AMPA and Kainate Receptors

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Abstract and Keywords

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate-type glutamate receptors (AMPARs and KARs) are dynamic ion channel proteins that govern neuronal excitation and signal transduction in the mammalian brain. The four AMPAR and five KAR subunits can heteromerize with other subfamily members to create several combinations of tetrameric channels with unique physiological and pharmacological properties. While both receptor classes are noted for their rapid, millisecond-scale channel gating in response to agonist binding, the intricate structural rearrangements underlying their function have only recently been elucidated. This chapter begins with a review of AMPAR and KAR nomenclature, topology, and rules of assembly. Subsequently, receptor gating properties are outlined for both single-channel and synaptic contexts. The structural biology of AMPAR and KAR proteins is also discussed at length, with particular focus on the ligand-binding domain, where allosteric regulation and alternative splicing work together to dictate gating behavior. Toward the end of the chapter there is an overview of several classes of auxiliary subunits, notably transmembrane AMPAR regulatory proteins and Neto proteins, which enhance native AMPAR and KAR expression and channel gating, respectively. Whether bringing an ion channel novice up to speed with glutamate receptor theory and terminology or providing a refresher for more seasoned biophysicists, there is much to appreciate in this summation of work from the glutamate receptor field.

Keywords: kainate receptor, AMPA receptor, ion channel, channel gating, receptor pharmacology, protein structure, auxiliary protein, TARP, Neto

Introduction to the Ionotropic Glutamate Receptor Family

Ionotropic glutamate receptors (iGluRs) are tetrameric proteins that mediate almost all fast-excitatory neurotransmission in the central nervous system. The arrival of an action potential in the presynaptic neuron of glutamatergic synapses triggers the vesicular re-

lease of the neurotransmitter L-glutamate, which binds to and activates iGluRs located on the postsynaptic neuron. The binding of the neurotransmitter triggers iGluRs to enter an activated state with the opening of a transmembrane ion channel pore that permits the rapid transport of mono- and divalent cations. Cation influx into the postsynaptic neuron causes membrane depolarization and, depending on its magnitude, may trigger action potential firing in the postsynaptic neuron (Figure 1A). Glutamatergic synaptic transmission occurs on a millisecond timescale, endowing neuronal circuits with the capability of responding rapidly to incoming signals, while giving rise to complex cognitive functions and behaviors, such as sensory perception, thought, movement, and memory.

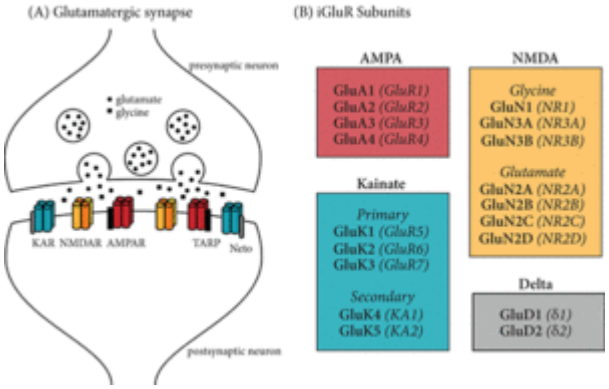

Figure 1. The iGluR family. (A) Depiction of a glutamatergic synapse, including AMPARs and KARs and their principal auxiliary subunits, as well as NMDARs, which require glutamate and glycine to activate. (B) List of iGluR subunits, according to the subfamily, with older nomenclature shown in brackets. Note that some NMDAR subunits bind glycine, rather than glutamate. Also, KARs are divided into primary subunits, capable of forming functional homomers, and secondary or high-affinity subunits, which must heteromerize with a primary subunit to form functional channels.

There are 18 mammalian iGluR subunits, which are grouped into four classes largely based on the names of agonists that selectively activate them (Figure 1B). Accordingly, iGluR subgroups sensitive to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainic acid (KA, or kainate), and *N*-methyl-D-aspartic acid (NMDA) are termed AMPA receptors (AMPARs), KA receptors (KARs), and NMDA receptors (NMDARs). Furthermore, there are two related, "nonconducting" subunits, the orphan/delta subunits (GluD1-2), which apparently fail to be activated in response to known iGluR agonists when expressed alone or with other iGluR subunits (Traynelis et al., 2010). The orphan class receptors appear to have other, non-ionotropic roles that are important in synapse signaling (Yuzaki & Aricescu, 2017). The current nomenclature was implemented beginning in 2009 (Collingridge, Olsen, Peters, & Spedding, 2009); previously, AMPARs were known as GluR1–4; KARs as GluR5–7, KA-1, and KA-2; NMDARs as NR1, NR2A-D, and

NR3A-B; and the orphan/delta receptors as δ1–2 (Dingledine, Borges, Bowie, & Traynelis, 1999) (Figure 1B).

AMPARs were originally identified as being selectively activated by the synthetic agonist AMPA (Krogsgaard-Larsen, Honore, Hansen, Curtis, & Lodge, 1980), which does not elicit responses at NMDARs though it does activate some homo- and heteromeric KARs (Swanson, Gereau, Green, & Heinemann, 1997). The AMPAR subunits can heteromerize interchangeably, though they also retain function as homomeric channels (Boulter et al., 1990; Keinänen et al., 1990). Despite the many possible combinations, it is generally agreed that the most abundant AMPARs in the mammalian brain are heteromers composed of either GluA1/GluA2 or GluA2/GluA3 subunits (Bowie, 2018). AMPAR subunits cannot assemble with KAR subunits to form functional channels (Bettler et al., 1990; Sommer et al., 1992), rendering the two iGluR subfamilies entirely separable at the molecular level.

The KAR subunits are discriminated based on their relatively high affinity for the agonist KA, first isolated from algae off the coast of Japan (Murakami, Takemoto, & Shimizu, 1953). These subunits only assemble with each other, rather than AMPAR or NMDAR subunits (Wenthold, Trumpy, Zhu, & Petralia, 1994). However, the functional distinction between AMPARs and KARs is blurred by the fact that most AMPAR subunits are responsive to KA, while some KAR subunits (e.g., GluK1) are responsive to AMPA (e.g., Sommer et al., 1992). KAR subunits are further divided into “primary” subunits (GluK1–3), capable of forming functional channels when expressed alone, and “secondary” subunits (GluK4–5), which require co-assembly with at least one primary subunit to exhibit functionality (reviewed in Contractor, Mulle, & Swanson, 2011). The secondary or “high-affinity” KAR subunits notably bind KA with 10-fold higher affinity than the primary subunits, possessing dissociation constant (K_d) values in the 10 nM range (Herb et al., 1992; Werner, Voigt, Keinanen, Wisden, & Seeburg, 1991).

In terms of antagonism, AMPARs and KARs are functionally separable from NMDARs based on their sensitivity to quinoxalinedione antagonists, such as 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) (Honore et al., 1988). These compounds do not inhibit the NMDAR-mediated response at glutamatergic synapses, where all three subfamilies are present. Likewise, the classic NMDAR competitive antagonist 2-amino-5-phosphonopentanoic acid (Davies & Watkins, 1982) does not affect AMPAR or KAR responses. Consistent with the similarity in their pharmacological profiles, the amino acid sequence identity between AMPAR and KAR subunits is roughly 40% but drops to 25% when either receptor subtype is compared to NMDARs (Hollmann & Heinemann, 1994). It is therefore unsurprising that NMDARs are distinguished from other iGluRs by a variety of functional properties, notably slower gating kinetics and distinct sites of allosteric regulation (Paoletti, Bellone, & Zhou, 2013).

An Overview of iGluR Topology

All iGluR subunits possess a similar overall topology, including a lengthy (>500 amino acid) N-terminal extracellular domain, four transmembrane (TM1–4) regions, and an in-

tracellular, largely disordered C-terminal tail (CTD) of variable length (Figure 2A). The extracellular region is further divided into two globular, self-interacting domains known as the amino-terminal domain (ATD; alternatively termed “NTD” in other publication) and the agonist or ligand-binding domain (LBD). Interestingly, TM2 partially enters the membrane and turns back into the intracellular space (Hollmann, Maron, & Heinemann, 1994). The LBD is formed of two discontinuous extracellular segments that are located between the ATD and TM1 (S1) as well as between TM3 and TM4 (S2) (Stern-Bach et al., 1994).

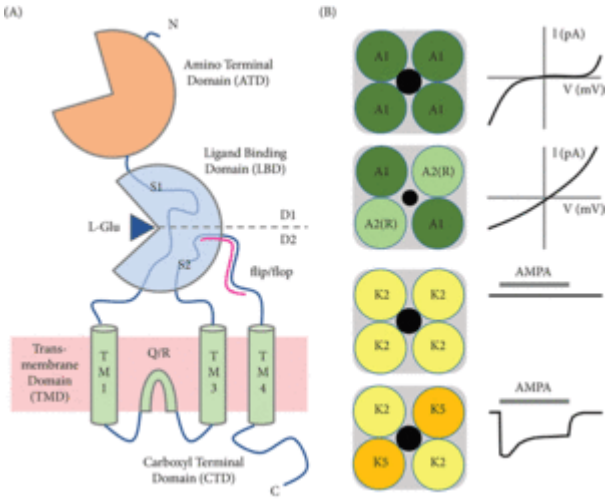

Figure 2. iGluR topology and subunit assembly. (A) Topological illustration of an iGluR subunit, including the four principal domains: ATD, LBD, TMD, and CTD. The upper and lower lobes of the LBD are referred to as *D1* and *D2*, while the discontinuous amino acid segments that form the LBD are *S1* and *S2*. Note the flip alternate splicing region is only found in AMPARs. (B) AMPAR subunits (green) and KAR subunits (yellow) can form into homomeric and heteromeric complexes. Ion channels formed by GluA2-containing AMPARs have lower conductance but are unaffected by polyamine block at positive membrane potentials, resulting in more linear current–voltage (*I–V*) plots. While GluK2 heteromers are unresponsive to AMPA, GluK2/GluK5 heteromers are activated by the same agonist.

Situated distal from the channel pore, the ATD contains approximately 400 amino acids, about half of the entire protein for AMPARs and KARs. Structurally, the ATD is divided into the upper R1 and lower R2 lobes, and the cleft in between plays an important role in the allosteric regulation of NMDARs, notably by inhibitory zinc ions (Hansen, Furukawa, & Traynelis, 2010). Despite its large size, the ATD is required for neither receptor assembly nor channel gating since the different iGluR subfamilies retain channel function if their respective ATDs have been deleted (e.g., Fayyazuddin, Villarroel, Le Goff, Lerma, & Neyton, 2000; Pasternack et al., 2002). At the same time, several studies suggest that the ATD facilitates the preferential assembly of specific combinations of subunits (Mayer, 2011), often resulting in 2:2 heteromeric arrangements (Figure 2B). In a physiological

context, the ATD also appears to mediate iGluR association with other pre- and postsynaptic proteins (Garcia-Nafria, Herguedas, Watson, & Greger, 2016).

Separated by a short linker from the ATD, the 300–amino acid LBD orchestrates the transduction of agonist binding into channel opening. The LBD is formed from both S1 and S2, which together define agonist selectivity (Stern-Bach et al., 1994). When considered in isolation, the LBD structure is divided into domains 1 and 2 (D1 and D2), representing the upper and lower lobes of the clamshell-shaped agonist-binding cleft (Armstrong, Sun, Chen, & Gouaux, 1998). The isolated LBD has, in fact, proven quite amenable to structural characterization, and a number of atomic resolution structures of this domain, particularly from GluA2 AMPARs, have contributed to the refinement of molecular mechanisms for iGluR channel gating (Pohlsgaard, Frydenvang, Madsen, & Kastrup, 2011).

The LBD is also an important site for both genetic and allosteric regulation in AMPARs and KARs. For example, alternative splicing of an exon encoding the S2–TM4 linker region produces either “flip” or “flop” isoforms for all AMPAR subunits (Sommer et al., 1990) (Figure 3). GluA2–4 flop isoform receptors are notable for desensitizing much more rapidly than their flip counterparts (e.g., Mosbacher et al., 1994). Furthermore, the flip/flop region is immediately preceded by an R/G RNA editing site (Lomeli et al., 1994). The conversion of arginine to glycine in flip and flop variants of GluA2–4 produces a modest slowing of desensitization and acceleration of recovery from desensitization (Lomeli et al., 1994). In contrast, a number of positive allosteric modulators, such as cyclothiazide, bind to the AMPAR LBD and greatly attenuate desensitization (Partin, Bowie, & Mayer, 1995; Sun et al., 2002). Recent work has shown that the flip/flop cassette regulates the nanoscale mobility of the apo state of the AMPAR, which in turn acts as a master switch to control channel gating, allosteric regulation, and regulation by auxiliary subunits (Dawe et al., 2019). For the KAR subfamily, allosteric ions acting at the LBD have a significant influence on desensitization and other gating properties of specific subunits (Bowie, 2002). Whether these effects of ions also work by regulating the mobility of the apo state remains to be investigated.

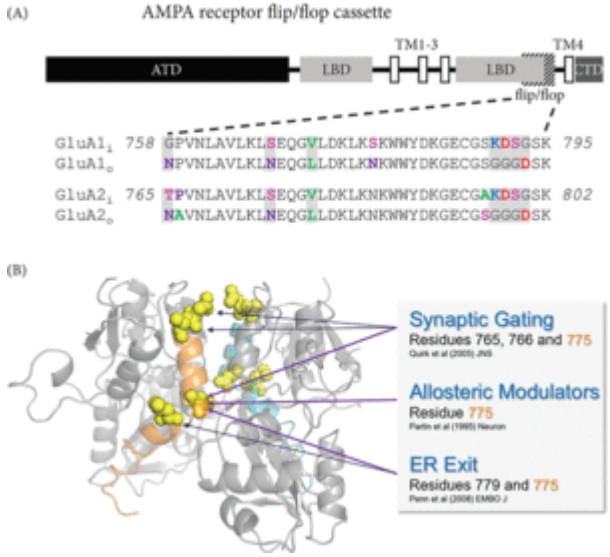

Figure 3. Importance of the flip/flop cassette in AMPAR gating and allosteric modulation. (A) Amino acid sequence alignment of the alternately spliced flip (i) and flop (o) cassettes of GluA1 and GluA2 AMPARs. Notable amino acid substitutions are highlighted. (B) Structural depiction of amino acid residues impacted by flip/flop alternative splicing. Situated at the apex of the LBD are residues that affect gating kinetics, whereas the 775 position influences sensitivity to cyclothiazide and allosteric anions. Positions lower in the subunit interface have been reported to affect subunit assembly and trafficking.

Adapted from G. B. Dawe et al. Nanoscale mobility of the apo state and TARP stoichiometry dictate the gating behavior of alternatively spliced AMPA receptors. *Neuron*, *102*(5), 976–992. © 2019, with permission from Elsevier.

The TM domain (TMD) is comprised of discontinuous tracts from TM1 to TM4 and is generally permeable to monovalent cations of varying diameter but less permeable to divalent cations (Bowie, 2018; Huettner, 2015). More specifically, TM1 and TM4 flank the outside of the pore, whereas the TM2 re-entrant loop forms the pore itself, and TM3 lines the upper segment of the permeation pathway (Sobolevsky, Rosconi, & Gouaux, 2009). The selectivity filter is thought to be located where TM2 bends back toward the intracellular face of the membrane (Kuner, Beck, Sakmann, & Seeburg, 2001).

A second locus of RNA editing found in TM2, known as the Q/R site, is critical for ion permeation in both AMPARs and KARs. Channels containing GluA2 subunits, in which the glutamine in the pore region is almost always substituted by an arginine, have a linear current–voltage (*I*–*V*) relationship, whereas channels comprised of unedited GluA1, GluA3, and GluA4 subunits yield whole-cell currents that inwardly rectify, unless forming heteromers with GluA2 (Verdoorn, Burnashev, Monyer, Seeburg, & Sakmann, 1991) (Figure 2B). This rectification is due to channel block by intracellular polyamines at more positive holding potentials (Bowie, 2018; Bowie & Mayer, 1995). Moreover, the presence of

an arginine at this position also reduces AMPAR calcium permeability (Burnashev, Monyer, Seeburg, & Sakmann, 1992; Hume, Dingledine, & Heinemann, 1991), leading to the classification of GluA2-lacking AMPAR complexes as “calcium-permeable.” Meanwhile in KARs, the Q/R position is edited to an intermediate extent (Sommer, Kohler, Sprengel, & Seeburg, 1991).

Below the plasma membrane, the iGluR CTD represents an important site of interaction with both kinases (e.g., Banke et al., 2000) and scaffolding proteins, which specify localization (Tomita, Nicoll, & Bredt, 2001). However, little structural information exists for this region, except within the NMDAR subfamily (Ataman, Gakhar, Sorensen, Hell, & Shea, 2007; Choi, Xiao, Wollmuth, & Bowen, 2011). On a functional level, AMPAR and KAR subtypes with a deleted C-terminal tail retain gating capabilities (Salussolia et al., 2011; S. Yan et al., 2004). As such, an in-depth discussion of the CTD is not provided in this chapter.

An Overview of iGluR Stoichiometry

To form functional channel complexes, iGluR subunits must first assemble as tetramers, whether homomeric or heteromeric in nature (Figure 2B). This stoichiometry was first derived indirectly using targeted mutations that altered agonist affinity (e.g., Laube, Kuhse, & Betz, 1998), as well as by analysis of single-channel conductance (Rosenmund, Stern-Bach, & Stevens, 1998). Nevertheless, more definitive proof came from the tetrameric subunit arrangement of the first intact iGluR structure to be obtained at atomic resolution (Sobolevsky et al., 2009). The *Y*-shaped GluA2 AMPAR structure revealed many insights into the modular organization of iGluR complexes, and intact NMDAR (Karakas & Furukawa, 2014; Lee et al., 2014) and KAR (Meyerson et al., 2016, 2014) structures have since been elucidated.

Adding to the complexity of iGluR signaling is the fact that synaptic AMPARs and KARs are often associated with auxiliary or regulatory proteins in the brain. Although there is an expanding list of iGluR auxiliary protein families (especially for AMPARs), they share the common ability to enhance membrane trafficking and/or positively modulate channel activity (Jackson & Nicoll, 2011). The precise stoichiometry between native iGluRs and different auxiliary subunits remains largely unresolved, especially because it is unclear how different classes of auxiliary proteins might occlude each other from association with pore-forming subunits. For the well-studied transmembrane AMPAR regulatory protein (TARP) family of AMPAR auxiliary subunits, there are emerging structural mechanisms that describe how they associate with and modulate pore-forming subunits. As discussed in the section *AMPAR-TARP Assembly & Stoichiometry*, recent work reports that the apparent TARP stoichiometry of native AMPARs varies in a cell-specific manner (Dawe et al., 2019).

Gating and Pharmacology of iGluRs: Theory of Ion Channel Gating

Much of the terminology used to describe iGluR gating originated from pioneering studies of voltage-gated ion channels (VGICs) and nicotinic acetylcholine receptors in the 1950s, long before the molecular identity of receptor–channel complexes could be elucidated. The term *gating* itself describes processes that open and close channels, while *permeation* reflects the transport of ions through an open channel (Horn, 1990). For ligand-gated ion channels (LGICs), the energy required for opening the channel pore (activation) is derived from the binding of one or more agonist molecules (Andersen & Koeppe, 1992). Meanwhile, there are two fundamental gating processes that result in the closure of ion channels. The first, deactivation, is observed upon removal of the agonist. The second, desensitization, can be described as a "progressive reduction in ionic flux in the prolonged presence of agonist" (Keramidas & Lynch, 2013). In other words, receptors become less sensitive over time to their chemical stimulus, favoring channel closure while agonist molecules are still bound.

It is also possible for iGluR desensitization to manifest in several other forms apart from decaying current responses. For instance, the high apparent affinity of desensitized receptors for certain agonists means that desensitization can persist long after an initial agonist application, preventing channel opening in response to subsequent agonist exposures. In this context, the extent of desensitization is reflected in the fractional current response following a second agonist pulse, relative to the maximal response after an initial pulse. Furthermore, iGluRs can become "pre-desensitized" by equilibration with low (inert) concentrations of an agonist, such that a rapid escalation of its concentration to saturating levels still cannot elicit any channel activity. Due to its various manifestations, it can be useful to define desensitization at the molecular level as the transition of an agonist-bound receptor into a nonconducting or (in some cases) poorly conducting state. Similarly, deactivation represents the transition(s) from an open to a closed, agonist-unbound state (Hinard et al., 2016).

The conceptualization of LGIC gating processes as transitions/reactions between states was inspired by earlier work on enzymes, which exist in multiple conformational states that are differentially occupied following the binding of a chemical substrate (Andersen & Koeppe, 1992). In this context, the binding of an agonist does not directly open the channel but rather reduces the energetic barrier for another state transition that corresponds to opening—indeed, in exceptionally rare instances, LGIC currents have been observed without any agonist present (Auerbach, 2015). Although definitions vary, one can reasonably say that affinity is determined by the initial agonist-binding reaction, whereas efficacy is the sum of all other gating transitions (Colquhoun, 1998). Accordingly, partial agonists are chemical compounds that display reduced efficacy at saturating concentrations compared to some maximally efficacious compound (i.e., L-glutamate), while competitive antagonists possess no efficacy (Stephenson, 1956). An additional category of ligands known as *noncompetitive antagonists* reduce receptor responsiveness by interacting

somewhere distinct from the orthosteric agonist-binding site and are thus said to act in an “allosteric” manner (Colquhoun, 1998).

Contributions of iGluR Subunits to Channel Gating

The binding of two agonist molecules is necessary for AMPAR and KAR channels to open (Clements, Feltz, Sahara, & Westbrook, 1998). This was proposed by examining the activation kinetics of non-NMDARs in cultured hippocampal neurons and comparing them with gating models that had one, two, or three binding sites (Clements et al., 1998). Though this study could not determine the exact number of binding sites, Rosenmund and colleagues (1998) further developed this idea and suggested a tetrameric stoichiometry. In this case, they engineered a nondesensitizing GluA3–GluK2 chimeric channel and studied single-channel responses. To observe the rapid transitions between the three observed subconductance states, the agonist binding rate was slowed by imposing a much slower step, namely the unbinding of the high-affinity antagonist 2,3-dioxo-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) (Rosenmund et al., 1998). The transition into the smallest conducting state was shaped by two time constants, consistent with the idea that two molecules are necessary for activation. Accordingly, the simplest explanation for the three-conductance observation was that receptors are tetramers with two additional agonist-binding steps accounting for the higher conductance levels (Rosenmund et al., 1998).

In AMPARs, the relative contribution of conductance states is agonist-dependent, with higher conductance levels being more abundant in higher agonist concentrations (Rosenmund et al., 1998; Smith & Howe, 2000). However, though the distribution of conductance levels in KARs does not appear to be agonist concentration–dependent, the results remain unclear due to the lack of pharmacological tools, such as cyclothiazide for AMPARs, to block KAR desensitization (though see Dawe et al., 2013).

Gating Behavior of Native AMPARs and KARs

AMPARs and KARs exhibit rapid gating behavior, particularly in comparison to the slower current rise times and decay properties of NMDARs. Generally, AMPAR state transitions occur on a scale of 10 ms or less (Baranovic & Plested, 2016), and this can be considered a good benchmark to divide fast and slow for most gating processes. The earliest observations of AMPAR and KAR gating behavior came from neurons as improvements in the voltage-clamp recording technique and the discovery of subfamily-selective agonists and antagonists made it possible to isolate these receptor populations (Mayer & Westbrook, 1987). At the same time, the accurate measurement of AMPAR and KAR currents also requires the delivery of a fixed agonist concentration over a precise time interval. Various “concentration-clamp” systems can be implemented (e.g., Franke, Hatt, & Dudel, 1987; Krishtal, Marchenko, & Pidoplichko, 1983) so that cells or membrane patches are quickly exposed to agonist-containing solutions on a submillisecond timescale. Without such systems, diffuse agonist release will obscure the visualization membrane current responses

because many AMPARs and KARs will rapidly desensitize before nearby receptors can themselves activate to generate a concerted peak response.

Neuronal recordings using the concentration-clamp technique demonstrate that non-NMDAR responses to glutamate almost completely desensitize within tens of milliseconds, with maximal response amplitudes occurring at very high (i.e., >10 mM) glutamate concentrations (Kiskin, Krishtal, & Tsyndrenko, 1986). In contrast, recovery from desensitization typically occurs over hundreds of milliseconds (Bowie & Lange, 2002; Trussell & Fischbach, 1989). Yet for many years, mixed populations of native AMPARs and KARs were unable to be studied in isolation, a problem recognized after KA was shown to activate recombinant AMPAR subunits (i.e., Keinänen et al., 1990). Fortunately, 2,3-benzodiazepine (GYKI) compounds are now used as noncompetitive antagonists, selective for AMPARs over KARs (Clarke et al., 1997; Wilding & Huettner, 1995), facilitating the isolation of KAR-mediated synaptic responses. Often, the residual agonist-evoked currents remaining after GYKI application are quite small (<20%) compared to those mediated by AMPAR populations (e.g., Wilding & Huettner, 1997).

Gating Behavior of Recombinant AMPARs and KARs

The most precise measurements of AMPAR and KAR gating have arguably come from recombinantly expressed GluA2 and GluK2 (Q/R unedited) receptors because these two subunits form homomeric channels with excellent exogenous expression in widely used cell lines (i.e., HEK 293 cells). Gating time constants of recombinant GluA2 receptors largely reflect those of neuronal AMPAR populations, whereas recombinant GluK2 properties often differ from native KAR behavior due to heteromerization with secondary and auxiliary subunits. A summary of GluA2 and GluK2 gating properties (see Table 1) entails rapid activation (~200 μs), deactivation (0.5–3 ms), and desensitization (5–10 ms), as well as low glutamate potency (~1 mM). The gating behavior of other iGluR subunits can be gleaned from more extensive reviews (e.g., Dingledine et al., 1999; Traynelis et al., 2010).

Table 1 Biophysical Properties of AMPAR and KAR Gating

	GluA2(Q), flip	**GluK2(Q)**
Gating Process	time constant (ms)	
Desensitization	5–10	5–8
Deactivation	0.5–1	2–3
Recovery from desensitization	20–25	2000–3000
Rise time (10–90%)	0.2–0.3	0.2
Equilibrium current (% of peak)	~1	0.3–0.4
Agonist potency	EC_{50} (μM)	
Peak current	1,000–2,000	~500
Equilibrium response	300–500 (in CTZ)	30

Sources. The following papers were referenced to develop this table: GluA2 (Carbone & Plested, 2012; Dawe et al., 2016; Horning & Mayer, 2004; Koike, Tsukada, Tsuzuki, Kijima, & Ozawa, 2000; MacLean et al., 2014; Robert, Armstrong, Gouaux, & Howe, 2005; Salazar, Eibl, Chebli, & Plested, 2017; Sun et al., 2002; Yu et al., 2016) and GluK2 (Bowie, 2002; Bowie, Garcia, Marshall, Traynelis, & Lange, 2003; Bowie & Lange, 2002; Carbone & Plested, 2012; Heckmann, Bufler, Franke, & Dudel, 1996). CTZ indicates cyclothiazide.

Single-channel activity of Q/R unedited AMPARs and KARs can be directly observed because unitary conductance (>5 pS) is greater than that of edited channels (0.2–0.5 pS) (Swanson, Kamboj, & Cull-Candy, 1997), where such values must be inferred from noise analysis (see Traynelis & Jaramillo, 1998). During sustained agonist applications, these receptors typically display a single burst of channel openings over a few milliseconds, prior to the rapid onset of desensitization, after which additional openings are extremely rare (e.g., Dawe et al., 2013). An interesting property of these bursts is that they exhibit fast transitions between multiple subconductance levels of approximately 8, 16, and 24 pS, with the mean open time at each level ranging between 0.3 and 0.9 ms (W. Zhang, Cho, Lolis, & Howe, 2008; W. Zhang et al., 2009). The occurrence of subconductance levels has been thought to correlate with the number of bound agonist molecules—two, three, or four (Rosenmund et al., 1998). A caveat to this interpretation is that GluA2 unitary activity in saturating glutamate still exhibits frequently occurring low and intermedi-

ate subconductance levels (Prieto & Wollmuth, 2010; W. Zhang et al., 2008), suggesting that other factors besides agonist occupancy dictate conductance level. Furthermore, a fourth, larger conductance level >30 pS is also occasionally visited, though it is more common if auxiliary subunits are present (Howe, 2015).

The Unique Pharmacology of AMPARs and KARs

From a functional sense, AMPARs are unique from KARs because they respond differently to certain agonists, excluding the indiscriminate neurotransmitter glutamate. Within the KAR subfamily, there are few other agonists that elicit maximal (>80% of glutamate) GluK2 peak current responses. Notably, the anthelmintic plant extract quisqualate (Biscoe, Evans, Headley, Martin, & Watkins, 1975), which the AMPAR subfamily was originally named after, and the synthetic molecule 2S,4R-4-methylglutamate (or SYM2081) fit this criterion (Fay, Corbeil, Brown, Moitessier, & Bowie, 2009). Somewhat less efficacious are KA as well as the related algal neurotoxin domoate, which produce GluK2 current responses that, respectively, comprise 50% and 15% of the peak amplitude evoked by glutamate (Fay et al., 2009). Interestingly, domoate responses are slow to desensitize, though deactivation following domoate removal is extremely sluggish, occurring over many seconds (Swanson et al., 1997), and consistent with high-affinity binding, common among toxins.

Further complicating the pharmacology of the KAR subfamily, there is an unusual divide between GluK1 and the other primary subunits (Table 2). Specifically, GluK1 is responsive to AMPA, while GluK2 and GluK3 are not, unless co-expressed with high-affinity KAR subunits (Herb et al., 1992; Schiffer, Swanson, & Heinemann, 1997). The same relationship holds for the AMPAR agonist (S)-5-iodowillardiine (Swanson, Green, & Heinemann, 1998), a modified relative of the amino acid willardiine found in plant seeds. A similar pattern also exists for the AMPA analogue (RS)-2-amino-3-(3-hydroxy-5-tert-butylisoxazol-4-yl)propanoic acid (ATPA), though this compound notably has greater potency at GluK1 than GluA1–4 subunits (Stensbol et al., 1999), enabling selective GluK1 KAR activation in neuronal preparations (Clarke et al., 1997). An added benefit of these agonists is that they have provided great templates from which to engineer multiple classes of GluK1-selective antagonists. In some cases, such antagonists (e.g., LY294486 and LY382884) are highly selective for GluK1 over AMPAR subunits, in addition to GluK2 and GluK3 (Bortolotto et al., 1999; Clarke et al., 1997). In other cases, antagonists like UBP310 inhibit GluK1 and GluK3 current responses more potently than those of GluK2 receptors (Mayer, Ghosal, Dolman, & Jane, 2006; Perrais, Pinheiro, Jane, & Mulle, 2009).

Table 2 Agonists and Antagonists of AMPARs and KARs

	GluA1–4	GluK1	GluK2	GluK3	GluK1/ GluK5	GluK2/ GluK5	GluK3/ Gluk5	NMDAR
Agonist								
L-Glutamate	+	+	+	+	+	+	+	+
Quisqualate	+(1–3)		+					+
AMPA	+	+	–	–	+	+	+	–
ATPA	+	+	–		+	+		
KA	+	+	+	+	+	+	+	–
Domoate	+(1,3)	+	+	–	+	+	–	
(S)-5-Iodowillardiine	+(1,2)	+	–	–	+	+	+	
Antagonist								

CNQX (10 µM)	+	+	+	+				–
NBQX	+	+	+					–
GYKI 52466 (100 µM)	+(1)		–			–		
LY 294486 (10 µM)	–(1–4)	+	–					
UBP 310 (30 µM)	–(2)	+	–	+				

Sources. The following papers were referenced to develop this table, though they do not represent the entirety of studies that examine AMPAR and KAR pharmacology: AMPAR agonists (Boulter et al., 1990; Jin et al., 2003; Keinänen et al., 1990; Kizelsztein, Eisenstein, Strutz, Hollmann, & Teichberg, 2000; Robert et al., 2005; Stensbol et al., 1999), KAR agonists (Alt et al., 2004; Herb et al., 1992; Schiffer et al., 1997; Swanson et al., 1997, 1998), NMDARs (Moriyoshi et al., 1991; Nakanishi, Shneider, & Axel, 1990), CNQX (Bettler et al., 1992; Egebjerg, Bettler, Hermans-Borgmeyer, & Heinemann, 1991; Sommer et al., 1992), NBQX (Bleakman et al., 1996), GYKI 52466 (Clarke et al., 1997; Stein, Cox, Seeburg, & Verdoorn, 1992), LY 294486 (Clarke et al., 1997), and UBP 310 (Mayer et al., 2006; Perrais et al., 2009).

Among AMPARs, there is a more consistent pharmacological profile between subunits (Table 2). AMPA and quisqualate are considered full agonists at recombinant receptors, and in comparison to glutamate they induce slower deactivation and produce left-shifted dose–response curves, suggestive of higher affinity (W. Zhang, Robert, Vogensen, & Howe, 2006). KA and domoate can also elicit AMPAR activation (Boulter et al., 1990), but KA has a much lower potency than glutamate (Armstrong, Mayer, & Gouaux, 2003) and yields minimal current responses (<1% of glutamate) that do not decay over time (e.g., Dawe et al., 2016). Moreover, the willardiine agonist series consists of moderate to weak partial agonists, with larger halogen substituents reducing efficacy (Jin, Banke, Mayer, Traynelis, & Gouaux, 2003). At present, there are no agonists with remarkable selectivity for a particular subset of AMPAR subunits because the amino acid residues forming the agonist-binding pocket are almost entirely conserved among GluA1–4. The compound 2-Bn-Tet-AMPA, which exhibits a half maximal effective concentration (EC_{50}) that is 10-fold lower at GluA4 versus GluA1–3 receptors (Jensen et al., 2007), is unique in this regard.

Structure and Function of the iGluR LBD

The molecular mechanism of agonist efficacy at iGluRs has been unraveled using a combination of pharmacological interrogation and high-resolution protein structures. The first of these structures to be resolved was a KA-bound GluA2 (flop) LBD, crystallized using an S1-/S2-linked construct (Armstrong et al., 1998). The individual LBD is “kidney-shaped,” containing upper and lower domain 1 (D1) and domain 2 (D2) lobes that both form contacts with the agonist molecule (Armstrong et al., 1998). In many cases, the LBD also crystallizes as a dimer, where the openings to the agonist-binding cleft are directed away from the central dimer interface (Armstrong & Gouaux, 2000). Interestingly, when full agonists like AMPA and glutamate bind to the isolated LBD they induce 20-degree closure of the agonist-binding cleft relative to the unliganded (apo) state, based on the angle between the D1 and D2 domains (Figure 4A). Meanwhile, the partial agonist KA and the competitive antagonist DNQX induce only 12 degrees and 5 degrees of additional cleft closure, respectively (Armstrong & Gouaux, 2000) (Figure 4B). Based on this spectrum of cleft closure, a structural model of agonist efficacy has developed, whereby more efficacious agonists are thought to induce greater closure, which in turn facilitates gating of the channel pore (Armstrong & Gouaux, 2000). Further validation of this model came from the willardiine agonist series, for which increasingly bulky substituent groups reduced cleft closure in crystal structures, as well as relative efficacy of steady-state activation in electrophysiological assays (e.g., Jin et al., 2003). In addition, single-molecule fluorescence-based measurements have reported a higher probability of cleft closure for agonist-bound versus apo-state AMPAR LBDs (Landes, Rambhadran, Taylor, Salatan, & Jayaraman, 2011).

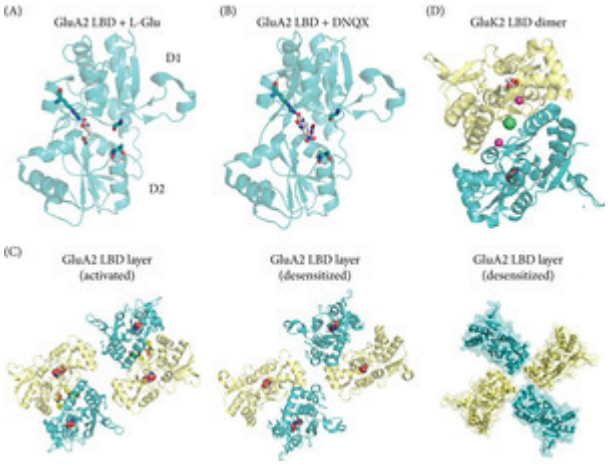

Figure 4 Dynamics of the AMPAR/KAR LBD underlie agonist efficacy and desensitization. (A, B) The isolated GluA2 LBD adopts a bilobed, clamshell-like arrangement with an agonist-binding cleft in between the D1 and D2 lobes. Binding of the full agonist glutamate (L-Glu; gray stick) induces closure of the cleft (PDB: 1FTJ), whereas binding of the competitive antagonist DNQX retains separation of residues on opposing faces of the cleft (PDB: 1FTL) (Armstrong & Gouaux, 2000). The same three residues are highlighted (cyan sticks) in each structure to provide perspective on the degree of cleft closure. (C) Top view of the GluA2 LBD layer from intact structures. In the presence of glutamate and cyclothiazide (yellow sticks), which attenuates desensitization, the subunits adopt an activated conformation, marked by closely held dimer pairs (left; PDB: 5WEO) (Twomey, Yelshanskaya, Grassucci, Frank, & Sobolevsky, 2017a). In a desensitized conformation, elicited by the agonist quisqualate, there is modest separation between subunits in each dimer pair (right, PBD: 5VHZ) (Meyerson et al., 2016; Twomey, Yelshanskaya, Grassucci, Frank, & Sobolevsky, 2017b). (D) Top view of the GluK2 LBD dimer (top), illustrating the binding pockets of allosteric sodium and chloride ions (PBD: 3G3F) (Chaudhry, Weston, Schuck, Rosenmund, & Mayer, 2009), as well as a similar view of complete GluK2 LBD layer (bottom) from an intact, desensitized structure (PDB: 4UQQ) (Meyerson et al., 2014). Note the extreme, >100-degree rotation of the B/D subunits (cyan) relative to those of the activated GluA2 structure.

Despite the one-dimensional nature of the cleft closure paradigm, the measurement strongly correlates with agonist efficacy (Pohlsgaard et al., 2011) and has provided a starting point for thinking about how iGluR structure regulates channel gating. However, among the various LBD structures published since 2000 it is clear that certain bound agonists have not induced a degree of cleft closure commensurate with their agonist activity (e.g., Venskutonyte et al., 2012). Indeed, it has even been postulated that efficacy may be governed by a twisting motion of the LBD, rather than cleft closure (Birdsey-Benson, Gill,

Henderson, & Madden, 2010). As more complete or intact receptor complexes are solved in different states, it will be appropriate to refine explanations of agonist efficacy to complement the observed changes in quaternary structure. For example, intersubunit crosslinking experiments indicate that the LBD layer explores more conformations, reducing its overall stability, when bound by partial, rather than full, agonists (Baranovic et al., 2016).

Allosteric Modulators of AMPARs

Several positive allosteric modulators of AMPARs have been identified from their effects on non-NMDAR responses in neuronal recordings. For example, the cognition-enhancing drug aniracetam potentiates AMPAR responses (Ito, Tanabe, Kohda, & Sugiyama, 1990) by slowing desensitization and excitatory postsynaptic current (EPSC) decay (Vyklicky, Patneau, & Mayer, 1991). Likewise, cyclothiazide, a benzothiadiazide compound originally developed as a diuretic, also enhances AMPAR responses (Yamada & Tang, 1993) but almost completely blocks agonist-induced desensitization, with minimal effect on deactivation kinetics (Patneau, Vyklicky, & Mayer, 1993; Yamada & Tang, 1993). In recombinant expression systems, cyclothiazide acts exclusively on AMPARs but not KARs (Partin, Patneau, Winters, Mayer, & Buonanno, 1993). Cyclothiazide exerts a greater modulation of flip AMPAR isoforms than flop versions, owing to the amino acid residue at position 775 (in GluA2), which is a serine in flip and asparagine in flop receptors (Partin et al., 1995; Partin, Fleck, & Mayer, 1996). As noted, this amino acid is positioned at the elbow point of an alpha-helical region on the interface between subunits of the dimer pairs in the LBD layer (Armstrong & Gouaux, 2000; Sobolevsky et al., 2009) (Figure 3B).

The LBD Dimer Interface and Receptor Desensitization

Slightly higher in the LBD dimer interface from the cyclothiazide binding site, residue 504 (in GluA2) can produce dramatic reductions in AMPAR desensitization when mutated from leucine to other aromatic-containing amino acids. In particular, the introduction of a tyrosine (known as the L/Y mutant) prevents desensitization almost entirely (Stern-Bach, Russo, Neuman, & Rosenmund, 1998). Because ultracentrifugation studies found that cyclothiazide-bound or L/Y mutant GluA2 LBDs exhibit greater protein dimerization, it was proposed that rupturing of the dimer interface is critical for desensitization to proceed for intact receptor complexes (Sun et al., 2002). In fact, a large number of residues along the AMPAR and KAR dimer interfaces appear to influence desensitization kinetics, based on the functional properties of receptors harboring mutations at these sites (e.g., Horning & Mayer, 2004; Y. Zhang, Nayeem, Nanao, & Green, 2006). Recent atomic resolution structures of intact GluA2 and GluK2 tetramers show that dimers within the LBD layer exhibit various degrees of subunit separation among proteins thought to be captured in desensitized states (e.g., Durr et al., 2014; Meyerson et al., 2014) (Figure 4C).

Within the KAR subfamily, for which there are no modulatory compounds able to disrupt desensitization to the extent of cyclothiazide at AMPARs, other approaches have been used to circumvent this gating process. Notably, the introduction of disulfide crosslinks

across the LBD dimer interface imparts the GluK2 receptor with nondecaying responses to glutamate (Priel, Selak, Lerma, & Stern-Bach, 2006; Weston, Schuck, Ghosal, Rosenmund, & Mayer, 2006). However, subsequent analysis of the Y512C/L783C mutant has shown single-channel openings to be sporadic, despite their occurring with equal probability during saturating agonist applications (Daniels, Andrews, Aurousseau, Accardi, & Bowie, 2013). A separate point mutation in GluK2 (D776K) also disrupts macroscopic current decay associated with desensitization (Nayeem, Zhang, Schweppe, Madden, & Green, 2009). From a structural perspective, the mutant lysine residue points across the apex of the LBD dimer interface, tethering into an electronegative pocket on the opposing subunit (Nayeem, Mayans, & Green, 2011). Accordingly, the D776K mutation acts in a similar manner to constrain the dimer interface as Y521C/L783C, though single-channel recordings demonstrate its open probability to be much higher in glutamate and thus truly nondesensitizing (Dawe et al., 2013).

The Requirement of External Ions for KAR Channel Gating

The gating properties of native and recombinant KARs are regulated by external anions and cations (reviewed in Bowie, 2010). Specifically, lowering the external ionic strength results in faster deactivation and desensitization rates of GluK2 receptors, while increasing ionic strength slows current decay kinetics (Bowie, 2002; Bowie & Lange, 2002). The cation and anion species composing the extracellular solution also influence gating. For GluK2 KARs, sodium produces the largest peak current and slowest desensitization, whereas substitution with progressively larger cations reduces peak amplitudes and accelerates desensitization (Bowie, 2002). Likewise, among anions, chloride and bromide yield maximal peak currents, but substitution with fluoride or iodide lowers peak amplitudes and accelerates desensitization (Bowie, 2002). Similar trends are also exhibited by GluK1 and GluK3 KARs (Plested & Mayer, 2007). Consistent with their vital role in channel gating, removal of all external monovalent ions eliminates detectable current responses from GluK2 KARs (even outward currents at positive holding potentials) but not of GluA1 or GluA2 AMPARs (Dawe et al., 2016; Wong, Fay, & Bowie, 2006), demonstrating that allosteric sodium and chloride ions are, in fact, required for GluK2 gating.

The site for allosteric ion binding at KARs is contained within the LBD dimer interface, where two sodium ions reside in electronegative pockets atop opposing subunits and a single chloride is located in between, at the middle of the interface (Plested & Mayer, 2007; Plested, Vijayan, Biggin, & Mayer, 2008) (Figure 4D). Mutations at residues surrounding the sodium pocket, notably M770K, limit the variability in GluK2 behavior between different ionic conditions (MacLean, Wong, Fay, & Bowie, 2011; Paternain, Cohen, Stern-Bach, & Lerma, 2003; Wong, MacLean, & Bowie, 2007). The nondesensitizing mutant D776K also mimics sodium binding by introducing a stably tethered charge into the sodium pocket (Nayeem et al., 2011), causing cation sensitivity to be lost (Dawe et al., 2013; Nayeem et al., 2009). Thus, it has been proposed that the role of ions at GluK2 KARs is to maintain the LBD dimer organization, preventing the separation of subunits that accompanies desensitization (Dawe et al., 2013). In this model, ion unbinding is a key

molecular event that triggers the onset of receptor desensitization (Dawe, Aurousseau, Daniels, & Bowie, 2015).

Surprisingly, heteromeric KARs are less sensitive to changes in external anion species than their homomeric counterparts (Paternain et al., 2003; Plested & Mayer, 2007). Assuming the LBD dimer pairs are composed of different subunits (e.g., GluK2 and GluK5), as in heteromeric AMPAR structures (Herguedas et al., 2016), the architecture of the dimer interface may account for the reduced sensitivity. In keeping with this, functional evidence shows that external lithium ions uniquely slow GluK2/GluK5 desensitization when substituted for sodium (Paramo, Brown, Musgaard, Bowie, & Biggin, 2017), an observation that is absent from homomeric channels. Interestingly, AMPARs also harbor the structural hallmarks of an allosteric cation-binding pocket as there are conserved electronegative residues in the equivalent location where sodium binds to KARs. Though ionic strength and external cation species generally do not influence AMPAR gating (Bowie, 2002; Bowie & Lange, 2002), an exception again occurs for lithium, which has been resolved in multiple GluA2 LBD structures (Assaf et al., 2013; Harms, Benveniste, MacLean, Partin, & Jamieson, 2013). Binding in a similar manner to sodium at KARs, lithium slows GluA2 desensitization, though without editing at the R/G position, as in GluA1, this effect is not observed (Dawe et al., 2016). Recent work has also shown that GluA2 flip receptors are exquisitely sensitive to external halide anions through a different binding site found at the LBD dimer interface, near position 775. Notably, larger halide species elicit faster entry into desensitization, though the effect is attenuated in GluA2 flop receptors (Dawe et al., 2019).

Structure and Function of the iGluR Amino-Terminal Domain

The ATD is not necessary for iGluR assembly and channel function, even though it encompasses roughly half of the entire protein (Kumar, Schuck, & Mayer, 2011). NMDARs (Fayyazuddin et al., 2000; Meddows et al., 2001), AMPARs (Pasternack et al., 2002), and KARs (Plested & Mayer, 2007) lacking their respective ATDs all remain capable of yielding current responses. Despite this apparent redundancy, it has long been known that the ATD is a regulatory site for gating, particularly in NMDARs (Hansen et al., 2010). For instance, variability within the ATD region of GluN2 subunits accounts for slower desensitization and deactivation in GluN2D versus GluN2A receptors (Gielen, Siegler Retchless, Mony, Johnson, & Paoletti, 2009; Monyer, Burnashev, Laurie, Sakmann, & Seeburg, 1994; Yuan, Hansen, Vance, Ogden, & Traynelis, 2009). The NMDAR ATD also contains binding sites for several allosteric modulators, including the divalent ion zinc (Peters, Koh, & Choi, 1987; Westbrook & Mayer, 1987) and the anti-ischemic drug ifenprodil (Carter et al., 1988), both of which antagonize responses. Among AMPARs, removal of the ATD slows desensitization roughly 2-fold and accelerates recovery from desensitization by a similar factor (Moykkynen, Coleman, Semenov, & Keinanen, 2014), though GluK2 KAR de-

sensitization and deactivation appear to be unaffected without the N-terminal region (Plested & Mayer, 2007).

The ATD in Receptor Assembly

The structures of isolated AMPAR (GluA2), KAR (GluK2), and NMDAR (GluN2B) ATDs all possess a similar architecture (Jin et al., 2009; Karakas, Simorowski, & Furukawa, 2009; Kumar, Schuck, Jin, & Mayer, 2009). The AMPAR and KAR ATDs crystallize as dimers, with each subunit displaying a bilobed, or clamshell-shaped, organization (Kumar et al., 2009). Though the ATD of iGluRs shows some homology with the binding domain of metabotropic glutamate receptors and bacterial amino acid–binding proteins (O'Hara et al., 1993), there is little structural indication of conserved ligand recognition. This is due to poor sequence conservation at key amino acid–binding residues, as well as several structural features hindering domain closure (Jin et al., 2009; Kumar et al., 2009). Consequently, the most interesting property of the ATD is arguably its propensity for dimerization. Consistent with earlier analytical ultracentrifugation experiments showing that the GluA4 ATD and LBD formed dimers and monomers, respectively (Kuusinen, Abele, Madden, & Keinanen, 1999), analysis of the GluA2 and GluK2 ATDs indicates that their monomer–dimer dissociation constants are orders of magnitude lower than the LBDs of the same subunits (Jin et al., 2009; Kumar et al., 2009). It has therefore been hypothesized that the ATD might facilitate the initial dimerization step during assembly of the tetrameric receptor complex (Gan, Salussolia, & Wollmuth, 2015). That being said, the structural template for tetramerization, at least for AMPARs, may be elsewhere, such as the TMD (Gan, Dai, Zhou, & Wollmuth, 2016)), since the AMPAR ATD cannot fully assemble on its own (H. Zhao et al., 2012). The ATD of GluK2/GluK5 KARs, however, is able to crystallize as a heterotetramer (Kumar et al., 2011), suggesting that some of the details of AMPAR and KAR assembly may be different.

A more refined interpretation of the ATD is that it biases AMPAR assembly in favor of specific subunit combinations, explaining the predominance of GluA1/GluA2 and/or GluA2/GluA3 heteromers at synapses (Henley & Wilkinson, 2016). This interpretation originated from investigation of AMPAR/KAR chimeras, from which it was concluded that a mismatched ATD region can prevent co-assembly of otherwise similar subunits (Ayalon & Stern-Bach, 2001; Leuschner & Hoch, 1999). Sedimentation velocity analysis of isolated AMPAR ATDs has revealed that the K_d values of ATD dimerization differ considerably between GluA1 (~100 nM), GluA2 (<10 nM), and GluA3 (>1 μM) (Rossmann et al., 2011; H. Zhao et al., 2012). However, the K_d for heterodimerization is reduced to around 1 nM for both GluA1/GluA2 and GluA2/GluA3 heteromers (Rossmann et al., 2011), implying that neither GluA1 nor GluA3 would be likely to assemble as a homomer in the presence of GluA2 subunits. Perhaps not coincidentally, it has been argued that most, if not all, AMPARs at the CA1 hippocampal synapse are GluA1/GluA2 (80%) or GluA2/GluA3 (15%) heteromers (Lu et al., 2009). Similarly, the GluK2/GluK5 heteromer has been described as the most common KAR complex in the brain (Petralia, Wang, & Wenthold, 1994). Not sur-

prisingly then, the ATD heterodimer comprised of GluK2 and GluK5 has a lower K_d value than homodimers of either subunit (Kumar et al., 2011).

Protein Interactions Mediated by the ATD

The ATD is the principal site where the iGluRs are glycosylated as they are trafficked through the endoplasmic reticulum (ER) and Golgi (Everts, Villmann, & Hollmann, 1997). Though N-linked glycosylation notably facilitates subunit assembly and surface expression, it can also influence gating behavior as some of the N-linked oligosaccharides are association sites for extracellular modulators like lectins (carbohydrate-binding proteins). For example, the plant lectin concanavalin-A modifies current responses in KARs to a greater extent than AMPARs (e.g., Everts et al., 1997; Partin et al., 1993), an effect that can be disrupted by ablation of the glycosylation sites (Everts et al., 1999; Fay & Bowie, 2006). Other exogenous lectins, like agglutinin (Yue, MacDonald, Pekhletski, & Hampson, 1995), as well as marine and vertebrate galectins, also modulate AMPAR and KAR function in a subunit-dependent manner (Copits, Vernon, Sakai, & Swanson, 2014; Ueda et al., 2013). More specifically, galectins slow AMPAR and KAR desensitization (Copits et al., 2014) to an extent that is proportional to the number of N-glycosylation sites (Garcia-Nafria et al., 2016). It has also been proposed that the iGluR ATD influences assembly of multiprotein complexes at synapses. In one case, the ATD of GluA2 has been shown to bind N-cadherin, an interaction which promotes spine formation in cultured hippocampal neurons (Saglietti et al., 2007). Likewise, neuronal pentraxin, a lectin protein expressed on axons, colocalizes with and clusters neuronal GluA4 AMPARs but not if the ATD is deleted (Sia et al., 2007).

Effect of Heteromerization on Channel Gating

iGluR heteromerization has profound effects on channel gating as the pharmacological and kinetic properties of heteromeric receptors often differ quite substantially from those of homomeric tetramers of their constituent subunits (summarized in Tables 1 and 2). That being said, the differing incorporation of the flip or flop cassettes into AMPAR complexes represents another type of heteromerization that can profoundly affect the gating properties of native receptors (Dawe et al., 2019). Interestingly, by comparing the kinetics and anion sensitivity of native AMPARs of the cerebellum with recombinant receptors, the authors were able to propose that cerebellar stellate and Purkinje cells express heteromers that contain both flip and flop isoforms (Dawe et al., 2019).

Heteromerization can also affect ion permeation through the channel pore region. As mentioned, the presence of the Q/R edited GluA2 subunit in heteromeric AMPAR complexes reduces calcium permeability and polyamine block (Burnashev et al., 1992; Geiger et al., 1995; Washburn, Numberger, Zhang, & Dingledine, 1997). While transcripts of the GluA2 subunit are entirely edited at the Q/R site (and other AMPAR subunits are not edited at all), editing of the KAR subunits is less extensive and less well understood (Puchalski et al., 1994; Schmitt, Dux, Gissel, & Paschen, 1996; Sommer et al., 1991). For GluK1

and GluK2 subunits, the extent of editing appears to depend on brain region and developmental regulation (Bernard et al., 1999; Sommer et al., 1991). Additional RNA editing at other residues in the TM1 segment of GluK2 can also determine divalent permeability and polyamine sensitivity, and these sites are also regulated to different extents (Köhler, Burnashev, Sakmann, & Seeburg, 1993).

Among KARs, the assembly of primary (GluK1–3) and secondary (GluK4–5) KAR subunits is a critical determinant of their functional properties. For example, KARs containing GluK4 or GluK5 are responsive to AMPA (see Figure 2B) and exhibit slower deactivation kinetics (Barberis, Sachidhanandam, & Mulle, 2008; Herb et al., 1992; Mott, Rojas, Fisher, Dingledine, & Benveniste, 2010). The secondary KAR subunits also have a higher affinity for glutamate than the primary subunits as the dose–response curve for GluK2/GluK5 channels is left-shifted compared to that of homomeric GluK2 (Barberis et al., 2008; Fisher & Mott, 2011). Within heteromeric KARs, the primary subunits are thought to drive channel desensitization, while activation of only the GluK4–5 subunits sustains channel activation because current responses to agonists selective for heteromers, as well as low concentrations of nonselective agonists, exhibit much weaker desensitization (Fisher & Mott, 2011; Mott et al., 2010).

Regulation of the AMPAR and KAR Channel Pore

The TMD is comprised of four TM regions, of which TM1 and TM4 reside on the outside of the pore, the TM2 re-entrant loop forms the pore, and TM3 lines the upper segment of the permeation pathway (Bowie, 2018; Sobolevsky et al., 2009; Twomey, Yelshanskaya, Vassilevski, & Sobolevsky, 2018). The precise location of the selectivity filter is the segment immediately following the Q/R site, from which TM2 bends back toward the intracellular face of the membrane (Kuner et al., 2001). Mutations in this region greatly reduce AMPAR channel permeability to large organic cations (Kuner et al., 2001). Interestingly, iGluRs are permeable to a range of different-sized monovalent cations from lithium to cesium, and non-NMDARs can even be permeated by some organic cations like tris(hydroxymethyl)aminomethane, resulting in estimates of minimal pore diameter of 5.5 Å for NMDARs (Villarroel, Burnashev, & Sakmann, 1995) and 7.5 to 8.0 Å for AMPARs and KARs (Burnashev, Villarroel, & Sakmann, 1996). At the same time, NMDAR permeability to divalent calcium ions is about 3-fold greater (~10% fractional current) than for AMPARs, even when the Q/R site is unedited (Burnashev, Zhou, Neher, & Sakmann, 1995).

Polyamine Block

Polyamines are organic nonprotein cations containing two or more charged amine groups. In mammals, the naturally occurring polyamines are putrescine, spermidine, and spermine, with the most abundant being the latter two (Pegg, 2009; Pegg & McCann, 1982). Polyamines are found in high concentrations in mammalian cells, with estimates ranging from 10 to 100 μM (Bowie & Mayer, 1995; Watanabe, Kusama-Eguchi, Kobayashi,

& Igarashi, 1991); but importantly, they are involved in a large number of cellular processes (Pegg, 2009). Given their cationic nature, they interact with negatively charged domains of biomolecules (Tabor & Tabor, 1984), including the pore regions of cation-selective VGICs and LGICs, where they bind with micromolar affinity and hinder ion flow (e.g., Bowie & Mayer, 1995; Haghighi & Cooper, 1998; Lopatin, Makhina, & Nichols, 1994). In this capacity, cytoplasmic polyamines are recognized as important determinants of neuronal signaling by regulating action potential firing rates (Fleidervish, Libman, Katz, & Gutnick, 2008) as well as the strength of neurotransmission (Aizenman, Munoz-Elias, & Cline, 2002; Rozov & Burnashev, 1999).

The inward rectification of *I–V* relationships obtained during AMPAR and KAR whole-cell recordings (e.g., Verdoorn et al., 1991) is due to channel block by intracellular polyamines (Bowie & Mayer, 1995; Donevan & Rogawski, 1995; Kamboj, Swanson, & Cull-Candy, 1995; Koh, Burnashev, & Jonas, 1995). As cations, polyamines are attracted into the channel pore, where, due to their larger cross-sectional diameter and slower permeation rates, they hinder the passage of other smaller cations such as sodium and calcium (Bowie, 2018). For example, the conductance of Q/R unedited GluK2 receptors at +50 mV is a mere 2% of the conductance at –100 mV, where virtually no polyamine block is detectable (Bowie & Mayer, 1995). At membrane potentials greater than +50 mV, the membrane electric field and cation permeation make conditions less favorable for polyamine binding (Bowie, Lange, & Mayer, 1998) such that the polyamines pass all the way through the pore (Bahring, Bowie, Benveniste, & Mayer, 1997). The ability of polyamines to both block and permeate unedited AMPARs and KARs defines them, in pharmacological terms, as permeant channel blockers, unlike the synthetic compounds MK-801 and phencyclidine, which block NMDARs in an effectively irreversible manner (Bowie, 2018). An added complication is that AMPAR and KAR pores are able to accommodate polyamines while in a closed state (Bowie et al., 1998). Interestingly, however, repetitive receptor activation can relieve much of this closed-channel block (Bowie et al., 1998; Rozov, Zilberter, Wollmuth, & Burnashev, 1998), which is thought to represent a novel mechanism of short-term plasticity in the mammalian brain (Rozov & Burnashev, 1999).

Given the prevalence of polyamines in the cytoplasm of almost all cells, most native AMPARs and KARs have evolved distinct mechanisms to prevent the occurrence of polyamine channel block. At AMPARs, two distinct mechanisms prevail that include (1) the formation of an electrostatic repulsion site at the apex of the pore, called the *Q/R site*, and (2) the co-assembly of AMPAR subunits with auxiliary proteins, such as TARPs and cornichon homologs (CNIHs; see below, Other AMPAR Auxiliary Proteins) (Bowie, 2018). Most native AMPARs are assembled with the GluA2 subunit, which contains a positively charged arginine residue at the Q/R site that repels polyamine from entering the pore. The *I–V* relationship of cells expressing GluA2-containing AMPARs is linear. In contrast, native AMPARs composed of GluA1, A3, and/or A4 subunits contain glutamine residues at the Q/R site, which favors polyamine block giving rise to inward rectification at positive membrane potentials. This difference in *I–V* relationships between GluA2-containing AMPARs, which exhibit a linear *I–V* relationship, and GluA2-lacking AMPARs, which exhibit a rectifying *I–V* relationship, is frequently used by synaptic physiologists as a marker to distin-

guish them in brain tissue. KARs have evolved three distinct mechanisms to attenuate polyamine block: (1) electrostatic repulsion at the Q/R site, (2) structural instability of pore helices via proline residues, and (3) the modulatory effect of neuropilin and tolloid-like (NETO) proteins (Bowie, 2018). Only GluK4 and GluK5 KAR subunits possess a proline residue in the pore helix, which means that native receptors assembled with either subunit lack cytoplasmic polyamine block. However, KARs assembled from GluK1, GluK2, and/or GluK3 all exhibit a high affinity for block by cytoplasmic polyamines.

Although polyamines are able to permeate AMPAR and KARs pores at extreme (>+50 mV) positive membrane potentials (Bahring et al., 1997), this has not been considered to be particularly significant in terms of cellular physiology. However, recent work has shown that the relief of polyamine block observed following auxiliary protein co-assembly with both AMPARs (e.g., TARP γ2 and CNIH3) and KARs (Neto1 and Neto2) (Soto, Coombs, Kelly, Farrant, & Cull-Candy, 2007) is achieved by facilitating polyamine permeation through the pore (Brown, Aurousseau, Musgaard, Biggin, & Bowie, 2016; Brown, McGuire, & Bowie, 2018). Surprisingly, polyamine flux can contribute to a significant conductance through the channel pore, particularly at positive membrane potentials. As mentioned, the lack of polyamine block of most native KARs containing either the GluK4 or GluK5 subunit (Barberis et al., 2008) is due to a single proline residue, conserved among GluK4 and GluK5 subunits, which is proposed to alter pore geometry around the selectivity filter (Brown et al., 2016).

Modulation by Fatty Acids

As with intracellular polyamines, externally applied fatty acids have also been shown to inhibit neuronal AMPARs and KARs, depending on their Q/R editing status (Huettner, 2015). Notably, arachidonic acid, a constituent of cell membranes, can attenuate neuronal AMPAR and KAR responses following several minutes of application (Kovalchuk, Miller, Sarantis, & Attwell, 1994; Wilding, Chai, & Huettner, 1998). Given that KAR inhibition occurs in a voltage-independent manner, it is noteworthy that increased susceptibility to inhibition occurs for Q/R edited receptors (Wilding, Zhou, & Huettner, 2005), the opposite of polyamine block. It remains unresolved whether fatty acids act as channel blockers or integrate into the membrane, altering the lipid environment around the TMD (Huettner, 2015).

Intact AMPAR and KAR Structures: Organization of Tetrameric Complexes

The first “intact” iGluR structure, resolved at atomic resolution (3.6 Å), was published in 2009, revealing the antagonist-bound GluA2 AMPAR to be a tall (180 Å), *Y*-shaped tetramer (Sobolevsky et al., 2009) (Figure 5A). Though earlier single-particle cryo-EM images had illustrated a “dimer of dimers” arrangement within the extracellular domains (Safferling et al., 2001; Tichelaar, Safferling, Keinanen, Stark, & Madden, 2004), the 2009 structure provided a wealth of new information regarding the arrangement of subunits,

while offering hints at the structural basis of activation. Notably, the A/B and C/D subunit pairs form ATD dimers, but the B and D subunits “cross over” to form closely packed pairs of LBD dimers comprised of A/D and B/C subunits. To achieve radial symmetry at the pore, the LBD–TMD linker orientations differ considerably between subunits, especially in the TM3–S2 linker, which is extended to reach the distal B and D subunit LBDs but compressed to connect with the more proximal A and C subunit LBDs (Sobolevsky et al., 2009).

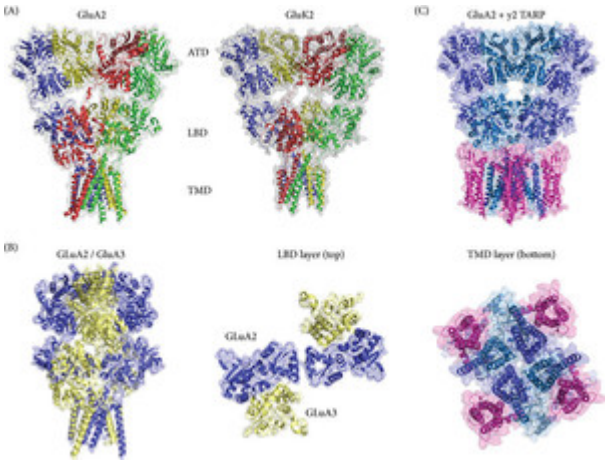

Figure 5. Arrangement of subunits in intact AMPAR and KAR complexes. (A) Side view of an intact tetrameric GluA2 AMPAR (left, PDB: 3KG2) in an antagonist-bound state (Sobolevsky et al., 2009) and a similarly arranged GluK2 KAR (right, PDB: 5KUF) in a desensitized state (Meyerson et al., 2016). Both structures are colored by subunit, as in the original 2009 paper: A, green; B, red; C, blue; D, yellow. Note the B and D subunits cross over between the ATD and LBD layers, forming dimer pairs with different opposing subunits in each layer. (B) Structure of an intact heterotetrameric AMPAR (left, PDB: 5IDE) formed by GluA2 (blue) and GluA3 (yellow) subunits (Herguedas et al., 2016). The arrangement of the subunits means that each LBD (and ATD) dimer comprises one GluA2 and one GluA3 subunit (right). (C) Structure of an intact AMPAR-TARP complex (top, PDB: 5WEO) formed by GluA2 (blue) and γ2 (magenta) subunits (Twomey et al., 2017a). Looking through the receptor from underneath the TMD layer (bottom), the channel pore can be resolved.

The first high-resolution cryo-EM structures of intact, recombinant GluA2/GluA3 and GluA1/GluA2 heteromers have exhibited a 2:2 subunit stoichiometry, within which one subunit occupied the A/C positions, while the other subunit occupied the B/D positions (Figure 5B). Consequently, the ATD and LBD layers were both comprised of heterodimers, and the pore region, which could not be resolved, was expected to be surrounded in a 2:3:2:3 or 1:2:1:2 configuration, placing like subunits opposite to one another, rather than adjacent (Herguedas et al., 2016, 2019). Recent data suggest a potentially more complicated situation with native AMPAR heteromers, which may also assemble with a 1:3 stoi-

chiometry, and with the apparent positional preference of specific subunits, such as GluA2, not necessarily fixed (Y. Zhao, Chen, Swensen, Qian, & Gouaux, 2019). There are not yet any intact structures of heteromeric KARs containing GluK4 or GluK5 subunits that would provide information regarding subunit position and its influence over their emergent gating properties. For NMDARs, which are obligate heteromers that are unable to form functional receptors from GluN1 or GluN2 subunits alone (Monyer et al., 1992), the intact structure reveals that the GluN1 subunits occupy the A/C positions, with the GluN2B subunits occupying the B/D positions (Karakas & Furukawa, 2014; Lee et al., 2014). Although representing an important advance, our understanding of the subunit arrangement within NMDA tetramers is likely to be revised as we learn more about the importance and abundance of triheteromeric NMDARs expressed in native brain tissue (Stroebel, Casado, & Paoletti, 2018; Yi, Bhattacharya, Thompson, Traynelis, & Hansen, 2019).

The GluA2/GluA3 apo state structures are also notable for their *O*-shaped conformation (Herguedas et al., 2016), featuring separated LBD dimers reminiscent of earlier EM reconstructions (i.e., Midgett, Gill, & Madden, 2012), rather than the *Y*-shaped crystal form. It should be noted that more recent structures where the tetramer is not constrained by stabilizing mutations do not exhibit this feature (Herguedas et al., 2019). Together, these structures suggest that the AMPAR apo state is capable of greater conformational flexibility than indicated by crystal structures. Fitting with this idea, EM images of native AMPAR complexes in unliganded and glutamate-bound conformations have illustrated that the compact organization of the ATD is lost during desensitization (Nakagawa, Cheng, Ramm, Sheng, & Walz, 2005; Nakagawa, Cheng, Sheng, & Walz, 2006). More recently, direct measurement of the conformational change induced by agonist binding and receptor desensitization was measured by atomic force microscopy (AFM) to represent almost 10% of the overall height of the receptor (Dawe et al., 2019). Another unexpected insight from AFM measurements has been the observation that nanoscale movement of the ATD in the apo state is regulated by the flip/flop cassette in the LBD (Dawe et al., 2019), which provides a mechanistic understanding of why the overall ATD architecture adopts several different conformations when the receptor is desensitized (Durr et al., 2014; Meyerson et al., 2014). This latter finding of bottom-up (LBD to ATD) conformational regulation of AMPARs differs from NMDARs, where it has been argued that top-down movements (ATD to LBD) dictate agonist open-channel probability and the degree of allosteric regulation (Gielen et al., 2008; Gielen et al., 2009; Yuan et al., 2009).

Mechanism of Pore Opening

A detailed, molecular model of iGluR activation has only recently come together, owing to the difficulty of capturing receptors in an open-channel state. Indeed, the brief, submillisecond open times measured during single-channel recordings of AMPARs and KARs (i.e., Swanson et al., 1997; W. Zhang et al., 2008) suggest that open states are inherently unstable. To better visualize AMPARs in an activated form, agonist-bound protein structures have been obtained with positive allosteric modulators also present (Durr et al., 2014; Meyerson et al., 2014). In such cases, AMPARs exhibit greater closure of the ago-

nist-binding cleft, along with 5–20 Å of vertical compression in the ATD and LBD layers, when compared to apo and antagonist-bound states. These structures also featured an outward expansion of the LBD–TMD linkers, proposed to generate the mechanical force that pulls open the channel pore (i.e., Dong & Zhou, 2011; Sobolevsky et al., 2009). Nevertheless, the first intact structure with the resolution of an open channel pore was obtained relatively recently by imaging GluA2–TARP complexes with cyclothiazide bound (Twomey et al., 2017a) (Figure 5C). Based on the architecture of the pore, it has been hypothesized that upward pulling by the LBD–TMD linkers (especially TM3–S2) flips apart pore-lining TM2 segments near the selectivity filter, along with TM3 residues that form an "upper gate," allowing ion permeation (Twomey et al., 2017a). Consistent with this framework, the insertion of amino acid residues into TM3–S2 linkers reduces NMDAR open probability, presumably counteracting the tension that pulls open the pore during gating (Kazi, Dai, Sweeney, Zhou, & Wollmuth, 2014).

LBD Layer Rotation During Desensitization

Several intact, agonist-bound AMPAR and KAR structures resolved in the absence of positive modulators have been presumed to represent desensitized conformations, on account of the high probability that these receptors are desensitized in the continued presence of the agonist. For GluK2 KARs, cryo-EM mapping of its resting and desensitized states indicates that the LBD layer undergoes dynamic rearrangements during the desensitization process (Schauder et al., 2013) (Figure 4C). Specifically, the resting state LBD is formed by two closely situated dimer pairs that separate into isolated domains as desensitization proceeds. Remarkably, this separation involves an extreme 125-degree rotation of the distal B/D subunits relative to the resting state (Meyerson et al., 2016, 2014). Rearrangements on a similar scale have not been observed for AMPARs, perhaps because their desensitized states are less stable and/or more short-lived (e.g., Bowie & Lange, 2002). In one agonist-bound GluA2 AMPAR structure, a 105-degree rotation was seen in one subunit within the LBD layer, though the other dimer was largely intact (Durr et al., 2014). Furthermore, a quisqualate-bound AMPAR complexed with the auxiliary protein GSG1L (which slows recovery from desensitization) displayed 14-degree rotation of the A/C subunits, relative to their position in another, presumably nondesensitized structure (Twomey et al., 2017a) (Figure 4C). These results suggest that structural arrangements during AMPAR desensitization might be complex and variable between agonists or with auxiliary protein association. Interestingly, despite some rotation of the overall ATD layer, ATD dimers remain intact in desensitized AMPAR structures (Meyerson et al., 2016; Twomey et al.,2017b).

Auxiliary Subunits

Though several proteins are considered auxiliary subunits of AMPARs and KARs, numerous other proteins may interact with or regulate these receptor families without being classified as auxiliary to pore-forming subunits. A useful framework defines auxiliary subunits as being unable of forming ion channels alone (1) but able to interact directly with a

pore-forming subunit (2) and modulate its trafficking and/or gating properties in heterologous cells (3), as well as have some effect in vivo (4) (D. Yan & Tomita, 2012). Extensive investigation into the modulation of AMPARs by TARPs (and other auxiliary proteins), as well as KARs by NETO1 and NETO2, has pointed to a fundamental role for auxiliary proteins in the physiology of glutamatergic synapses (Jackson & Nicoll, 2011).

TARPs as AMPAR Chaperones

The first known AMPAR auxiliary protein (TARP γ2) was originally referred to as *stargazin* because it is encoded by the gene disrupted in the stargazer mutant mouse, noted for its absence seizures (Letts et al., 1998; Noebels, Qiao, Bronson, Spencer, & Davisson, 1990). Interestingly, synaptic AMPAR-mediated responses are almost entirely absent in stargazer mice in cerebellar granule cells (Hashimoto et al., 1999). This observation led to the discovery that AMPAR surface expression is enhanced in the presence of stargazin and that their association is required for synaptic localization of AMPARs, which depends on interactions between stargazin and the scaffolding protein PSD-95 (L. Chen et al., 2000; Schnell et al., 2002). Further to this point, interactions between AMPAR and γ2 subunits have been detected in the plasma membrane and ER (Bedoukian, Weeks, & Partin, 2006), suggesting that TARP augmentation of receptor trafficking might stem from early intervention in protein folding or subunit assembly (Figure 6A).

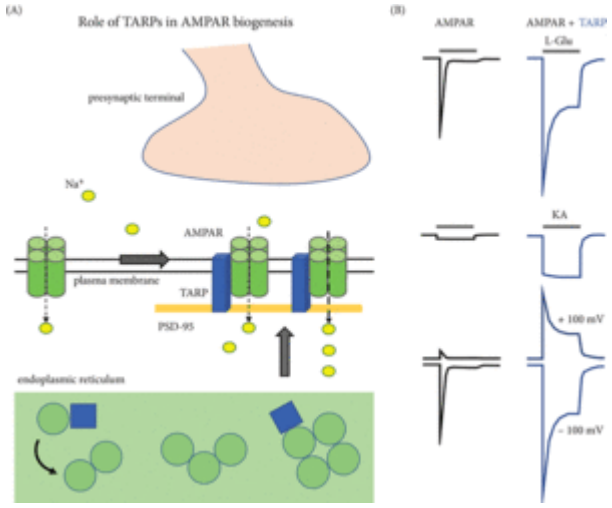

Figure 6. Role of auxiliary subunits in AMPAR expression and gating. (A) Cartoon illustration of the various roles that TARPs (and other auxiliary proteins) are thought to play in AMPAR biogenesis. TARPs associate with AMPARs in the ER and promote forward trafficking to the plasma membrane. At the membrane, TARPs help AMPARs to anchor in the postsynaptic density through binding to PSD-95. Finally, TARPs also enhance AMPAR gating. (B) TARPs modulate AMPAR function in numerous ways, including slowing agonist-induced desensitization (top), increasing the efficacy of KA (middle), and relieving polyamine block at positive membrane potentials (bottom).

The cloning of additional TARP subunits has led to the grouping of γ2, γ3, γ4, and γ8 (type I) based on a conserved TTPV amino acid motif at the intracellular C terminus, while two smaller subgroups of γ5 and γ7 (type II), as well as γ1 and γ6, also exist (Burgess, Gefrides, Foreman, & Noebels, 2001). Protein expression of these type I TARP subunits occurs differentially throughout the brain, with a predominance of γ2 in the cerebellum, γ3 in the cerebral cortex, and γ8 in the hippocampus (Tomita et al., 2003). On the whole, there is minor variability in the functional phenotype imparted onto AMPARs by the type I TARP subunits (Kott, Werner, Korber, & Hollmann, 2007; Milstein, Zhou, Karimzadegan, Bredt, & Nicoll, 2007), and they all generally modulate surface trafficking and the duration of channel gating in a positive manner (summarized in Jackson & Nicoll, 2011). For the type II subunits γ5 and γ7, which possess a shorter intracellular C terminus, less modulation of AMPAR gating occurs, despite the fact that both can immunoprecipitate with AMPAR subunits from brain tissue (Kato et al., 2007). The γ5 subunit actually modestly accelerates the deactivation and desensitization of recombinant GluA2 receptors (with no effect on GluA1), despite simultaneously increasing their current response (Kato, Siuda, Nisenbaum, & Bredt, 2008). This current understanding is likely to be revised as we learn more about the regional and cell-type expression of TARPs in the mammalian brain and their effect on recombinant receptors.

Functional Modulation of AMPARs by TARPs

Numerous gating and permeation properties of recombinant AMPARs are positively modulated by stargazin and other type I TARPs. Notably, deactivation and desensitization are slower, the relative efficacy of KA versus glutamate is increased, the glutamate dose–response curve is leftward-shifted, and single-channel properties such as channel conductance and burst length are increased (Priel et al., 2005; Tomita et al., 2005; Turetsky, Garringer, & Patneau, 2005) (Figure 6B). In keeping with their ability to favor and stabilize the open state, TARP binding to AMPARs has also been shown to convert quinoxalinedione antagonists (i.e., CNQX and DNQX) into weak partial agonists (MacLean & Bowie, 2011; Menuz, Stroud, Nicoll, & Hays, 2007), promote “resensitization” of the channel during long agonist pulses (Kato et al., 2007), reduce the ability of cyclothiazide to potentiate AMPARs (Cho, St-Gelais, Zhang, Tomita, & Howe, 2007), trigger the occurrence of modal gating behavior (W. Zhang, Devi, Tomita, & Howe, 2014), and uncover enhanced recovery from desensitization, or “superactivation,” where the test response amplitude surpasses the initial/conditioning response (Carbone & Plested, 2016). Other than their effects on channel gating, TARPs also affect the channel pore by attenuating channel block by cytoplasmic polyamines (Soto et al., 2007) by enhancing polyamine permeation (Brown et al., 2018). Importantly, the appreciation of TARP-mediated effects has helped explain the biophysical properties of native AMPARs that differed from findings on recombinant receptors. As a result, it is generally assumed that almost all native AMPARs expressed in the mammalian brain are associated either fully or partially with TARP subunits (reviewed in Kato, Gill, Yu, Nisenbaum, & Bredt, 2010; see also Dawe et al., 2019).

AMPAR–TARP Assembly and Stoichiometry

Because AMPAR–TARP fusion proteins retain the same altered biophysical properties as observed during co-expression (Morimoto-Tomita et al., 2009; Shi, Lu, Milstein, & Nicoll, 2009), it was initially assumed that one TARP subunit associated with every pore-forming AMPAR subunit. Without the constraint of protein fusion, experimental approaches quantifying the molecular weight of AMPAR complexes or counting fluorescent-tagged TARP subunits have suggested a variable stoichiometry of between one and four TARPs per channel (Hastie et al., 2013; Kim, Yan, & Tomita, 2010). Such estimates have also been supported by cryo-EM studies of GluA2–γ2 complexes, consisting of one, two, or four TARPs per receptor (Twomey, Yelshanskaya, Grassucci, Frank, & Sobolevsky, 2016; Y. Zhao, Chen, Yoshioka, Baconguis, & Gouaux, 2016) (Figure 7). A recent study exploring AMPAR–TARP stoichiometry in the cerebellum has shown that some cells (i.e., Purkinje cells) behave as though having a full TARP contingent, whereas other cells, such as inhibitory stellate cells, are only partially TARP-modified (Dawe et al., 2019). Whether these observations can be extended to other brain regions, such as the hippocampus, where γ8 rather than γ2 expression predominates, remains to be investigated.

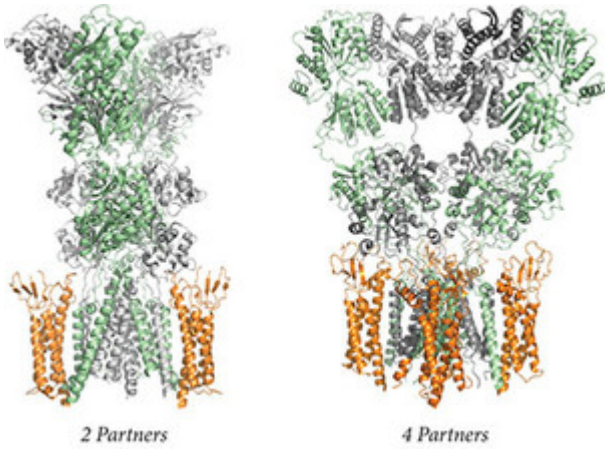

Figure 7. Variable stoichiometry of AMPAR auxiliary proteins. Synaptic AMPARs display current responses consistent with intermediate and full TARP association, depending on the cell type recorded from. Variable auxiliary protein stoichiometry represents an additional layer to the regulation of glutamatergic signaling.

The structural basis for TARP enhancement of AMPAR gating likely involves multiple interaction sites within the LBD, TMD, and CTD since TARPs have four TM segments with relatively small exterior regions. Indeed, deletion of the ATD still permits functional modulation by γ2 (Cais et al., 2014; Tomita, Shenoy, Fukata, Nicoll, & Bredt, 2007). Yet closer to the membrane, γ2 induces domain closure of the AMPAR agonist-binding cleft, presumably through some extracellular contact point, accounting for the increased efficacy of partial agonists (MacLean, Ramaswamy, Du, Howe, & Jayaraman, 2014). Likewise, the mutation of positively charged LBD residues, namely the KGK motif, predicted to interact with the extracellular loop of TARPs, greatly attenuates the effect of γ2 on GluA2 gating (Dawe et al., 2016). Full polyamine block is not recovered by this mutation, suggesting that other regions of the TARP structure are responsible for its effects on the channel pore (Dawe et al., 2016). On this note, evidence for the TMD being the principal region of TARP association has come from the study of GluK2–GluA3 chimeras. In this case, substitution of the AMPAR TM segments and CTD into the KAR backbone confers TARP association and modulation of some gating properties (Ben-Yaacov et al., 2017).

Whether AMPAR–TARP contact is maintained over time or more transient in nature has been debated, following initial reports that TARPs can dissociate from the AMPAR during agonist binding/activation in a process termed *autoinactivation* (Morimoto-Tomita et al., 2009). Given insight from recent work, the dissociation may simply represent disengagement from the KGK motif (Dawe et al., 2016), although this possibility would need to be formally investigated. For synaptic AMPARs, tracked at the single-molecule level, glutamate exposure appears to induce greater mobility, and the effect can be prevented by fusion to TARPs, suggesting that TARP uncoupling may occur (Constals et al., 2015). However, various indicators of TARP modulation (i.e., enhanced KA-evoked currents, reduced polyamine block) remain intact following long, desensitizing agonist applications, sup-

porting the idea that dissociation does not occur (Coombs, MacLean, Jayaraman, Farrant, & Cull-Candy, 2017). Clearly, more work is needed if these apparent differences are to be resolved.

Other AMPAR Auxiliary Proteins

Cornichon homologs 2 and 3 (CNIH-2 and CNIH-3) have also been identified as AMPAR auxiliary proteins (Schwenk et al., 2009). Although initial studies suggested that these proteins are likely to have three TM helices (Schwenk et al., 2009), a recent cryo-EM structure has shown that they possess four TM regions with little or no extracellular domain (Nakagawa, 2019). In agreement with the recent full-length structure (Nakagawa, 2019), CNIHs have been shown to associate with the TMD of AMPARs through membrane-proximal residues of the extracellular and intracellular domains (Shanks et al., 2014). Like TARPs, heterologously expressed CNIH-2 and CNIH-3 enhance AMPAR surface expression but have an even more profound impact on slowing current decay kinetics (Brown et al., 2018; Coombs et al., 2012; Schwenk et al., 2009). In addition, they increase single-channel conductance and attenuate voltage-dependent polyamine block of unedited AMPARs (Coombs et al., 2012) through a mechanism that promotes polyamine permeation (Brown et al., 2018). CNIH proteins are important in the brain, where conditional knockout of both CNIH-2 and CNIH-3 can greatly reduce synaptic AMPAR-mediated currents (Herring et al., 2013). Interestingly, CNIH-2, but not CNIH-3, is found in high relative abundance throughout the rodent brain (Schwenk et al., 2014).

Germ cell-specific gene 1-like (GSG1L) is another four-pass TM protein found in native AMPAR complexes (Schwenk et al., 2012; Shanks et al., 2012) and shown to associate with multiple AMPAR subunits in vitro (Shanks et al., 2012). When co-expressed with GluA2, GSG1L slows desensitization and recovery from desensitization (Shanks et al., 2012), though interestingly, unlike TARP and CNIH subunits, it reduces single-channel conductance and modestly enhances polyamine block (McGee, Bats, Farrant, & Cull-Candy, 2015; though see Bowie, 2018). In neurons, GSG1L impairs membrane trafficking to reduce the amplitude of AMPAR-mediated EPSCs (Gu et al., 2016; McGee et al., 2015). The full-length AMPAR–GSG1L cryo-EM structure suggests that GSG1L modulates AMPAR gating via an extracellular loop in a manner similar to γ2 TARPs, which is surprising given that GSG1L dramatically slows recovery from desensitization, whereas stargazin speeds it up.

Another class of AMPAR auxiliary proteins is known as cystine-knot AMPAR-modulating proteins (CKAMPs) or Shisa proteins (Haering, Tapken, Pahl, & Hollmann, 2014). CKAMP members are thought to possess a single-pass TM topology with an intracellular PDZ domain-binding motif (Farrow et al., 2015). Two members (CKAMPs 44 and 52 or Shisas 9 and 6, respectively) have been specifically identified as co-localizing with AMPARs at excitatory synapses (Klaassen et al., 2016; von Engelhardt et al., 2010) and are thought to stabilize AMPARs in the postsynaptic density through PDZ interactions (Khodosevich et al., 2014; Klaassen et al., 2016). Biophysical investigation in recombinant systems has revealed variable effects on current amplitudes and gating properties, depending on the

AMPAR and CKAMP subunits being expressed (Farrow et al., 2015; Khodosevich et al., 2014; Klaassen et al., 2016), though the most striking effect is to slow recovery from desensitization much like GSG1L. Since AMPAR behavior in CKAMP44 and CKAMP52 knockout mice is not particularly perturbed (Khodosevich et al., 2014; Klaassen et al., 2016; von Engelhardt et al., 2010), it was initially questioned whether CKAMPs play a fundamental role in AMPAR physiology. However, more recent work on knockout mice has demonstrated the importance of CKAMP44 in modulating synaptic short-term depression and input integration of the visual pathway in the lateral geniculate nucleus (X. Chen, Aslam, Gollisch, Allen, & von Engelhardt, 2018).

Other putative AMPAR auxiliary subunits have also been identified, including SynDIG1 (Kalashnikova et al., 2010), Porcupine (Erlenhardt et al., 2016), and SOL-1,2 in *Caenorhabditis elegans* (Wang et al., 2012; Zheng et al., 2006); but less is known about their role in AMPAR function at synapses. For a review of the modulatory effects of some of these auxiliary proteins, see Haering et al. (2014).

KAR Auxiliary Proteins

Neto1 and Neto2 are the two principal KAR auxiliary subunits (Tang et al., 2011; W. Zhang et al., 2009). They are predicted to be single-pass TM proteins containing two extracellular complement C1r/C1s, Uegf, Bmp1 domains and a low-density lipoprotein receptor domain A domain (Stöhr, Berger, Froehlich, & Weber, 2002). Both Neto1 and Neto2 interact with the GluK1 and GluK2 KAR subunits, enhancing trafficking to synaptic membranes (Sheng, Shi, Lomash, Roche, & Nicoll, 2015; Tang et al., 2011) as well as modulating functional properties (Copits, Robbins, Frausto, & Swanson, 2011; W. Zhang et al., 2009) in a subunit-dependent manner (Fisher, 2015). At present, the stoichiometry of Neto1 and Neto2 with homomeric and heteromeric KARs remains unknown.

Generally speaking, Neto1 and Neto2 slow the desensitization and deactivation kinetics of recombinant and synaptic KARs (Straub et al., 2011; W. Zhang et al., 2009). For Neto2, this effect results from an increase in the open probability and burst length of GluK2 single-channel currents (W. Zhang et al., 2009). Structurally, interactions of Neto2 with the GluK2 M3–S2 linker, as well as with the D1–D1 dimer interface, are critical for its modulatory effects on gating (Griffith & Swanson, 2015). Like TARPs, the Neto proteins also attenuate voltage-dependent polyamine block of KARs (Fisher & Mott, 2012) by facilitating polyamine permeation through the channel pore (Brown et al., 2016) in much the same way that TARPs and CNIHs attenuate channel block of AMPARs.

Conclusion

The vast majority of iGluR subunits were initially cloned in the early 1990s. Since that time, the first decade of research uncovered the complex gating behavior and pharmacology of recombinantly expressed receptors and rules governing heteromeric assembly. The second decade heralded the first atomic resolution structures of individual iGluR domains, as well as the discovery of auxiliary proteins that modulate AMPAR and KAR activi-

ty at the synapse. Finally, the years leading up to 2020 have been accompanied by structures of intact receptor complexes and models that relate protein movements to gating processes. Looking ahead, several fundamental questions connecting the biophysical properties of iGluRs with their physiological functions remain to be answered. Specifically, what combinations of auxiliary proteins accompany iGluRs in different cell types, and is their stoichiometry important in distinguishing responses between synapses? Moreover, how are iGluR–auxiliary protein interactions temporally regulated, and can such interactions contribute to synaptic plasticity? On a separate note, can the now extensive contingent of iGluR-regulating proteins provide novel routes for pharmacological regulation of glutamatergic signaling? An even more fundamental question involves the variability in AMPAR, KAR, and TARP subunit expression between brain regions. What is the need for local enrichment of specific subunits that generally exhibit the same gating properties as other subunits? And does the utility of flip/flop alternative splicing extend beyond the fine-tuning of desensitization, affecting native receptor assembly and function in other ways? Even after so many breakthrough advances in our understanding of AMPAR and KAR biology, it is still true, both literally and metaphorically, that exciting times remain ahead.

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