminus, while delta2's role in synaptogenesis is mediated by other parts of the protein [44]. Consistently, a decoy peptide representing the C terminus of the protein that was intracellularly perfused during patch clamp experiments could abrogate LTD in acute cerebellar slices, suggesting that the correct interaction of intracellular proteins with the extreme C terminus of delta2 is sufficient for mediating the receptor's role in LTD induction [43].

By contrast, LTD could be normally induced in cultured delta2-deficient Purkinje cells that had been transfected with mutant delta2 receptors lacking the C terminus [52]. An explanation for this divergent report might lie in the different techniques used: transfected cultured Purkinje cells and iontophoretic application of glutamate to induce LTD were used in one case [52], as opposed to recordings from acute cerebellar slices of delta2-deficient mice that had been virus-infected to introduce mutant delta2 receptors and depolarization to induce LTD in other studies [41–44].

The LBD Structure of Delta2 Reveals D-Serine Binding

Recently, the crystal structure of a soluble construct representing the putative LBD of delta2 was reported [53]. This part of the receptor had already been crystallized for all other major iGluR subtypes, revealing that S1 and S2 form a bi-lobed structure, with each segment contributing to both lobes [96–100]. Ligands bind in a deep cleft between the two lobes which are referred to as domain 1 (D1) and domain 2 (D2) (Fig. 3a). Agonist binding activates the receptor by inducing a switch from a relaxed, open-cleft conformation to a constrained, closed-cleft, agonist-bound form. First, glutamate docks to D1 via its α-amino and α-carboxyl groups. Next, D2 is attracted by the γ-carboxyl group of glutamate and rotates (approximately 21° in GluR2 [101]) toward D1 to close the cleft, locking the agonist in its binding pocket. In the majority of crystals, two LBDs assemble to form a twofold symmetric dimer in which the backsides of D1 form an extensive interface between the two monomers.

Similarly, the *apo* structure of delta2 shows a twofold symmetric dimer in which each monomer forms the typical bi-lobed structure comprised of D1 and D2 (Fig. 3). A unique feature of the delta2 LBD is the tight coordination of two calcium ions at the dimer interface by four residues in D1. While overall structural similarity is highest to the AMPA receptor GluR2 LBD, the ligand-binding cavity most closely resembles that of the glycine/D-serine-binding NR1 subunit. Indeed, D-serine and glycine were found to bind to the soluble delta2 LBD protein, however, with a very high K_D value (in the millimolar range) and thus a very low affinity [53].

The structure of the D-serine-bound form revealed a ligand coordination similar to that of glycine in NR1 with two remarkable differences [53]. First, a tyrosine residue (Y543) located in the D1–D2 hinge region provides a hydrogen bond to the β-hydroxyl group of D-serine. By contrast, a serine residue (S688) from a completely different receptor region coordinates the equivalent β-hydroxyl group of glycine in NR1. Secondly, in NR1 three water molecules are coordinated with D-serine in the ligand-binding cavity, while D-serine completely fills the cavity in delta2. The degree of domain closure induced by D-serine binding to delta2 is 9° larger than the 21° observed in crystal structures of the AMPA receptor GluR2 [53, 96, 101]. In contrast to the *apo* structure, the D-serine-bound form of the delta2 LBD did not crystallize as a dimer. Furthermore, the calcium coordination observed in the dimer of the *apo* structure is absent. Instead, a sodium ion occupies the corresponding position. Notably, receptor modulation by extracellular ions that bind at the dimer interface of iGluR LBDs has also been reported for kainate receptors [102–104].

Electrophysiology with Spontaneously Gating Orphan Receptors

Electrophysiological tests using typical glutamatergic agonists all failed to activate delta1 or delta2 in commonly used expression systems (Table 3). Likewise, early inves-

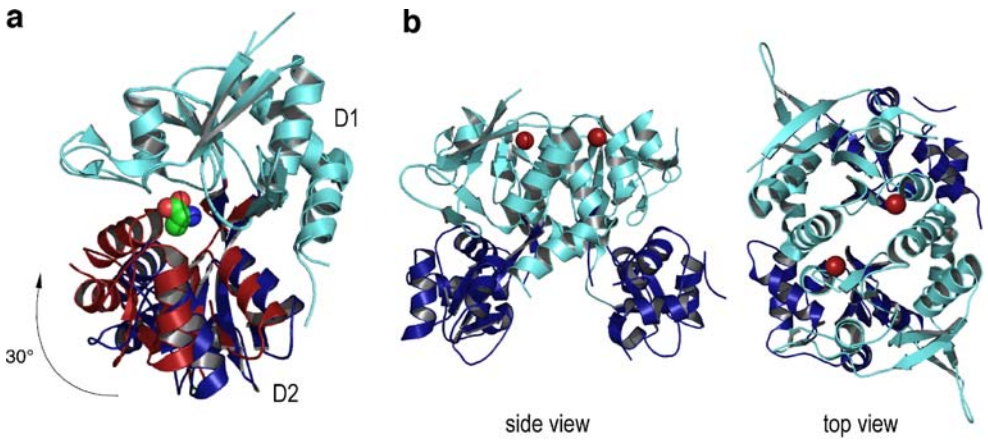

Fig. 3 LBD structure of delta2. **a** Monomeric structure of the LBD of delta2 (PDB code 2V3U). Shown is an overlay of the *apo* and D-serine-bound forms, aligned are the D1 lobes (cyan); D2 movement is indicated by the *arrow*. The open conformation of D2 is indicated in *blue*, the closed conformation in *red*. **b** Dimeric structure of the LBD of delta2 in its *apo* conformation both in *side* and *top view* (PDB code 2V3T). The coordinated calcium ions are highlighted in *red*

tigations using ion pore domain transplantation failed to demonstrate that the two delta receptor pores are able to form functional ion channels when transplanted into AMPA, kainate, or NMDA receptor subunits [105].

This lack of ionotropic function and pharmacological tools to manipulate delta receptors has prevented the elucidation of their specific involvement in synaptic transmission until today. Even though the recently obtained crystal structure revealed D-serine and glycine binding to delta2, both substances fail to elicit any current responses at delta2 wild-type receptors [53]. Thus, all electrophysiological characterization of delta receptor properties remains restricted to the analysis of the spontaneous currents through delta-*lurcher* channels (Table 4). However, even this indirect approach proved inconclusive for delta1. The introduction of the *lurcher* mutation at the corresponding site in the delta1 subunit yielded only weak spontaneous activation in one case [29], while another group could not find spontaneous activation at all [28]. As a consequence, all delta ion channel properties have been inferred from delta2-*lurcher* channels only. The results of these studies revealed both AMPAR- and NMDAR-like features.

The spontaneous currents through delta2-*lurcher* show a doubly rectifying I/V relationship and a calcium permeability comparable to that of AMPA and kainate receptors (Q variants) [27]. Mutation of the naturally occurring gluta-

Table 3 Pharmacological experiments with wild type delta receptors

Wild-type delta subunit	Co-expressed subunit	Substances tested	Expression system	Reference
delta2	–	Not specified	*Xenopus* oocytes	Araki et al. [2]
delta1	–	Glu, KA, AMPA	HEK293	Lomeli et al. [3]
delta1	GluR7			
delta1	delta2	D-Ser, D-Ser+Glu, Gly, Gly+Glu	*Xenopus* oocytes	Naur et al. [53]
delta2	–	Glu, KA, AMPA	HEK293	Lomeli et al. [3]
delta2	GluR1, 2, 3			
delta2	GluR5			
delta2	KA1			
delta2	NR1			
delta2	NR2A, B, C			
delta2	–	Gly, L-cysteic acid, D- and L-forms of the following amino acids: A, V, L, I, P, S, T, C, M, Y, F, W, H, E, D, N, K, R	*Xenopus* oocytes	Naur et al. [53]
delta2	GluR1, 2	D-Ser, D-Ser+Glu, Gly, Gly+Glu		
delta2	GluR6			
delta2	KA1, 2			
delta2	NR1			
delta2	NR2A, B, C, D			
delta2	NR3B			
delta2	–	D-Ser, D-Ser+Ca^{2+}, Gly, Gly+Ca^{2+}	HEK293	Naur et al. [53]
delta2	GluR2	D-Ser, D-Ser+Glu, Gly, Gly+Glu		

Reported receptor-ligand combinations tested with delta receptors in *Xenopus* oocytes or HEK293 cells. Either no current responses were observed, or in case of coexpression with other iGluR subunits, responses did not deviate from controls expressing the other iGluR subunits alone. Agonist concentrations used were: Glu (300 μM), KA (300 μM), AMPA (100 μM), D-Ser (1 mM oocytes, 10 mM HEK293), Gly (1 mM oocytes, 10 mM HEK293), Ca^{2+} (0 mM or 10 mM), other amino acids (1 mM).

Table 4 Electrophysiological experiments with delta *lurcher* mutants

Mutant delta subunit	Q/R site amino acid	Substances/characteristics tested	Expression system	Reference
delta2-*lurcher*	Q	No response to agonists: 1 mM Glu, 100 μM KA, 1 mM Asp; each ±10 μM Gly No response to antagonists: 100 μM CNQX, 100 μM APV, 100 μM 7-CKA Potassium-permeable pore	*Xenopus* oocytes	Zuo et al. [25]
delta2-*lurcher*	Q	Doubly rectifying current-voltage relationship Moderate calcium permeability (P_{Ca}/P_{mono}=0.44) Spontaneous currents NASP-sensitive (10 μM) No modulation by: CTZ (50 μM), GYKI (50–100 μM), MK801, ConA (300 μg/μl), NBQX (10–20 μM)	HEK293	Kohda et al. [26]
	R	Linear current-voltage relationship Calcium-impermeable Spontaneous currents NASP-insensitive (10 μM) No modulation by: CTZ (50 μM), GYKI (50–100 μM), MK801, ConA (300 μg/μl), NBQX (10–20 μM)		
delta2-*lurcher*	Q	Doubly rectifying current-voltage relationship Calcium-permeable (2–3%, fura-2 fluorescence) Potentiation by extracellular calcium	HEK293	Wollmuth et al. [27]
	R	Linear current-voltage relationship Calcium-impermeable		
delta1-*lurcher*	Q	Spontaneous currents Calcium-permeable (qualitative test) Modulation by PKC activator	*Xenopus* oocytes	Ikeno et al. [29]
delta2-*lurcher*	Q	Spontaneous currents Various pore blockers, most sensitive to pentamidine and 9-THA Sensitivity to extracellular pH, IC_{50}=7.5	*Xenopus* oocytes	Williams et al. [28]
delta1-*lurcher*	Q	No spontaneous current		
delta2-*lurcher*	Q	Reduction of spontaneous current (in order of magnitude): D-Ser, Gly, D-Ala, L-Ala, D-Cys, L-Asp (minimal reduction: L-cysteic acid, D-Thr, L-Cys, D-Met, D-Asp, L-Thr) No response to L-Met and the D- and L-forms of (all 1 mM): V, L, I, P, Y, F, W, H, E, N, K, R	*Xenopus* oocytes	Naur et al. [53]

Reported electrophysiological tests with delta receptor *lurcher* mutants in *Xenopus* oocytes or HEK293 cells; the main results are given under substances/characteristics tested. The amino acid at the position corresponding to the Q/R site is indicated. Wild type delta receptors both feature a Q at this site.

mine at the Q/R site of delta2 to arginine diminishes rectification and calcium permeability, analogous to AMPA and kainate receptors [27]. A unique feature of delta2-*lurcher* is that extracellular calcium potentiates spontaneous currents with a maximal potentiation at physiological calcium concentrations [27]. This finding is particularly interesting with regard to the recently discovered coordination of two calcium ions at the dimer interface in the *apo* structure of the delta2 LBD [53].

Analogous to currents through NMDA receptors, the spontaneous current through delta2-*lurcher* is reduced by extracellular protons, with the maximal inhibition of 70% occurring at pH 6.5 to 6.0 [28]. Open delta2-*lurcher* channels are blocked by 1-naphthylacetyl spermine (NASP), *N*-dansylspermine, *N*-dansylspermidine, pentamidine, and 9-tetrahydroaminoacridine (9-THA) [26, 28]. Both pentamidine and 9-THA are open channel blockers of NMDA receptors, where they block currents in a voltage-dependent manner [28]. Application of glutamate (1 mM), aspartate (1 mM), or kainate (100 μM) had no effect on spontaneous currents through delta2-*lurcher*, regardless of the simultaneous presence of glycine (10 μM), CNQX (100 μM), AP-5 (100 μM), or 7-chlorokynurenic acid (7-CKA) (100 μM) [25]. Although the spontaneous gating of delta2-*lurcher* channels shows that the protein is able to form homomeric ion channels, the spontaneous activity could, until recently, only be manipulated by substances acting on the open channel and has remained insensitive to substances that targeted the LBD of delta2 [26].

The first evidence that the LBD of delta2 does communicate its conformational status to the ion pore came from the application of an antibody that was designed to specifically recognize amino acids 505–514 in the ligand-binding cleft of delta2 [84]. This antibody blocks the spontaneous currents through delta2-*lurcher* and hence was categorized as antagonistic. Injection of the antibody into the supracerebellar space of adult mice transiently caused

ataxia and blocked LTD [84]. Additional evidence for communication between LBD and ion pore of delta2 stems from the effect of D-serine or glycine on delta2-*lurcher*. Both substances inhibit the spontaneous current through delta2-*lurcher* when applied at high concentrations [53]. Contrary to the initial report by Zuo et al. [25], the competitive NR1 antagonist 7-CKA was now found to reduce the spontaneous current through delta2-*lurcher* channels [106]. Hence, the profile of substances acting on delta2-*lurcher* shows similarities to the pharmacological profile of NR1. However, none of the substances was able to evoke current responses at delta2-*lurcher* channels in addition to the basal spontaneous currents, regardless of their agonistic or antagonistic nature with respect to NR1.

The only compound that can increase the amplitude of spontaneous activation therefore remains extracellular calcium. Interestingly, the presence of extracellular calcium decreases the potency of D-serine to reduce the spontaneous current through delta2-*lurcher* [107]. Extracellular calcium and D-serine thus seem to counteract each other's influence on the LBD of delta2. However, despite all recent progress, it is still unclear whether wild-type delta receptors contribute to EPSCs at synapses or rather exert their physiological functions via protein interactions at their C termini.

Can Delta Subunits Form Heteromers with Other iGluR Subunits?

Although homomeric delta receptors reach the plasma membrane in heterologous expression systems [3], delta receptors might exert their function in vivo by forming heteromers with other iGluRs. A number of coexpression experiments with iGluRs from all major subtypes have been reported (Table 3). None of the tested combinations, however, showed any indication arguing for the formation of functional ionotropic heteromers.

Coimmunoprecipitation experiments yielded ambiguous results: Antibodies against delta2 did not co-precipitate GluR2/3, GluR6, or KA2 from solubilized cerebellar membranes [4], whereas delta2 partially co-precipitated from cerebellar synaptosomal fractions with antibodies against GluR2 or KA2 [108]. Recombinant heteromeric expression of the artificial R variant of delta2-*lurcher* with Q variants of GluR1-*lurcher* and GluR6-*lurcher* yielded non-rectifying channels, indicative of delta2 incorporation into both GluR1- and GluR6-*lurcher* channels [108]. Coexpression of GluR1 and GluR6 with the wild-type delta2 subunit, however, reduced current amplitudes compared to homomeric GluR1 and GluR6 responses. Thus, if heteromeric wild-type receptors assemble in vivo, they will very likely not participate in glutamate-evoked EPSCs.

At the parallel fiber synapse of Purkinje cells, delta2 is colocalized with mGluR1, GluR2/3, and GluR5–7, but only with minimal amounts of GluR1 and NR1 [7]. Furthermore, delta2 is linked to mGluR1 and AMPA receptors via Shank proteins. Both findings hint at an intimate but perhaps feeble interaction with AMPA and kainate receptors, but incorporation into heteromeric assemblies remains to be shown in vivo.

Open Questions and Future Directions

One of the big open questions with respect to delta receptor physiological function is still whether these subunits serve as ion channels in vivo. In this regard, arguments for a mere metabotropic function of delta2 receptors have been brought forward that stem from two lines of evidence [39, 40, 42]: First, an early pharmacological study had shown that AMPA receptor antagonists such as CNQX can completely block EPSCs at the parallel fiber Purkinje cell synapse in the cerebellum [109]. This was taken as an indication that the entire EPSC is carried by AMPA receptors with no contribution by delta2. This argument, however, assumes that delta2 is not susceptible to block by CNQX, which might not be the case in light of recent findings that the LBD has a pharmacological profile similar to NR1, which was shown to be sensitive to CNQX [53, 106]. Furthermore, the amplitudes and kinetics of mEPSCs are normal in delta2-deficient mice [15, 52]. Both these findings have led to the conclusion that delta2 receptors likely do not contribute to EPSCs at the parallel fiber Purkinje cell synapse [39, 40, 42]. The second line of evidence stems from transgenic rescue studies showing that the apparent disruption of delta2 ligand binding or ion permeability does not interfere with the mutant subunit's ability to rescue the abrogated synaptic plasticity in delta2-deficient mice [39–42]. Hence, ligand-gated ion channel function seems not to be required for the subunit's involvement in cerebellar synaptic plasticity.

On the other hand, the spontaneous current through homomeric delta2-*lurcher* channels clearly shows that the mutant receptor is capable of fluxing ions, although in this case only passively and not ligand-induced. Furthermore, two findings showed that the LBD of delta2 does communicate its conformation to the ion pore under certain circumstances: D-serine and an antibody directed against the LBD of delta2 reduced spontaneous current through delta2-*lurcher* channels [53, 84].

Similar to the NR1 and NR3 subunits of NMDA receptors, delta2 binds D-serine and glycine [53]. However, D-serine application does not evoke current responses at recombinant wild-type delta2 receptors, and the spontaneous current through delta2-*lurcher* is blocked by D-serine, rather than

activated. One major difference between delta2 and the NR1 and NR3 subunits appears to be the affinity for D-serine and glycine. As determined by isothermal titration calometry, the D-serine affinity of the isolated delta2 LBD is three orders of magnitude lower than typical values reported for the NMDA receptor subunits [53]. If D-serine or glycine were the activating signals for delta2, such a low affinity would require concentrations of D-serine and glycine that are hard to reconcile with those found in vivo (extracellular D-serine concentration in the adult cerebellum is 0.5 μM as determined by in vivo microdialysis [110]).

With regard to the availability of D-serine in sufficiently high concentration, two findings are particularly interesting: First, D-serine concentrations are high in the developing cerebellum and restricted to Bergmann glia cells that ensheath parallel fiber to Purkinje cell synapses [110, 111]. Concentrations peak at P14 and then decline to negligible levels in the adult. In comparison, the expression of delta2 in Purkinje cells starts as early as E15 and is maintained through adulthood [4]. Second, the effective concentration necessary to reduce spontaneous current through delta2-*lurcher* channels varies with the concentration of extracellular calcium. D-Serine is more potent in calcium-free solutions (~300 μM at 0 mM calcium), while at higher calcium concentrations, the potency declines to values in the millimolar range (~3 mM at 3 mM calcium) [107]. It is worth mentioning that the introduction of the *lurcher* mutation into other iGluR subunits decisively increases agonist potency [26, 112]. Likewise, the reported potency of D-serine to reduce spontaneous current at delta2-*lurcher* channels might be different from actual D-serine potencies of wild-type delta2 receptors. Hence, although the report by Naur and colleagues [53] represented the first demonstration that a ligand can bind to and induce a functional effect on delta2, the findings also suggest that in vivo D-serine levels might not be sufficient to affect delta2—at least not in the adult.

As an additional or alternative possibility, extracellular calcium may be required to modulate and/or activate delta receptors. It is not unprecedented that extracellular calcium can be a first messenger. The human Ca^{2+}-sensing receptor (CaR) is a G-protein-coupled receptor (GPCR) that is activated by rising extracellular calcium [113]. Most interestingly, it belongs to the same family of GPCRs as the mGluRs [114], the $GABA_B$ receptor [115, 116], and the GPRC6A receptor [117]. Furthermore, the ectodomain of the CaR is related to bacterial periplasmic binding proteins, just like the NTD of iGluRs [113, 118].

It is not intuitively obvious that calcium may serve as an important first messenger. The ion occurs at least in a 10,000-fold higher concentration in the extracellular space than in the intracellular space. Nevertheless, there is accumulating evidence that locally confined changes of extracellular calcium are sensed by cells and have profound consequences for synaptic transmission [119–121]. Simulations indicate that substantial depletion of extracellular calcium (from 1.5 to 0.8 mM) in the synaptic cleft can occur during high-frequency stimulations [122]. The delta receptors might therefore be involved in sensing depletion of extracellular calcium. However, how such a signal might modulate the delta2 receptor and cerebellar synaptic plasticity remains to be elucidated.

The recent crystallization of the LBD of delta2 and identification of ligands that modulate delta2-*lurcher* function [53] as well as transgenic rescue studies [40–42, 44] have provided exciting progress toward clarifying delta receptor function. However, many open questions remain unanswered. Do delta receptors form homomeric complexes, or do they require other iGluRs or accessory proteins to function? Do delta receptors function as ion channels or as metabotropic receptors? Do they contribute to EPSCs? How different is delta1 from delta2?

Especially this last question has not been addressed at all, as all work focused on delta2 after the *lurcher* mutation had been described in the delta2 subunit. Though it becomes increasingly difficult to imagine delta receptors as 'old-fashioned' ligand-gated ion channels, the question why both subunits retain a conserved channel domain certainly requires a convincing answer.

Acknowledgments We thank Daniel Tapken, Nora Cavara, and Kasper B. Hansen for critical reading of this manuscript, and Thomas Hülsken for calculating the phylogenetic tree.

References

1. Yamazaki M, Araki K, Shibata A, Mishina M (1992) Molecular cloning of a cDNA encoding a novel member of the mouse glutamate receptor channel family. Biochem Biophys Res Commun 183:886–892
2. Araki K, Meguro H, Kushiya E, Takayama C, Inoue Y, Mishina M (1993) Selective expression of the glutamate receptor channel δ2 subunit in cerebellar Purkinje cells. Biochem Biophys Res Commun 197:1267–1276
3. Lomeli H, Sprengel R, Laurie DJ, Köhr G, Herb A, Seeburg PH (1993) The rat δ1 and δ2 subunits extend the excitatory amino acid receptor family. FEBS Lett 315:318–322
4. Mayat E, Petralia RS, Wang Y, Wenthold RJ (1995) Immunoprecipitation, immunoblotting, and immunocytochemistry studies suggest that glutamate receptor δ subunits form novel postsynaptic receptor complexes. J Neurosci 15:2533–2546
5. Safieddine S, Wenthold RJ (1997) The glutamate receptor subunit δ1 is highly expressed in hair cells of the auditory and vestibular systems. J Neurosci 17:7523–7531
6. Takayama C, Nakagawa S, Watanabe M, Mishina M, Inoue Y (1996) Developmental changes in expression and distribution of the glutamate receptor channel δ2 subunit according to the Purkinje cell maturation. Dev Brain Res 92:147–155

7. Zhao H, Wenthold RJ, Wang Y, Petralia RS (1997) δ-glutamate receptors are differentially distributed at parallel and climbing fiber synapses on Purkinje cells. J Neurochem 68:1041–1052
8. Landsend AS, Amiry-Moghaddam M, Matsubara A, Bergersen L, Usami S, Wenthold RJ, Ottersen OP (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-spine synapses. J Neurosci 17:834–842
9. Petralia RS, Zhao H, Wang Y, Wenthold RJ (1998) Variations in the tangential distribution of postsynaptic glutamate receptors in Purkinje cell parallel and climbing fiber synapses during development. Neuropharmacol 37:1321–1334
10. Yatsushiro S, Hayashi M, Morita M, Yamamoto A, Moriyama Y (2000) Glutamate receptor subunit δ2 is highly expressed in a novel population of glial-like cells in rat pineal glands in culture. J Neurochem 75:1115–1122
11. Jakobs TC, Ben Y, Masland RH (2007) Expression of mRNA for glutamate receptor subunits distinguishes the major classes of retinal neurons, but is less specific for individual cell types. Mol Vis 13:933–948
12. Kashiwabuchi N, Ikeda K, Araki K, Hirano T, Shibuki K, Takayama C, Inoue Y, Kutsuwada T, Yagi T, Kang Y, Aizawa S, Mishina M (1995) Impairment of motor coordination, Purkinje cell synapse form, and cerebellar long-term depression in GluRδ2 mutant mice. Cell 81:245–252
13. Hirano T, Kasono K, Araki K, Shinozuka K, Mishina M (1994) Involvement of the glutamate receptor δ2 subunit in the long-term depression of glutamate responsiveness in cultured rat Purkinje cells. Neurosci Let 182:172–176
14. Hirano T, Kasono K, Araki K, Mishina M (1995) Suppression of LTD in cultured Purkinje cells deficient in the glutamate receptor δ2 subunit. NeuroReport 6:524–526
15. Jeromin A, Huganir RL, Linden DJ (1996) Suppression of the glutamate receptor δ2 subunit produces a specific impairment in cerebellar long-term depression. J Neurophysiol 76:3578–3583
16. Funabiki K, Mishina M, Hirano T (1995) Retarded vestibular compensation in mutant mice deficient in δ2 glutamate receptor subunit. NeuroReport 7:189–192
17. Kurihara H, Hashimoto K, Kano M, Takayama C, Sakimura K, Mishina M, Inoue Y, Watanabe M (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
18. Kishimoto Y, Kawahara S, Suzuki M, Mori H, Mishina M, Kirino Y (2001) Classical eyeblink conditioning in glutamate receptor subunit δ2 mutant mice is imparied in the delay paradigm but not in the trace paradigm. Eur J Neurosci 13:1249–1253
19. Hashimoto K, Ichikawa R, Takechi H, Inoue Y, Aiba A, Sakimura K, Mishina M, Hashikawa R, Konnerth A, Watanabe M, Kano M (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
20. Ichikawa R, Miyazaki T, Kano M, Hashikawa T, Tatsumi H, Sakimura K, Mishina M, Inoue Y, Watanabe M (2002) Distal extension of climbing fiber territory and multiple innervation caused by aberrant wiring of adjacent spiny branchlets in cerebellar Purkinje cells lacking glutamate receptor δ2. J Neurosci 22:8487–8503
21. Takatsuki K, Kawahara S, Kotani S, Fukunaga S, Mori H, Mishina M, Kirino Y (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
22. Yoshida T, Katoh A, Ohtsuki G, Mishina M, 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
23. Katoh A, Yoshida T, Himeshima Y, Mishina M, Hirano T (2005) Defective control and adaption of reflex eye movements in mutant mice deficient in either the glutamate receptor δ2 subunit or Purkinje cells. Eur J Neurosci 21:1315–1326
24. Gao J, Maison SF, Wu X, Hirose K, Jones SM, Bayazitov I, Tian Y, Mittleman G, Matthews DB, Zakharenko SS, Liberman MC, Zuo J (2007) Orphan glutamate receptor delta1 subunit required for high-frequency hearing. Mol Cell Biol 27:4500–4512
25. Zuo J, DeJager PL, Takahashi KA, Jiang W, Linden DJ, Heintz N (1997) Neurodegeneration in *Lurcher* mice caused by mutation in delta2 glutamate receptor gene. Nature 388:769–773
26. Kohda K, Wang Y, Yuzaki M (2000) Mutation of a glutamate receptor motif reveals its role in gating and δ2 receptor channel properties. Nat Neurosci 3:315–322
27. Wollmuth LP, Kuner T, Jatzke C, Seeburg PH, Heintz N, Zuo J (2000) The *lurcher* mutation identifies δ2 as an AMPA/kainate receptor-like channel that is potentiated by Ca^{2+}. J Neurosci 20:5973–5980
28. Williams K, Dattilo M, Sabado TN, Kashiwagi K, Igarashi K (2003) Pharmacology of delta2 glutamate receptors: effects of pentamidine and protons. J Pharm and Exp Ther 305:740–748
29. Ikeno K, Yamakura T, Yamazaki M, Sakimura K (2001) The *lurcher* mutation reveals Ca^{2+} permeability and PKC modification of the GluRδ channels. Neurosci Res 41:193–200
30. Heintz N, Jager PLD (1999) GluRδ2 and the development and death of cerebellar Purkinje neurons in *lurcher* mice. Ann NY Acad Sci 868:502–514
31. Norman DJ, Feng L, Cheng SS, Gubbay J, Chan E, Heintz N (1995) The *lurcher* gene induces apoptotic death in Purkinje cells. Development 121:1183–1193
32. Selimi F, Doughty M, Delhaye-Bouchaud N, Mariani J (2000) Target-related and intrinsic neuronal death in *Lurcher* mutant mice are both mediated by caspase-3 activation. J Neurosci 20:992–1000
33. Zanjani HS, Vogel MW, Martinou JC, Delhaye-Bouchaud N, Mariani J (1998) Postnatal expression of hu-Bcl-2 gene in *Lurcher* mutant mice fails to rescue Purkinje cells but protects inferior olivary neurons from target-related cell death. J Neurosci 18:319–327
34. Selimi F, Vogel MW, Mariani J (2000) Bax inactivation in *Lurcher* mutant rescues cerebellar granule cells but not Prukinje cells or inferior olivary neurons. J Neurosci 20:5339–5345
35. Doughty ML, Jager PLD, Korsmeyer SJ, Heintz N (2000) Neurodegeneration in *lurcher* mice occurs via multiple cell death pathways. J Neurosci 20:3687–3694
36. Lu W, Tsirka SE (2002) Partial rescue of neural apoptosis in the *Lurcher* mutant mouse through elimination of tissue plasminogen activator. Development 129:2043–2050
37. Yue Z, Horton A, Bravin M, DeJager PL, Selimi F, Heintz N (2002) A novel protein complex linking the delta2 glutamate receptor and autophagy: implications for neurodegeneration in *Lurcher* mice. Neuron 35:921–933
38. Selimi F, Lohof AM, Heitz S, Lalouette A, Jarvis CI, Bailly Y, Mariani J (2003) *Lurcher* GRID2-induced death and depolarization can be dissociated in cerebellar Purkinje cells. Neuron 37:813–819
39. Hirai H, Miyazaki T, Kakegawa W, Matsuda S, Mishina M, Watanabe M, Yuzaki M (2005) Rescue of abnormal phenotypes of the δ2 glutamate receptor-null mice by mutant δ2 transgenes. EMBO Rep 6:90–95
40. Yuzaki M (2005) Transgenic rescue for characterizing orphan receptors: a review of δ2 glutamate receptor. Transgenic Res 14:117–121
41. Kakegawa W, Kohda K, Yuzaki M (2007) The δ2 'ionotropic' glutamate receptor functions as a non-ionotropic receptor to control cerebellar synaptic plasticity. J Physiol 584:89–96

42. Kakegawa W, Miyazaki T, Hirai H, Motohashi J, Mishina M, Watanabe M, Yuzaki M (2007) Ca^{2+} permeability of the channel pore is not esssential for the δ2 glutamate receptor to regulate synaptic plasticity and motor coordination. J Physiol 579:729–735
43. Kohda K, Kakegawa W, Matsuda S, Nakagami R, Kakiya N, Yuzaki M (2007) The extreme C-terminus of GluRdelta2 is essential for the induction of long-term depression in cerebellar slices. Eur J Neurosci 25:1357–1362
44. Kakegawa W, Miyazaki T, Kyoichi E, Matsuda K, Kohda K, Motohashi J, Mishina M, Kawahara S, Watanabe M, Yuzaki M (2008) Differential regulation of synaptic plasticity and cerebellar motor learning by the C-terminal PDZ-binding motif of GluRδ2. J Neurosci 28:1460–1468
45. Roche KW, Ly CD, Petralia RS, Wang YX, McGee AW, Bredt DS, Wenthold RJ (1999) Postsynaptic density-93 interacts with the delta2 glutamate receptor subunit at parallel fiber synapses. J Neurosci 19:3926–3934
46. Hironaka K, Umemori H, Tezuka T, Mishina M, Yamamoto T (2000) The protein-tyrosine phosphatase PTPMEG interacts with glutamate receptor δ2 and ε subunits. J Biol Chem 275:16167–16173
47. Ly CD, Roche KW, Lee H, Wenthold RJ (2002) Identification of rat EMAP, a δ-glutamate receptor binding protein. Biochem Biophys Res Comm 291:85–90
48. Miyagi Y, Yamashita T, Fukaya M, Sonoda T, Okuno T, Yamada K, Watanabe M, Nagashima Y, Aoki I, Okuda K, Mishina M, Kawamoto S (2002) Delphilin: A novel PDZ and formin homology domain-containing protein that synaptically colocalizes and interacts with glutamate receptor δ2 subunit. J Neurosci 22:803–814
49. Yap CC, Murate M, Kishigami S, Muto Y, Kishida H, Hashikawa T, Yano R (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
50. Yap CC, Muto Y, Kishida H, Hashikawa T, Yano R (2003) PKC regulates the δ2 glutamate receptor interaction with S-SCAM/MAGI-2 protein. Biochem Biophys Res Comm 301:1122–1128
51. Uemura T, Mori H, Mishina M (2004) Direct interaction of GluRδ2 with Shank scaffold proteins in cerebellar Purkinje cells. Mol Cell Neurosci 26:330–341
52. Yawata M, Tsuchida H, Kengaku M, Hirano T (2006) Membrane-proximal region of glutamate receptor delta2 subunit is critical for long-term depression and interaction with protein interacting with C kinase 1 in a cerebellar Purkinje neuron. J Neurosci 26:3626–3633
53. Naur P, Hansen KB, Kristensen AS, Dravid SM, Pickering DS, Olsen L, Verstergaard B, Egebjerg J, Gajhede M, Traynelis SF, Kastrup JS (2007) Ionotropic glutamate-like receptor δ2 binds D-serine and glycine. Proc Natl Acad Sci USA 104:14116–14121
54. Boulter J (1994) Nucleotide sequence of rat glutamate receptor subunit gene delta1. GenBank U08255
55. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D (2002) The human genome browser at UCSC. Genome Res 12:996–1006
56. Boulter J (1994) Nucleotide sequence of rat glutamate receptor subunit gene delta2. GenBank U08256
57. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W et al (2001) Initial sequencing and analysis of the human genome. Nature 409:860–921
58. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA et al (2001) The sequence of the human genome. Science 291:1304–1351
59. Consortium IHGS (2004) Finishing the euchromatic sequence of the human genome. Nature 431:931–945
60. Klein SL, Strausberg RL, Wagner L, Pontius J, Clifton SW, Richardson P (2002) Genetic and genomc tools for *Xenopus* research: The NIH *Xenopus* initiative. Dev Dyn 225:384–391
61. Rasooly RS, Henken D, Freeman N, Tompkins L, Badman D, Briggs J, Hewitt AT et al (2003) Genetic and genomic tools for zebrafish research: The NIH zebrafish initiative. Dev Dyn 228:490–496
62. Hu W, Zuo J, de Jager PL, Heintz N (1998) The human glutamate receptor δ2 gene (GRID2) maps to chromosome 4q22. Genomics 47:143–145
63. Treadaway J, Zuo J (1998) Mapping of the mouse glutamate receptor δ1 subunit (Grid1) to chromosome 14. Genomics 54:359–360
64. Wang Y, Matsuda S, Drews V, Torashima T, Meisler MH, Yuzaki M (2003) A hot spot for *hotfoot* mutations in the gene encoding the delta2 glutamate receptors. Eur J Neurosci 17:1581–1590
65. Ayalon G, Stern-Bach Y (2001) Functional assembly of AMPA and kainate receptors is mediated by several discrete protein-protein interactions. Neuron 31:103–113
66. Hollmann M, Heinemann S (1994) Cloned glutamate receptors. Annu Rev Neurosci 17:31–108
67. Hollmann M, Maron C, Heinemann S (1994) N-glycosylation site tagging suggests a three transmembrane domain topology for the glutamate receptor GluR1. Neuron 13:1331–1343
68. Crépel F (1982) Regression of functional synapses in the immature mammalian cerebellum. Trends Neurosci 5:266–269
69. Takayama C, Nakagawa S, Watanabe M, Mishina M, Inoue Y (1995) Light- and electron-microscopic localization of the glutamate receptor channel δ2 subunit in the mouse Purkinje cell. Neuroscience Letters 188:89–92
70. Morando L, Cesa R, Rasetti R, Harvey R, Strata P (2001) Role of glutamate δ-2 receptors in activity-dependent competition between heterologous afferent fibers. Proc Natl Acad Sci USA 98:9954–9959
71. Cesa R, Morando L, Strata P (2003) Glutamate receptor δ2 subunit in activity-dependent heterologous synaptic competition. J Neurosci 23:2363–2370
72. Takeuchi T, Miyazaki T, Watanabe M, Mori H, Sakimura K, Mishina M (2005) Control of synaptic connection by glutamate receptor δ2 in the adult cerebellum. J Neurosci 25:2146–2156
73. Yuzaki M (2004) The delta2 glutamate receptor: A key molecule controlling synaptic plasticity and structure in Purkinje cells. Cerebellum 3:89–93
74. Hirano T (2006) Cerebellar regulation mechanisms learned from studies on GluRdelta2. Mol Neurobiol 33:1–16
75. Saglietti L, Dequidt C, Kamieniarz K, Rousset MC, Valnegri P, Thoumine O, Beretta F, Fagni L, Choquet D, Sala C, Sheng M, Passafaro M (2007) Extracellular interactions between GluR2 and N-cadherin in spine regulation. Neuron 54:461–477
76. Ohtsuki G, Kawaguchi S, Mishina M, Hirano T (2004) Enhanced inhibitory synaptic transmission in the cerebellar molecular layer of the GluRδ2 knock-out mouse. J Neurosci 24:10900–10907
77. Hirai H, Matsuda S (1999) Interaction of the C-terminal domain of delta glutamate receptor with spectrin in the dendritic spines of cultured Purkinje cells. Neurosci Res 34:281–287
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. Hirai H (2000) Clustering of delta glutamate receptors is regulated by the actin cytoskeleton in the dendritic spines of cultured rat Purkinje cells. Eur J Neurosci 12:563–570

80. Matsuda S, Hannen R, Matsuda K, Yamada N, Tubbs T, Yuzaki M (2004) The C-terminal juxtamembrane region of the d2 glutamate receptor controls its export from the endoplasmatic reticulum. Eur J Neurosci 19:1683–1690
81. Matsuda I, Mishina M (2000) Identification of a juxtamembrane segment of the glutamate receptor δ2 subunit required for the plasma membrane location. Biochem Biophys Res Comm 275:565–571
82. Matsuda S, Matsuda K, Yuzaki M (2006) A new motif necessary and sufficient for stable localization of the δ2 glutamate receptors at postsynaptic spines. J Biol Chem 281:17501–17509
83. Ito M (2001) Cerebellar long-term depression: characterization, signal transduction, and functional roles. Physiol Rev 81:1143–1195
84. Hirai H, Launey T, Mikawa S, Torashima T, Yanagihara D, Kasaura T, Miyamoto A, Yuzaki M (2003) New role of δ2-glutamate receptors in AMPA receptor trafficking and cerebellar function. Nat Neurosci 6:869–876
85. Kondo T, Kakegawa W, Yuzaki M (2005) Induction of long-term depression and phosphorylation of the delta2 glutamate receptor by protein kinase C in cerebellar slices. Eur J Neurosci 22:1817–1820
86. Phillips R (1960) '*Lurcher*', a new gene in linkage group XI of the house mouse. J Genet 57:35–42
87. Cheng SS, Heintz N (1997) Massive loss of mid- and hindbrain neurons during embryonic development of homozygous *lurcher* mice. J Neurosci 17:2400–2407
88. Caddy KW, Biscoe TJ (1979) Structural and quantitative studies on the normal C3H and *Lurcher* mutant mouse. Philos Trans R Soc Lond B Biol Sci 287:167–201
89. Wetts R, Herrup K (1982) Interaction of granule, Purkinje and inferior olivary neurons in *Lurcher* chimeric mice. II. Granule cell death. Brain Res 250:358–362
90. Fortier PA, Smith AM, Rossignol S (1987) Locomotor deficits in the mutant mouse, *Lurcher*. Exp Brain Res 66:271–286
91. Wetts R, Herrup K (1982) Interaction of granule, Purkinje and inferior olivary neurons in *Lurcher* chimeric mice. I. Qualitative studies. J Embryol Exp Morphol 68:87–98
92. Lalouette A, Guenet JL, Vriz S (1998) *Hotfoot* mouse mutations affect the delta2 glutamate receptor gene and are allelic to *lurcher*. Genomics 50:9–13
93. Lalouette A, Lohof A, Sotelo C, Guenet J, Mariani J (2001) Neurobiological effects of a null mutation depend on genetic context: comparison between two *hotfoot* alleles of the delta2 ionotropic glutamate receptor. Neurosci 105:443–455
94. Matsuda S, Yuzaki M (2002) Mutation in *hotfoot*-4J mice results in retention of delta2 glutamate receptors in ER. Eur J Neurosci 16:1507–1516
95. Motohashi J, Kakegawa W, Yuzaki M (2007) Ho15J: a new *hotfoot* allele in a hot spot in the gene encoding the delta2 glutamate receptor. Brain Res 1140:153–160
96. Armstrong N, Sun Y, Chen GQ, Gouaux E (1998) Structure of a glutamate-receptor-ligand-binding core in complex with kainate. Nature 395:913–917
97. Furukawa H, Gouaux E (2003) Mechanisms of activation, inhibition and specificity: crystal structures of NR1 ligand-binding core. EMBO J 22:1–13
98. Furukawa H, Singh SK, Mancusso R, Gouaux E (2005) Subunit arrangement and function in NMDA receptors. Nature 438:185–192
99. Mayer ML (2005) Crystal structures of the GluR5 and GluR6 ligand binding core: molecular mechanisms underlying kainate receptor selectivity. Neuron 45:539–552
100. Nanao MH, Green T, Stern-Bach Y, Heinemann SF, Choe S (2005) Structure of the kainate receptor subunit GluR6 agonist-binding domain complexed with domoic acid. Proc Natl Acad Sci USA 102:1708–1713
101. Armstrong N, Gouaux E (2000) Mechanism for activation and antagonism of an AMPA-sensitive glutamate receptor: crystal structure of the GluR2 ligand binding core. Neuron 28:165–181
102. Plested AJ, Mayer ML (2007) Structure and mechanism of kainate receptor modulation by anions. Neuron 53:829–841
103. Wong AY, Fay AL, Bowie D (2006) External ions are coactivators of kainate receptors. J Neurosci 26:5750–5755
104. Wong AY, MacLean DM, Bowie D (2007) Na^+/Cl^- dipole couples agonist binding to kainate receptor sactivation. J Neurosci 27:6800–6809
105. Villmann C, Strutz N, Morth T, Hollmann M (1999) Investigation by ion channel domain transplantation of rat glutamate receptor subunits, orphan receptors and putative NMDA receptor subunit. Eur J Neurosci 11:1765–1778
106. LeVasseur NL, Hansen KB, Kristensen AS, Naur P, Kastrup JS, Traynelis SF (2007) Identification of ligands capable of modulating the activity of the orphan glutamate-like receptor GluRδ2 *Lurcher* mutant. Society for Neuroscience, San Diego 876.1:F41
107. Hansen KB, LeVasseur NL, Kristensen AS, Naur PKJS, Traynelis SF (2007) Modulation of GluRδ2 function by extracellular calcium. Society for Neuroscience, San Diego, 440.3
108. Kohda K, Kamiya Y, Matsuda S, Kato K, Umemori H, Yuzaki M (2003) Heteromer formation of δ2 glutamate receptors with AMPA and kainate receptors. Mol Brain Res 110:27–37
109. Kano M, Kato M (1987) Quisqualate receptors are specifically involved in cerebellar synaptic plasticity. Nature 325:276–279
110. Hashimoto A, Oka T, Nishikawa T (1995) Extracellular concentration of endogenous free D-serine in the rat brain as revealed by *in vivo* microdialysis. Neuroscience 66:635–643
111. Schell MJ, Brady RO Jr, Molliver ME, Snyder SH (1997) D-serine as a neuromodulator: Regional and developmental localization in rat brain glia resemble NMDA receptors. J Neurosci 17:1604–1615
112. Taverna F, Xiong Z, Brandes L, Roder JC, Salter MW, MacDonald JF (2000) The *lurcher* mutation of an a-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
113. Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J, Hebert SC (1993) Cloning and characterization of an extracellular Ca^{2+}-sensing receptor from bovine parathyroid. Nature 366:575–580
114. Francesconi A, Duvoisin RM (2004) Divalent cations modulate the activity of metabotropic glutamate receptors. J Neurosci Res 75:472–479
115. Wise A, Green A, Main MJ, Wilson R, Fraser N, Marshall FH (1999) Calcium sensing properties of the $GABA_B$ receptor. Neuropharmacol 38:1647–1656
116. Galvez T, Urwyler S, Prezeau L, Mosbacher J, Joly C, Malitschek B, Heid J, Brabet I, Froestl W, Better B, Kaupmann K, Pin JP (2000) Ca^{2+} requirement for high-affinity gamma-aminobutyric acid (GABA) binding at $GABA_B$ receptors: involvement of serine 269 of the $GABA_BR1$ subunit. Mol Pharmacol 57:419–426
117. Christiansen B, Hansen KB, Wellendorph P, Bräuner-Osborne H (2007) Pharmacological characterization of mouse GPRC6A, an L-alpha-amino-acid receptor modulated by divalent cations. Br J Pharmacol 150:798–807
118. Hu J, Spiegel AM (2007) Structure and function of the human calcium-sensing receptor: insights from natural and engineered mutations and allosteric modulators. J Cell Mol Med 11:908–922
119. Brown EM, Vassilev M, Hebert SC (1995) Calcium ions as extracellular messengers. Cell 83:679–682

120. Rusakov DA (2001) The role of perisynaptic glial sheaths in glutamate spillover and extracellular Ca^{2+} depletion. Biophys J 81:1947–1959
121. Rusakov DA, Fine A (2003) Extracellular Ca^{2+} depletion contributes to fast activity-dependent modulation of synaptic transmission in the brain. Neuron 37:287–297
122. Vassilev PM, Mitchel J, Vassilev M, Kanazirska M, Brown EM (1997) Assessment of frequency-dependent alterations in the level of extracellular Ca^{2+} in the synaptic cleft. Biophys J 72:2103–2116
123. Sonoda T, Mochizuki C, Yamashita T, Watanabe-Kaneko K, Miyagi Y, Shigeri Y, Yazama F, Okuda K, Kawamoto S (2006) Binding of glutamate receptor delta2 to its scaffold protein, Delphilin, is regulated by PKA. Biochem Biophys Res Comm 350:748–752