

TOPICAL REVIEW

Glutamate receptor pores

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Abstract Glutamate receptors are ligand-gated ion channels that mediate fast excitatory synaptic transmission throughout the central nervous system. Functional receptors are homo- or heteromeric tetramers with each subunit contributing a re-entrant pore loop that dips into the membrane from the cytoplasmic side. The pore loops form a narrow constriction near their apex with a wide vestibule toward the cytoplasm and an aqueous central cavity facing the extracellular solution. This article focuses on the pore region, reviewing how structural differences among glutamate receptor subtypes determine their distinct functional properties.

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Abbreviations AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNG, cyclic nucleotide gated; iGluR, ionotropic glutamate receptor; KA, kainate; MTS, methanethiosulfonate; NMDA, *N*-methyl-D-aspartate; TMD, transmembrane domain; TRP, transient receptor potential.

Ionotropic glutamate receptors (iGluRs) are members of the pore loop superfamily of ion channels that also includes potassium channels, voltage-gated sodium and calcium channels, cyclic nucleotide gated (CNG) channels and transient receptor potential (TRP) channels (Hille, 2001). In contrast to most members of this family, in iGluRs the re-entrant pore loops dip into the plane of the membrane from the cytoplasmic side, while the central cavity and the inner helix bundle crossing, thought to form the gate for ion flow, face the extracellular side (Sobolevsky *et al.* 2009). Each iGluR subunit contributes exclusively to one of three distinct iGluR subtypes that are named for the agonists *N*-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate (KA) (Hollmann & Heinemann, 1994). These subtypes serve distinct roles at excitatory synapses and aberrant activation of each has been

implicated in pathologies of the nervous system (Traynelis *et al.* 2010).

Functional AMPA and KA receptors may be homo- or heteromeric tetramers, whereas NMDA receptors are obligate heteromers, requiring a GluN1 subunit combined with either a GluN2 or GluN3 subunit (Traynelis *et al.* 2010). Figure 1 displays a protein alignment for the transmembrane domain (TMD) of the four AMPA receptor subunits (GluA1–A4), five KA receptor subunits (GluK1–K5) and seven NMDA receptor subunits (GluN1, GluN2A–2D, GluN3A–3B) from rat. Each TMD comprises one pore loop (M2) that includes a short helix and random coil selectivity filter flanked by outer (M1) and inner (M3) transmembrane helices (Fig. 1A and C; Fig. 2A–C; Sobolevsky *et al.* 2009). All eukaryotic iGluR subunits also include an additional (M4) transmembrane helix (Fig. 1B and C; Fig. 2A–C) that is required for receptor assembly

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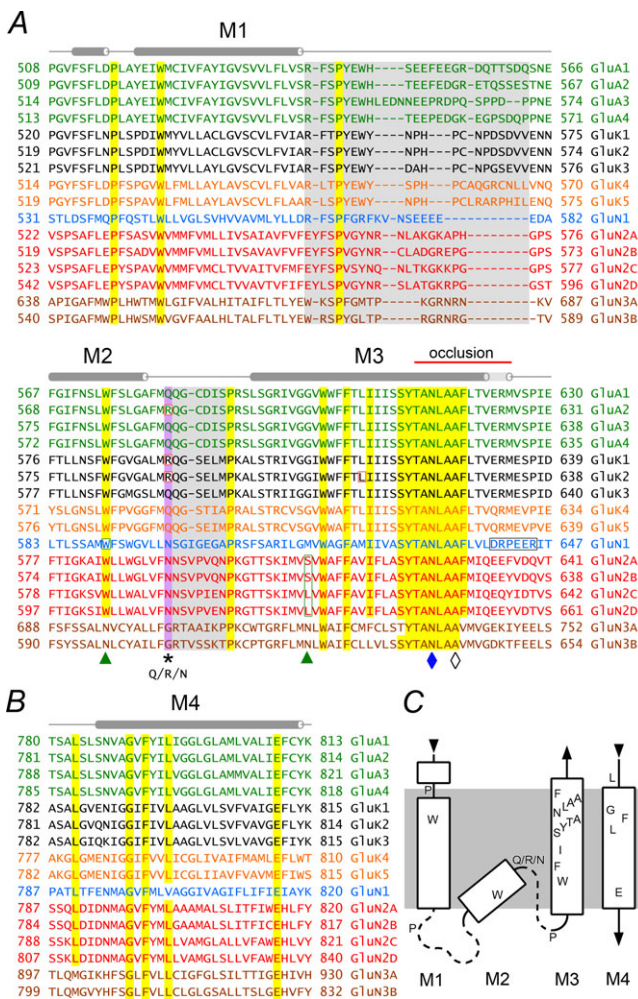


Figure 1. Sequence alignment of glutamate receptor TMDs
A, alignment of the M1–M3 segment for the four AMPA receptor subunits (green, UniProt numbers: P19490–19493), three low affinity KA receptor subunits capable of forming homomeric channels (black, P22756, P42260, P42264), two high affinity KA receptor subunits that do not form functional homomers (orange, Q01812, Q63273), the GluN1 subunit essential for all NMDA receptors (blue, P35439), four GluN2 subunits (red, Q00959–009561, Q62645) and two GluN3 subunits (brown, Q9R1M7, Q8VHN2). All sequences are from rat. Highly conserved residues are highlighted in yellow. Sequence that was not resolved in the GluA2 crystal structure is highlighted in grey. The asterisk indicates the Q/R/N site. GluA2, GluK1 and 2 are shown in the edited (R) form (red boxes). Green triangles indicate the M3 S/L site of GluN2 and the conserved M2 Trp of GluN1 that interact (green boxes). The GluN1 DRPEER motif is boxed in black. The conserved Asn (N5) and *Lurcher* site Ala (A8) in the SYTNLAALF motif are indicated by a filled blue diamond and open black diamond, respectively. Residue numbers in **A** and **B** are for the mature proteins after signal sequence removal. Secondary structure is shown as cylinders (α -helices) and lines (random coil loops) above the alignment. The red line above M3 indicates the occlusion at the inner helix bundle crossing in the GluA2 crystal structure. **B**, alignment of the M4 helix. **C**, diagram of TMD topology displaying the approximate position of the conserved residues highlighted in yellow in **A** and **B**. Dashed lines indicate segments that were not resolved in the GluA2 crystal structure.

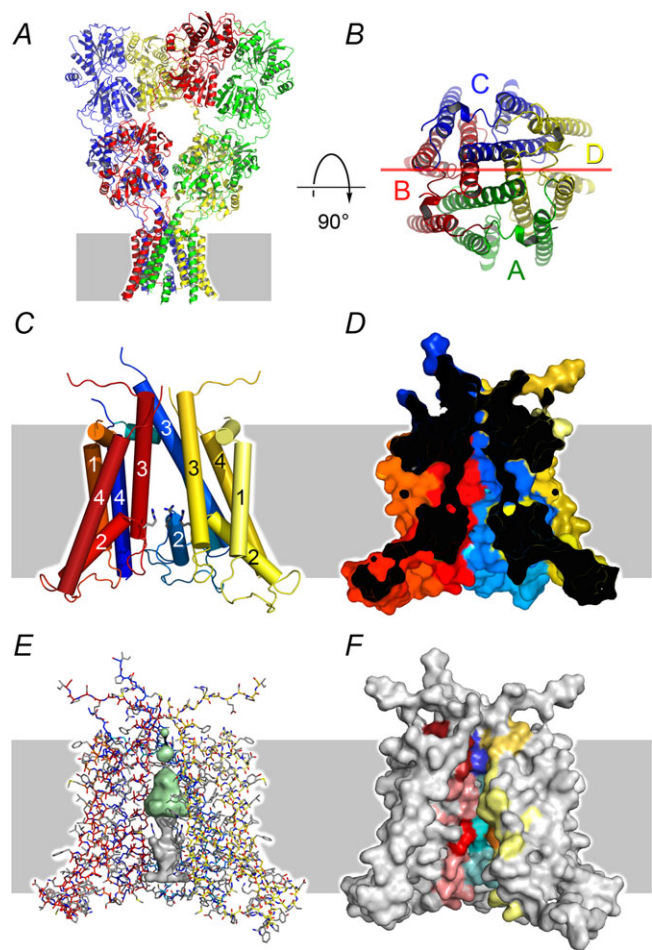


Figure 2. TMD structure

A, X-ray crystal structure of the homomeric GluA2 AMPA receptor (PDB 3KG2) from Sobolevsky *et al.* (2009). Grey background indicates the approximate position of the membrane. **B**, view down the pore axis from the extracellular membrane surface. The A/C (green/blue) and B/D (red/yellow) subunits are labelled. The horizontal red line indicates the plane of section through the TMD shown in side view in panel **D**. **C–F**, homology model of the rat GluK2(R) TMD from Lopez *et al.* (2013) based on the GluA2 crystal structure. For clarity, the A (green) subunit has been removed to provide a view into the pore. All four panels are shown in the same orientation. **C**, M1, M3 and M4 transmembrane helices and the M2 pore-loop helix shown as numbered cylinders. **D**, surface rendering sectioned along the axis of the pore as indicated in **B**. **E**, the pore cavity rendered as a surface generated by the program Hollow (Ho & Gruswitz, 2008). The central cavity is shaded green. The narrow constriction and cytoplasmic vestibule in grey are more speculative because residues in this region were disordered in the GluA2 crystal structure and were modelled without a template. **F**, coloured residues on this surface rendering are homologous to AMPA receptor locations that can be modified by extracellular or intracellular MTS reagents in the presence of agonist (Kuner *et al.* 2001; Sobolevsky *et al.* 2003). Darker colouration near the extracellular surface indicates residues within the bundle crossing occlusion zone.

and function (Schorge & Colquhoun, 2003; Terhag *et al.* 2010; Salussolia *et al.* 2011, 2013). Opening of the pore is controlled by four extracellular ligand binding domains (LBD), with each LBD connected to the transmembrane helices by three short random coil linkers (Sobolevsky *et al.* 2009).

Amino acids throughout the TMD contribute to determining the distinctive pore properties of NMDA, AMPA, and KA receptors but the Q/R/N site near the apex of the pore loop plays a particularly important role (Traynelis *et al.* 2010). Genes for all AMPA and KA receptor subunits encode a glutamine (Q) at this position, but mRNA for the GluA2 AMPA receptor subunit and GluK1 and GluK2 KA receptor subunits can undergo RNA editing that changes the amino acid at this location to arginine (R) (Traynelis *et al.* 2010). GluN1 and GluN2 NMDA receptor subunits all encode asparagine (N) at this position whereas rodent GluN3 subunits have a glycine (G) (Fig. 1A and C) and human GluN3B has an Arg.

The only crystal structure that includes an iGluR TMD is of the homomeric GluA2 AMPA receptor (Sobolevsky *et al.* 2009), which was generated with a competitive antagonist bound and is presumed to represent the closed state (Fig. 2A and B). In this structure the M2 pore helix and transmembrane M1, M3 and M4 helices are resolved; but, the random coil 'turret' domain connecting M1 to M2 and the selectivity filter connecting the M2 helix to the cytoplasmic end of M3 are disordered (Sobolevsky *et al.* 2009). Such disorder in the crystal structure suggests greater intrinsic mobility for these segments in iGluRs (see also Kuner *et al.* 1996), particularly as compared to the relatively rigid selectivity filter of potassium-selective channels (Doyle *et al.* 1998). In contrast to potassium channels (Hille, 2001), iGluRs are unlikely to be long, single file, multi-ion pores (Zarei & Dani, 1994, 1995). Instead, glutamate receptor pores have a short narrow constriction just below the Q/R/N site at the apex of the pore loop (Kuner *et al.* 1996; Wollmuth *et al.* 1996), with a relatively wide vestibule toward the cytoplasm and a water-filled central cavity facing the extracellular solution (Fig. 2D–F; Zarei & Dani, 1995; Kuner *et al.* 2001). In addition, the homomeric GluA2 structure exhibits 2-fold symmetry in the extracellular domains with distinct A/C and B/D subunit conformations arranged diagonally across from each other within the 4-fold symmetric TMD (Fig. 2B; Sobolevsky *et al.* 2009).

NMDA receptor pores

For conventional NMDA receptors, made up of GluN1 and GluN2 subunits, there is substantial evidence supporting an N1–N2–N1–N2 alternating arrangement (Sobolevsky *et al.* 2009; Salussolia *et al.* 2011; Riou *et al.* 2012), although a few studies instead favor an N1–N1–N2–N2 adjacent

organization (Schorge & Colquhoun, 2003; Balasuriya *et al.* 2013). Detailed molecular architecture within the pore is currently lacking; however, inferences about structure can be made from the permeability of organic cations, from the accessibility of substituted cysteine residues to modification by methanethiosulfonate (MTS) reagents, and from the effects of amino acid substitutions on pore properties, all of which suggest that NMDA receptors have asymmetric pores. Based on the relative permeability of organic cations, NMDA receptors have a rectangular minimal cross-section estimated at 0.45 by 0.55 nm (Villarroel *et al.* 1995; Zarei & Dani, 1995), which is somewhat larger than the narrow selectivity filters defined for sodium (~0.31 by 0.51 nm) and potassium (~0.33 nm diameter) selective channels but smaller than the ~0.65 nm diameter pore of pentameric acetylcholine receptors (Hille, 2001). Cysteine substitutions along the M3 helix have demonstrated asymmetric modification for homologous positions of GluN1 (Beck *et al.* 1999) versus GluN2 subunits (Sobolevsky *et al.* 2002), suggesting an offset and asymmetry of the A–C (GluN1) relative to B–D (GluN2) subunit pairs. For Cys substitutions in the pore loop, several positions can be modified by cytoplasmic application of MTS reagents, whereas modification of GluN1 and GluN2 by extracellular MTSET only occurs for Cys substitution at the Q/R/N site (Kuner *et al.* 1996), suggesting that the Asn side-chain at the Q/R/N position of each subunit extends up into the central cavity while other pore loop residues face away from the central cavity and in many cases contribute to the cytoplasmic vestibule.

NMDA receptor channels are permeable to Na⁺, K⁺ and Ca²⁺, but are subject to voltage-dependent block by Mg²⁺ (Traynelis *et al.* 2010). This unique permeation profile underlies their role as coincidence detectors in triggering synaptic plasticity (Bliss & Collingridge, 1993). Calcium entry through NMDA receptors activates cytoplasmic biochemical pathways that regulate synaptic strength (Lisman *et al.* 2012) but only when cells are sufficiently depolarized to relieve channel block by Mg²⁺ (Mayer *et al.* 1984; Nowak *et al.* 1984). Single amino acid substitutions have shown that calcium permeability and magnesium block both involve interactions at or near the Q/R/N site (Burnashev *et al.* 1992a,b; Mori *et al.* 1992; Cavara *et al.* 2010), as does channel block by organic inhibitors such as MK-801 and memantine (Mori *et al.* 1992; Sakurada *et al.* 1993; Kashiwagi *et al.* 2002; Chen & Lipton, 2005). However, homologous substitutions in GluN1 and GluN2 indicate that the two subunits make asymmetric contributions. For example, Asn replacement with Gln in GluN1, when co-expressed with wild-type GluN2A or 2C, reduces calcium permeability and increases channel block by calcium. This substitution also slightly reduces block by extracellular magnesium (Burnashev *et al.* 1992b) and strongly decreases block by

intracellular magnesium (Kupper *et al.* 1998; Wollmuth *et al.* 1998b). In contrast, substitutions at the Q/R/N site, or the adjacent N+1 Asn, of GluN2 cause only a modest decline in calcium permeability but substantially increase magnesium permeability and reduce block by magnesium applied extracellularly (Burnashev *et al.* 1992b; Sakurada *et al.* 1993; Wollmuth *et al.* 1996, 1998a) and, to a lesser extent, intracellularly (Kupper *et al.* 1998; Wollmuth *et al.* 1998b). Similar evidence for asymmetry at the pore loop comes from studies of channel block by memantine, which is reduced to a greater extent by substitutions at the Q/R/N site in GluN1 than by homologous substitution in GluN2 or by substitutions at the GluN2 N+1 Asn (Kashiwagi *et al.* 2002; Chen & Lipton, 2005). Not all organic blockers display such asymmetry, however. For example, N to Q substitution at the Q/R/N site of GluN1 and GluN2B, as well as at the N+1 Asn of GluN2B all produce a similar reduction in potency of channel block by MK-801 (Kashiwagi *et al.* 2002).

The changes in minimal pore dimensions produced by substitutions at or near the Q/R/N site do not directly correlate with their effects on permeation and block (Wollmuth *et al.* 1998a), indicating that the dimensions of the narrow constriction put an upper limit on the size of ions that can pass through the pore but do not directly determine either unitary conductance or relative permeability. Indeed, single channel analysis of receptors with the Asn to Gln substitution at the Q/R/N site in GluN1 revealed two main conductance levels with distinct relative permeability to sodium and caesium (Schneggenburger & Ascher, 1997), suggesting that subtle differences in side-chain orientation may differentially affect the energy barriers for passage of each ion species. The Q/R/N site residue is critical to normal function *in vivo*, as mice engineered with pore loop N to Q or N to R point mutations in the GluN1 subunit exhibit drastic abnormalities in behaviour and physiology (Single *et al.* 2000).

In addition to the narrow constriction near the tip of the pore loop, there are additional sites along the NMDA receptor permeation pathway that influence ion flux (Premkumar & Auerbach, 1996; Antonov *et al.* 1998) as well as channel inhibition by organic (Kashiwagi *et al.* 2002; Chang & Kuo, 2008a; Jin *et al.* 2008; Nelson *et al.* 2009; Limapichat *et al.* 2013) and inorganic (Kupper *et al.* 1996; Sharma & Stevens, 1996; Williams *et al.* 1998) blockers. On the cytoplasmic side of the narrow constriction, substitutions of the negatively charged Glu located five residues C-terminal to the GluN1 Q/R/N site reduce calcium permeability (Schneggenburger, 1998) and block by internal Mg^{2+} (Kupper *et al.* 1996). In addition, mutation in GluN2 of the highly conserved M2 Trp residue reduces barium permeability and channel block by organic compounds (Kashiwagi *et al.* 2002) and by external Mg^{2+} (Williams *et al.* 1998).

Mutation experiments (Watanabe *et al.* 2002) on the extracellular side of the narrow constriction suggest that two components of GluN1 near the external end of the pore contribute to calcium permeation: the Asn in the conserved SYTANLAAF motif and residues in the DRPEER motif just past the end of M3 (Fig. 1A and C). Interestingly, analogous mutations in GluN2A either are non-functional or have little effect on calcium permeability (Watanabe *et al.* 2002). Such differences are consistent with an asymmetric contribution of pore-lining residues from GluN1 and GluN2 (Sobolevsky *et al.* 2007). An extensive study of mutations that influence pore block by spermine also reveals substantial differences between GluN1 and GluN2B (Jin *et al.* 2008). On the other hand, analysis of M3 residues essential for channel block by memantine (Limapichat *et al.* 2013) and felbamate (Chang & Kuo, 2008a) indicates a similar contribution to antagonist binding by specific homologous residues along M3 in both GluN1 and GluN2, which argues against a drastic asymmetry (see also Dai & Zhou, 2013). Final determination of how GluN1 and GluN2 subunits are arranged within the pore will likely require a high resolution NMDA receptor structure that includes the TMD.

Many aspects of NMDA receptor function depend on the identity of the GluN2 subunit (Paoletti *et al.* 2013). Properties thought to be determined by structural differences in the pore and surrounding transmembrane domain include unitary conductance, relative permeability to calcium, and potency of block by magnesium, all of which are quantitatively greater in channels formed by GluN1 plus GluN2A or GluN2B than in receptors made up of GluN1 with GluN2C or GluN2D (Burnashev *et al.* 1995; Schneggenburger, 1998; Cull-Candy & Leszkiewicz, 2004). Although a number of structural elements contribute to these differences (Kuner & Schoepfer, 1996; Wrighton *et al.* 2008), the main determinant is a single amino acid difference near the cytoplasmic end of the GluN2 M3 transmembrane helix (Siegler Retchless *et al.* 2012; Clarke *et al.* 2013). GluN2A and 2B have a polar serine at this location where 2C and 2D encode a more hydrophobic leucine (Fig. 1A). Substituting L for S in GluN2A reduces unitary conductance, calcium permeability, and the potency of Mg^{2+} block, thus converting the phenotype from GluN2A to that of GluN2C or 2D. Similarly, the reverse S for L substitution in GluN2D has the opposite effect, yielding conductance, permeability and block expected for receptors with GluN2A or 2B (Siegler Retchless *et al.* 2012). In addition, double mutant cycle analysis indicates that this location in M3 interacts with a conserved M2 helix tryptophan in the adjacent GluN1 subunit (Siegler Retchless *et al.* 2012; see Fig. 1A). Interestingly, substitution of the conserved tryptophan residue in M2 of GluN1 causes only modest changes in magnesium block. In contrast, as mentioned above

mutations at the homologous tryptophan residue in GluN2B (or 2A) dramatically reduce both Mg^{2+} block and permeability to barium (Williams *et al.* 1998; Siegler Retchless *et al.* 2012). Substitution of the conserved GluN2B M2 Trp with unnatural amino acids provides evidence that this side-chain is unlikely to interact directly with divalent cations in the pore (McMenimen *et al.* 2006) but it remains to be determined whether the conserved M2 Trp of GluN2 forms an intersubunit interaction with residues along M3 of GluN1.

AMPA and KA receptor pores

Editing at the Q/R/N site alters many properties of AMPA and KA receptors (Traynelis *et al.* 2010). Channels that include an edited (R) subunit exhibit lower unitary conductance and reduced permeability to calcium as compared to channels made up only of unedited (Q) subunits (Sommer *et al.* 1991; Burnashev *et al.* 1992a, 1995, 1996; Dingledine *et al.* 1992; Howe, 1996; Swanson *et al.* 1996). In addition, recombinant homomeric edited (R) channels exhibit finite permeability to chloride ions, with P_{Cl}/P_{Cs} estimated from reversal potential measurements at 0.14 for GluA2(R) and 0.74 for GluK2(R) (Burnashev *et al.* 1996). Editing of GluA2 mRNA is highly efficient such that nearly all GluA2 subunits are in the edited form (Isaac *et al.* 2007). Indeed, mutant mice unable to make unedited GluA2 appear completely normal (Kask *et al.* 1998), whereas mice with defects in GluA2 editing are prone to seizures and die prematurely (Feldmeyer *et al.* 1999; Higuchi *et al.* 2000). By contrast, GluK1 and GluK2 editing increases over the course of development (Bernard *et al.* 1999) but even in the adult, reaches only ~80–90%. Mutant animals with greatly reduced GluK2 editing exhibit enhanced synaptic plasticity and greater susceptibility to seizures but are otherwise normal (Vissel *et al.* 2001). In most brain cells, native AMPA and KA receptors are likely to be heteromeric combinations that include both edited and unedited subunits (Traynelis *et al.* 2010); however, calcium permeable receptors that only contain unedited subunits have been demonstrated in a number of cell types where they are postulated to play specific functional roles (Lee *et al.* 2001; Isaac *et al.* 2007).

As for NMDA receptors (Wollmuth *et al.* 1998a), the estimated minimal cross-section of non-NMDA receptor pores does not directly correlate with permeability or conductance (Burnashev *et al.* 1996). For example, both edited (R) and unedited (Q) KA receptors are estimated to have similar minimal pore diameters of 0.76 and 0.75 nm, respectively, comparable to the 0.78 nm diameter estimated for homomeric GluA1 AMPA receptors (Burnashev *et al.* 1996). Homomeric edited (R) channels are essentially impermeable to calcium (Burnashev *et al.* 1995) and have unitary conductance less

than 1 pS (Howe, 1996; Swanson *et al.* 1996, 1997), whereas unedited (Q) channels exhibit multiple conductance levels greater than 1 pS (Swanson *et al.* 1996, 1997; Rosenmund *et al.* 1998) and are calcium permeable (Burnashev *et al.* 1995), with estimated fractional calcium current of 1.5 to 2% for KA receptors and 3 to 4% for unedited AMPA receptors (Burnashev *et al.* 1995; Wollmuth & Sakmann, 1998). In contrast, NMDA receptors have a smaller minimal pore constriction (0.45 by 0.55 nm; Villarroel *et al.* 1995; Zarei & Dani, 1995) but exhibit larger unitary conductance and even higher fractional calcium current 8 to 18% (Burnashev *et al.* 1995; Schneggenburger, 1998; Wollmuth & Sakmann, 1998). Thus, Q/R site editing, or differences in primary sequence between NMDA and non-NMDA receptor channels, present distinct energetic barriers to ion flux without necessarily constricting or enlarging the minimal dimensions of the pore.

In non-NMDA receptors, as in NMDA receptors (Kuner *et al.* 1996), evidence suggests that the narrowest constriction is just below the apex of the pore loop (Kuner *et al.* 2001) and only the Q/R/N site side-chain extends up from the pore loop into the central cavity. For example, MTSES in the extracellular solution only reacts with Cys substituted at the Q/R/N site of GluA4 AMPA receptors and not at any other pore loop location (Kuner *et al.* 2001). In addition, substantial permeability to chloride is only observed for homomeric edited GluK2(R) KA receptors (Burnashev *et al.* 1996), and for unedited (Q) channels with positively charged Arg or Lys substitutions along M3 at the level of the central cavity (Wilding *et al.* 2010) but not for Arg substitutions at any other position in the pore loop (Wilding *et al.* 2008). Thus, chloride permeability appears to depend on electrostatic interactions on the extracellular side of the narrowest pore constriction with the Q/R/N site being the only pore loop residue capable of influencing ion stability in the central cavity. As for NMDA receptors (Watanabe *et al.* 2002), calcium permeability through non-NMDA receptors depends on other amino acids lining the pore including the Asn residue in the conserved SYTANLAAF motif (Jatzke *et al.* 2003); however, residues homologous to the GluN1 DRPEER motif appear to play less of a role in AMPA and KA receptors (Jatzke *et al.* 2003).

In addition to permeability and conductance, Q/R site editing also determines susceptibility to inhibition by polyamines (Bowie & Mayer, 1995; Donevan & Rogawski, 1995; Isa *et al.* 1995; Kamboj *et al.* 1995; Koh *et al.* 1995) and *cis*-unsaturated fatty acids (Wilding *et al.* 2005). Voltage-dependent block of outward current by cytoplasmic polyamines underlies the strong inward rectification of fully unedited (Q) channels. With depolarization past approximately +50 mV, outward current increases, suggesting that extreme voltages force polyamines to pass through the channel and relieve the block (Bowie & Mayer, 1995; Koh *et al.* 1995). Cytoplasmic

polyamines can also interact with closed channels (Bowie *et al.* 1998; Rozov *et al.* 1998), resulting in use-dependent unblocking that underlies short term plasticity at some synapses (Rozov & Burnashev, 1999). In contrast to open channels, blockade of closed channels exhibits very little voltage dependence (Bowie *et al.* 1998; Rozov *et al.* 1998), suggesting that in the closed state, the binding may be to superficial sites in the cytoplasmic vestibule followed by entry into the narrowest portion of the pore when channels open (Bowie *et al.* 1998). Consistent with this idea, polyamine block of GluK2(Q) is eliminated by neutral substitutions for a negatively charged Glu side-chain in the selectivity filter located four residues C-terminal to the Q/R site (Panchenko *et al.* 2001; see also Dingedine *et al.* 1992). Block is restored in double mutants that reintroduce a Glu residue at several nearby positions (Panchenko *et al.* 2001). A number of substitutions along the M2 helix and adjacent portions of M1 and M3 also reduce or eliminate polyamine block (Panchenko *et al.* 2001; Wilding *et al.* 2010), possibly by inducing subtle changes in pore helix orientation. In addition to changes in the pore loop, block by cytoplasmic polyamines of GluA1 AMPA receptors (Sobolevsky *et al.* 2005) and GluK2 KA receptors (Wilding *et al.* 2010; Lopez *et al.* 2013) can be influenced by mutations at several locations along M3 extracellular to the narrow pore loop constriction at the level of the central cavity. Introduction of positively charged side-chains reduces block of GluK2(Q) while negatively charged side-chains increase block of GluK2(R), suggesting a direct interaction between the Q/R site residue and the M3 helix (Lopez *et al.* 2013).

Lipid-derived modulators differentially regulate NMDA, AMPA, and kainate receptors. NMDA receptors are potentiated by long-chain *cis*-unsaturated Ω -3 and Ω -6 compounds including fatty acids and endocannabinoids (Miller *et al.* 1992; Nishikawa *et al.* 1994; Hampson *et al.* 1998), whereas neuronal AMPA (Kovalchuk *et al.* 1994) and KA (Wilding *et al.* 1998) receptors exhibit weak and strong inhibition, respectively. Interestingly, although native KA receptors are likely to be heteromeric and include both edited (R) and unedited (Q) subunits (Roche & Haganir, 1995), wild-type recombinant KA receptors expressed in heterologous cells only exhibit strong inhibition if all four subunits are edited (Wilding *et al.* 2005). Mutation studies have shown that in addition to the Q/R site, KA receptor modulation can be modified dramatically by amino acid substitutions throughout the TMD (Wilding *et al.* 2008, 2010; Lopez *et al.* 2013) including numerous gain of function mutations that render unedited channels susceptible to inhibition (Wilding *et al.* 2008) as well as loss of function mutations that eliminate or in some cases reverse the inhibition of fully edited channels (Lopez *et al.* 2013). Most gain of function positions are in the interface between the M2 pore loop helix and portions of

the transmembrane M1 and M3 helices that contact M2 (Wilding *et al.* 2008, 2010) whereas M3 locations where substitution converts inhibition of fully edited channels into potentiation are at the level of the central cavity (Lopez *et al.* 2013). Recent work (Wilding *et al.* 2014) on chimeric subunits has shown that the GluK2 KA receptor TMD, fused to GluN1 and GluN2B NMDA receptor extracellular domains, is sufficient to recapitulate the modulation observed in homomeric GluK2 receptors, suggesting that *cis*-unsaturated compounds act directly on the TMD. Because modulation exhibits little or no voltage dependence (Wilding *et al.* 2005), negatively charged fatty acids are unlikely to enter the pore along the conduction pathway but instead may partition into the membrane and affect channel operation either by altering membrane mechanical properties (Patel *et al.* 2001; Bruno *et al.* 2007) or by direct interaction with the TMD, possibly via lateral fenestrations that may expose the pore to membrane constituents (Payandeh *et al.* 2011; Mayer, 2011).

Importantly, double mutant cycle experiments on both KA receptors (Lopez *et al.* 2013) and NMDA receptors (Siegler Retchless *et al.* 2012) provide support for the idea that channel opening may involve significant movement of the M2 helix relative to M1 and M3 (see Sobolevsky *et al.* 2005). In both cases, the strength of interaction from double mutant cycle analysis (Siegler Retchless *et al.* 2012; Lopez *et al.* 2013) predicts much closer contact between residue pairs than the 8–12 Å separation that is observed in homology models based on the GluA2 closed state (Sobolevsky *et al.* 2009). In NMDA receptors the interaction occurs across the boundary between adjacent subunits with the highly conserved M2 Trp in GluN1 interacting with an M3 Ser on the adjacent GluN2 subunit (green triangles in Fig. 1A; Siegler Retchless *et al.* 2012). In KA receptors, however, the Q/R site and central cavity M3 residues interact within the same subunit, with nearly equivalent interactions for subunits in the A–C and B–D configurations (Wilding *et al.* 2014).

Although native Q/R site editing, and other pore substitutions, clearly affect channel properties, the extent of regulation for any given parameter may vary in individual receptors (Swanson *et al.* 1997) depending on additional factors such as the subunit composition and stoichiometry, alternative splicing or editing at other locations, post-translational covalent modifications, or interactions with other proteins including the growing number of auxiliary subunits (Isaac *et al.* 2007). For example, although block by endogenous polyamines, or polyamine toxins, has been widely used to identify channels that are likely to exhibit high calcium permeability, there is increasing evidence that some cell types express calcium permeable AMPA receptors that are much less sensitive to polyamine block (see Bowie, 2012).

Opening the gate

As for other pore loop channel family members, the inner helix (M3) bundle crossing is thought to form the gate for ion flux through the pore (Sobolevsky, 2013). In the closed state GluA2 crystal structure occlusion of the pore involves several helical turns near the extracellular end of M3 (Sobolevsky *et al.* 2009), beginning in the highly conserved SYTANLAAF motif but extending six residues beyond, where there is structural divergence between the A–C and B–D subunit conformations as well as primary sequence divergence among the different iGluR subtypes (Figs 1A and 2C–F). Earlier studies had suggested that the gate for ion flow might involve a narrowing of the pore loop constriction rather than a tight seal at the bundle crossing because extracellularly applied MTS reagents were found to modify Cys residues substituted deep into the central cavity in the absence of added agonist (Beck *et al.* 1999; Sobolevsky *et al.* 2002, 2003). In retrospect, such modification may have resulted from entry and trapping of MTS reagents in the pore during brief channel openings (Phillips *et al.* 2003) that either occurred spontaneously (Turecek *et al.* 1997) or were triggered by trace agonist levels in control conditions (Johnson & Ascher, 1987; Kleckner & Dingledine, 1988).

Observation of tight occlusion at the bundle crossing is consistent with the fact that some pore blocking drugs only bind and unbind when channels are open (Huettner & Bean, 1988; Qian & Johnson, 2002). However, bundle crossing occlusion does not rule out the possibility that closed state constriction might also occur at the selectivity filter, which was not resolved in the crystal structure (Sobolevsky *et al.* 2009). One argument against occlusion at the pore loop selectivity filter comes from single channel analysis of NMDA receptors with the GluN1 Q/R/N site Asn mutated to Gln, which showed that cytoplasmic ions rapidly equilibrate with the apex of the pore loop in closed channels, (Schneggenburger & Ascher, 1997).

Mutations in the SYTANLAAF motif significantly alter normal iGluR gating (Klein & Howe, 2004; Blanke & VanDongen, 2008; Chang & Kuo, 2008*b*) as first observed for the *Lurcher* mutant mouse, which has an Ala to Thr (A8T) substitution in the orphan GluD2 subunit (Kohda *et al.* 2000). Mutations at A8 in AMPA and KA receptor subunits slow desensitization and deactivation and promote constitutive activation (Kohda *et al.* 2000; Taverna *et al.* 2000; Schwarz *et al.* 2001; Klein & Howe, 2004) whereas NMDA receptor gating is more strongly affected by A7 substitutions (Jones *et al.* 2002; Blanke & VanDongen, 2008; Chang & Kuo, 2008*b*; Murthy *et al.* 2012). Although a detailed description of iGluR gating is not yet available (Sobolevsky, 2013), analysis of spatial relations and timing of pore movements that underlie gating remains an active area of research, including both experimentation (Murthy *et al.* 2012; Kazi *et al.* 2013;

Wilding *et al.* 2014) and modelling (Dong & Zhou, 2011; Dai & Zhou, 2013).

Conclusions

In almost every central neuron, iGluR activation at excitatory synapses leads to depolarization and in some cases entry of calcium. The ability of iGluRs to conduct monovalent cations, calcium or chloride ions depends on the specific amino acid side-chain at the apex of the pore loop as well as at several other critical positions along the pore axis. Better understanding of iGluR gating and permeation should aid in design of drugs and allosteric modulators that may have therapeutic benefit in the treatment of seizures, memory disorders and other pathologies. Much of the recent research on iGluRs has been fueled by insights gleaned from the homomeric GluA2 AMPA receptor crystal structure (Sobolevsky *et al.* 2009). Further progress toward understanding the operation of glutamate receptor pores will clearly benefit as additional iGluR structures are solved, including structures for NMDA and KA receptors as well as additional conformational states representing open and desensitized channels (Sobolevsky, 2013).

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Additional information

Competing interests

None declared.

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