Accessories assist AMPA receptors to close pockets

Gabriela K. Popescu

Department of Biochemistry, University at Buffalo, The State University of New York, Buffalo, NY 14214

Stargazin (STZ), the prototypical member of the transmembrane AMPA receptor regulatory protein (TARP) family, can boost AMPA receptor activity to enhance synaptic currents as much as one third. The effects of STZ on microscopic and macroscopic AMPA receptor currents are consistent with a mechanism in which STZ facilitates opening of the AMPA receptor channel. However, the mechanism whereby this occurs is unclear. One hypothesis, largely based on structural models of AMPA receptor activation, is that STZ promotes closure of the cleft in the ligand-binding domain (LBD) of AMPA receptor, a conformation that more closely resembles that of the agonist-bound receptor structure (Armstrong and Gouaux, 2000). Two groups with complementary expertise, Howe and Jayaraman, teamed up to test this hypothesis (MacLean et al., 2014). They performed a series of challenging electrophysiological analyses and spectroscopic measurements and report the first direct evidence that indeed, in the presence of STZ, the agonist-binding cleft of AMPA receptor is more closed, regardless of whether or not glutamate is present. In addition to providing valuable evidence for a physiologically important neuromodulatory mechanism, this study is noteworthy for the elegance with which it bridges the still sizeable gap between structural and kinetic models of AMPA receptor activation. Such combined approaches hold the key to achieving an integrated view of how synaptic responses in the central nervous system arise and are modulated.

The Journal of General Physiology

AMPA receptors mediate excitatory neurotransmission in the brain

AMPA receptors are glutamate-gated ion channels and a hallmark of central excitatory synapses. Their kinetics are finely tuned to detect the rise and fall of the glutamate concentration in the synaptic cleft; to integrate this chemical signal with extracellular and intracellular cues; and to produce a postsynaptic current whose amplitude and kinetics reflect the totality of this information. The molecular diversity of tetrameric AMPA receptors and the constellation of modulators present at each synapse give rise to the broad diversity of synaptic electric signatures required to meet the specific needs of the trillion excitatory synapses that coexist in an adult (human) brain. Evidence is accumulating that STZ modulates several aspects of AMPA receptor physiology and thus regulates major aspects of the synaptic response.

The kinetic properties of the AMPA receptor response are determined primarily by the receptor's specific molecular composition (Fig. 1 A; Sobolevsky et al., 2009; Traynelis et al., 2010). Functional AMPA receptors can be homotetramers or heterotetramers of four subunit types (GluA1–GluA4), with native preparations expressing predominantly GluA1/GluA2 and GluA3/GluA4 diheterotetramers. Posttranscriptional (RNA splicing and editing) and posttranslational (covalent) modifications, which are in many cases under dynamic physiological control, produce additional molecular forms, each with distinctive functional features. An even wider diversity of AMPA receptor signals is achieved by modulating the activity of each channel protein through allosteric control of its pharmacologic and gating properties. This layer of regulation is dynamic and is achieved by the specific and reversible binding of various ligands, which can be ions, small molecules, or interacting proteins. In the late 1990s, STZ and a series of related proteins were found to regulate AMPA receptors.

STZ is required for proper expression of AMPA receptor currents in the cerebellum

STZ was identified as the culprit for the neurological and behavioral deficits of the Stargazer mouse, which is an inbred mouse line that arose spontaneously at The Jackson Laboratory, and was so designated for its distinctive head-tossing habit and ataxic gait (Noebels et al., 1990). When these mice are still, their electrocorticograms show recurrent spike–wave seizures characteristic of absence epilepsy (Di Pasquale et al., 1997). This defect was traced to a single (recessive) genetic locus on mouse chromosome 15 (Letts et al., 1997) that encodes a protein designated STZ to commemorate the phenotype that led to its discovery, or γ2, to indicate the (modest) similarity of its sequence to that of the γ subunit of Ca_v channels. Six additional proteins, γ3–γ8, were subsequently found to have similar sequences, thus defining a family of γ subunits (Klugbauer et al., 2000; Burgess et al., 2001).

Correspondence to Gabriela K. Popescu: popescu@buffalo.edu

The Rockefeller University Press
J. Gen. Physiol. Vol. 145 No. 1 17–21
www.jgp.org/cgi/doi/10.1085/jgp.201411320

Like all γ subunits, STZ is a transmembrane protein with four membrane-spanning helical regions, two extracellular loops, and cytosolic N and C termini (Fig. 1 B). It is broadly expressed in the adult brain, with the highest levels found in the cerebellum, olfactory bulb, cerebral cortex, thalamus, and the CA1 and DG regions of the hippocampus (Letts et al., 1998). Defects in the cerebellum and inner ear may lead to the ataxic gait and head-tossing behaviors that distinguish Stargazers from other mouse lines with epileptic features. STZ localizes at synapses, consistent with a role in synaptic transmission; however, at the time of its discovery, its function was unknown. Electrophysiological recordings revealed that, in Stargazer mice, the synapses that connect cerebellar mossy fibers with granule cells lack the fast AMPA receptor–mediated component of the excitatory postsynaptic current, directly implicating STZ in AMPA receptor–mediated synaptic transmission (Hashimoto et al., 1999). This landmark observation concentrated subsequent research on the possible mechanisms by which STZ might influence the expression of AMPA receptor currents at these—and potentially at other—synapses in the brain (Sharp et al., 2001).

STZ modulates AMPA receptor activity

STZ binds directly to AMPA receptors, a process that is dynamic and physiologically regulated (Chen et al., 2000; Sharp et al., 2001; Chetkovich et al., 2002; Tomita et al., 2004; Vandenberghe et al., 2005; Cais et al., 2014). Early studies indicated that STZ plays an essential role in AMPA receptor trafficking (Hashimoto et al., 1999; Chen et al., 2000), whereas later research revealed that it also modulates receptor function (Yamazaki et al., 2004; Priel et al., 2005; Tomita et al., 2005). Different regions of the STZ protein mediate its distinct roles in AMPA receptor physiology; a portion of the γ2 ectodomain (loop1) and the TM2 helix (Fig. 1 B), when transposed into a modulatory inert γ subunit (γ5), enhance AMPA receptor gating, whereas the cytosolic C-terminal tail increases the receptor's surface expression (Tomita et al., 2005). This is the discovery that ushered the idea that TARPs may have synaptic functions beyond escorting AMPA receptors into synapses, and may also serve to sculpt the kinetic signatures of synaptic receptors in situ (Tomita et al., 2005). This hypothesis gained further support with the observation that synaptic AMPA receptors can exist as distinct TARPed and TARPless populations (Bats et al., 2012). If indeed TARP association with AMPA receptors can, in a subunit-dependent and activity-dependent manner, control the shape of the synaptic current, which in turn controls neuronal firing probability, it becomes important to determine how TARPs change the response and what mechanisms are in play. These questions are currently under intense investigation.

TARPing controls pharmacologic and gating properties of AMPA receptors

The electrophysiological properties of AMPA receptors and the mechanisms that regulate them are incompletely understood. This is largely because of the receptor's fast and complex kinetics, and the difficulty of obtaining high resolution structures, as is typical for large membrane-embedded proteins. The available information has led to a scheme in which agonist binding to the extracellular LBD initiates intramolecular rearrangements that lead to the opening of the transmembrane channel or to receptor desensitization. Therefore, functionally, a receptor can be found in one of four states: resting unliganded (R), resting liganded (RA), active liganded (OA), or desensitized liganded (DA) (Fig. 2 A). The associated equilibria denote the agonist binding reaction: $R + A \rightleftarrows RA$ (association/dissociation), and two isomerization reactions: $RA \rightleftarrows OA$ (opening/closing) and $RA \rightleftarrows DA$ (desensitization/resensitization). Various approaches have been developed to extract the rate and equilibrium constants that govern this reaction, with

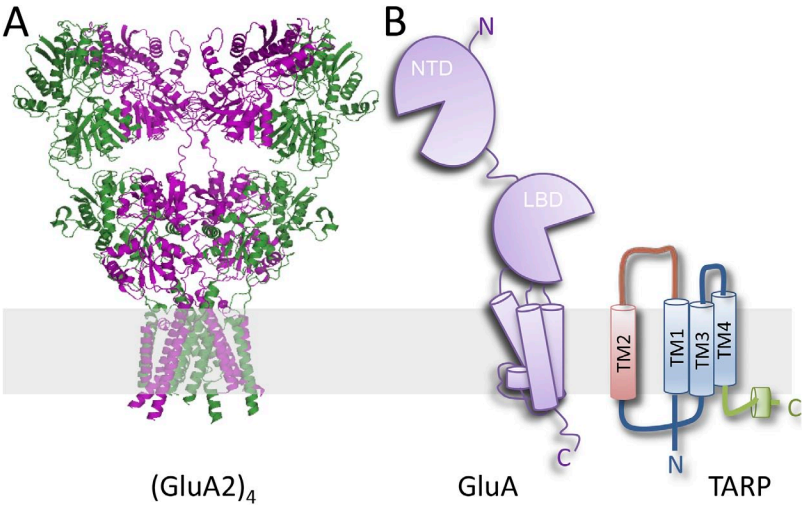

Figure 1. AMPA receptor structure. (A) Structural model of a GluA2 homotetramer (Protein Data Bank accession no. 3KG2) illustrates pore-forming subunits with distinct conformations (purple, green). (B) Cartoons illustrate the topology of one pore-forming subunit (GluA, left) and of one auxiliary subunit (TARP, right).

the goal of describing numerically the synaptic current and the effect of synaptic modulators. Despite several caveats inherent in the complexity of the process observed, these approaches have revealed that STZ modulates both the pharmacologic properties and the gating kinetics of AMPA receptors.

STZ makes glutamate a more effective agonist (shifts EC_{50} to left); makes partial agonists such as kainate much more effective by increasing their efficacy (increasing the maximal response); and can convert antagonists (such as CNQX) into partial agonists (Yamazaki et al., 2004; Priel et al., 2005; Tomita et al., 2005). These observations indicate that STZ may affect the structure of the agonist-binding site, to which all of these drugs bind. However, a simple change in the binding equilibrium does not explain the robust effects that STZ has on the time course of macroscopic currents, which desensitize more slowly, resensitize more rapidly, and also deactivate more slowly. Collectively, these observations suggest that STZ likely increases AMPA receptor opening. In fact, the change in gating kinetics represents the major effect of STZ on AMPA receptor currents and can result in an up to 30% increase in the charge transferred after a single synaptic response (Tomita et al., 2005). At present, the challenge is to understand how STZ produces these pronounced effects on receptor responses.

TARPing may promote LBD conformations that are more compact

How STZ changes AMPA receptor structure is unknown. However, given the functional observations summarized above and the emerging knowledge about the structures that support distinct functional states, we can begin to imagine likely hypotheses and approaches to test them. AMPA receptors project into the extracellular space two stacked domains: the N-terminal domain (NTD) and LBD, which have similar globular structures (Fig. 1, A and B). The LBD is proximal to the membrane and is directly connected, through short linkers, to three transmembrane helices, one of which, M3, directly gates the channel. Agonists bind in a cleft formed by two hinged lobes that constitute the LBD. The clefts of resting receptors oscillate around an equilibrium position that is relatively extended and easily accessible to diffusible ligands. All agonists and competitive antagonists bind in the same general area, but because they have distinct shapes and sizes, and straddle the cleft by contacting the two lobes through somewhat different contacts, they produce liganded structures in which the dimensions of the ligand-binding pocket differ. Each ligand's efficacy correlates strongly with its ability to close the binding pocket, an observation that led to the current structural model of channel activation, which states that energetic perturbations (mutations, ligands, etc.) that promote closed-cleft conformations also promote channel opening, presumably by pulling on the short linkers that connect the lower lobe of the LBD with the transmembrane helices (Fig. 2 B). Alternatively, the tension generated by cleft closure, rather than pulling on M3 to open the gate, may relax by structural rearrangements elsewhere to produce desensitized structures, i.e., receptors with closed clefts and closed pores. Given that AMPA receptor agonists are more efficacious in the presence of STZ and that receptors desensitize less and deactivate more slowly, it is reasonable to hypothesize that in the presence of STZ, the agonist-binding clefts tend to be more closed. MacLean et al. (2014) now present functional and structural evidence that directly supports this scenario.

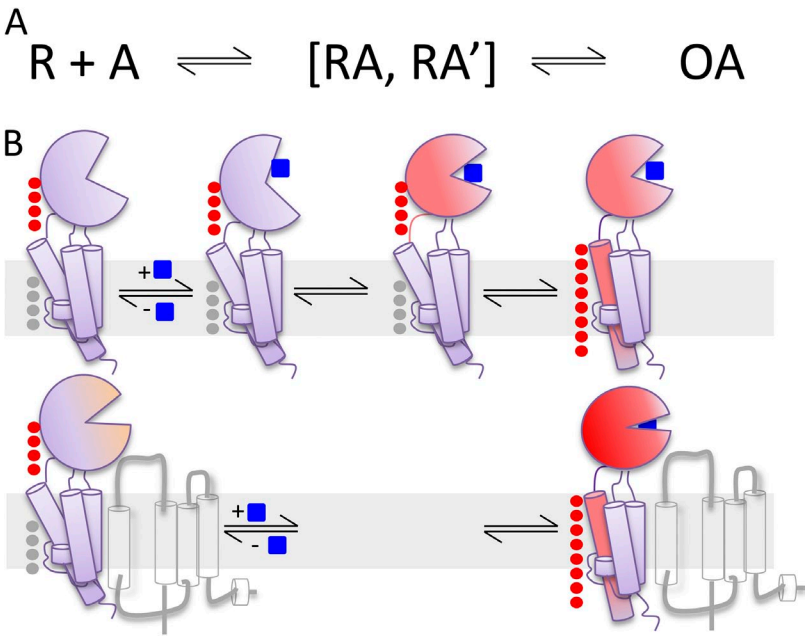

Figure 2. AMPA receptor activation. (A) Kinetic model represents stepwise receptor transitions from resting (apo, R) into liganded closed (RA, RA') and finally into liganded open (OA) kinetic states; each state aggregates a family of conformations with similar energy; desensitized states are omitted. R, receptor; A, agonist. (B) Cartoon illustrates structural changes that accompany activation by agonist of TARPless (top) and TARPed (bottom) receptors; pore-forming subunit (GluA2, NTD module omitted) is in color, and the accessory subunit (STZ) is in gray. Color change from purple to red illustrates a continuum of more active receptor conformations (narrower cleft, more stable open gate). Spheres illustrate permeation of external (red) Na^+ through open receptors.

First, they reasoned that if STZ indeed assists receptors in adopting conformations in which the agonist-binding clefts are narrower, it should do the same for mutant receptors that have difficulty in keeping their pockets closed, and in doing so should rescue their pharmacologic and gating deficits. Considerable work on two cleft mutants at threonine 686, T/S and T/A, has demonstrated that this minute single-residue perturbation, by preventing a native cross cleft contact, produces receptors that spend less time in closed-cleft conformations (Robert et al., 2005; Landes et al., 2011). Consistent with these observations, these mutants are also less responsive to glutamate (relative to quisqualate) and take longer to open (have slower rise times) (Robert et al., 2005; Zhang et al., 2008). MacLean et al. (2014) recorded currents elicited by brief agonist (glutamate or quisqualate) applications onto excised patches of cells expressing wild-type or mutant (T/S or T/A) receptors and observed that, in all cases, when STZ was coexpressed, cleft mutants behaved more like wild-type receptors: their responses to glutamate relative to quisqualate increased, and they produced currents that peaked faster. Thus, it appears that STZ assists agonist-bound receptors that cannot keep their pockets closed to behave more like their healthy relatives. However, it was unclear whether STZ also affected unliganded receptors. To answer this question, the authors turned to a clever pharmacologic approach.

If indeed STZ causes resting receptors to close their pockets, and thus to visit extended-cleft conformations less often, then bulkier ligands, which necessarily require extended cleft confirmations to access the binding site, will take longer to bind. Such a bulky ligand is NBQX. However, NBQX is an antagonist and does not produce currents; therefore, its association rate cannot be evaluated directly by measuring current. Instead, MacLean et al. (2014) exposed receptors to NBQX for increasing time periods, to increase the fraction of occupied and thus unresponsive receptors, and then evaluated the fraction of unoccupied (responsive) receptors with brief pulses of agonist (glutamate). By monitoring the decrease in glutamate-elicited responses with increasing NBQX preincubation times, they extracted an apparent binding rate constant for NBQX. When STZ was coexpressed, receptors had to be exposed to NBQX for longer periods to become unresponsive to glutamate. Thus, STZ reduced the apparent association rate of NBQX, consistent with a less accessible cleft. However, these results cannot show just how much more closed the cleft becomes when STZ is present. To answer this question, the authors turned to spectroscopic approaches, which can monitor with sufficient accuracy the average distance between two labeled residues.

The labeled residues were chosen to report on the position of the two lobes relative to each other: one was located at the N terminal of the LBD, thus on the upper, or membrane distal lobe, and the other was at the external tip of the binding cleft, on the lower, membrane proximal lobe. Results show that when STZ was present, this distance was consistently 2–3 Å shorter, whether the receptor was in its resting (apo) or liganded (glutamate or kainate) state. These measurements provide the first direct evidence that when STZ is present, AMPA receptors adopt conformations in which their agonist-binding lobes are closer together and thus their binding cleft is narrower. Within the currently accepted activation theory, which equates a narrower cleft with a more active receptor, the new results explain the potentiating effects of STZ on AMPA receptor currents as a capacity to promote closed-cleft receptor conformations (Fig. 2 B).

A way forward

Many questions pertaining to both mechanism and physiology remain. It will be important to delineate the precise contacts between STZ and AMPA receptors and determine how these modify function and structure: to answer questions related to stoichiometry (how many TARP molecules per AMPA receptor) and to specificity (which TARP works with which receptor and how). The literature is still uneven in the care with which the effects of individual TARPs on homomeric AMPA receptor isoforms (GluA1 through GluA4) have been delineated. Given that the predominant forms of native AMPA receptors are heterodimeric GluA1/2 and GluA2/3 subunits, it will be important to bolster the currently sparse literature on the basic gating properties of heterodimeric receptors and add to this how TARPs modulate their gating kinetics. Importantly, it is unknown whether the mechanism described here (pocket closure) is also responsible for the effects of TARPs on desensitization. The experiments that will address these topics will likely be challenging; monitoring function and intramolecular movement requires sophisticated expertise, technology, and equipment unlikely to be found in the same laboratory. Instead, collaborative approaches such as that of MacLean et al. (2014) can be the way forward to filling the current knowledge gap between what AMPA receptors do and how they look while doing it.

REFERENCES

Armstrong, N., and E. Gouaux. 2000. Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: Crystal structures of the GluR2 ligand binding core. *Neuron.* 28:165–181. http://dx.doi.org/10.1016/S0896-6273(00)00094-5

Bats, C., D. Soto, D. Studniarczyk, M. Farrant, and S.G. Cull-Candy. 2012. Channel properties reveal differential expression of TARPed and TARPless AMPARs in stargazer neurons. *Nat. Neurosci.* 15:853–861. http://dx.doi.org/10.1038/nn.3107

Burgess, D.L., L.A. Gefrides, P.J. Foreman, and J.L. Noebels. 2001. A cluster of three novel Ca^{2+} channel γ subunit genes on chromosome 19q13.4: Evolution and expression profile of the γ subunit gene family. *Genomics.* 71:339–350. http://dx.doi.org/10.1006/geno.2000.6440

Cais, O., B. Herguedas, K. Krol, S.G. Cull-Candy, M. Farrant, and I.H. Greger. 2014. Mapping the interaction sites between AMPA

receptors and TARPs reveals a role for the receptor N-terminal domain in channel gating. *Cell Reports.* 9:728–740. http://dx.doi.org/10.1016/j.celrep.2014.09.029

Chen, L., D.M. Chetkovich, R.S. Petralia, N.T. Sweeney, Y. Kawasaki, R.J. Wenthold, D.S. Bredt, and R.A. Nicoll. 2000. Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature.* 408:936–943. http://dx.doi.org/10.1038/35050030

Chetkovich, D.M., L. Chen, T.J. Stocker, R.A. Nicoll, and D.S. Bredt. 2002. Phosphorylation of the postsynaptic density-95 (PSD-95)/discs large/zona occludens-1 binding site of stargazin regulates binding to PSD-95 and synaptic targeting of AMPA receptors. *J. Neurosci.* 22:5791–5796.

Di Pasquale, E., K.D. Keegan, and J.L. Noebels. 1997. Increased excitability and inward rectification in layer V cortical pyramidal neurons in the epileptic mutant mouse Stargazer. *J. Neurophysiol.* 77:621–631.

Hashimoto, K., M. Fukaya, X. Qiao, K. Sakimura, M. Watanabe, and M. Kano. 1999. Impairment of AMPA receptor function in cerebellar granule cells of ataxic mutant mouse stargazer. *J. Neurosci.* 19:6027–6036.

Klugbauer, N., S. Dai, V. Specht, L. Lacinová, E. Marais, G. Bohn, and F. Hofmann. 2000. A family of γ-like calcium channel subunits. *FEBS Lett.* 470:189–197. http://dx.doi.org/10.1016/S0014-5793(00)01306-5

Landes, C.F., A. Rambhadran, J.N. Taylor, F. Salatan, and V. Jayaraman. 2011. Structural landscape of isolated agonist-binding domains from single AMPA receptors. *Nat. Chem. Biol.* 7:168–173. http://dx.doi.org/10.1038/nchembio.523

Letts, V.A., A. Valenzuela, J.P. Kirley, H.O. Sweet, M.T. Davisson, and W.N. Frankel. 1997. Genetic and physical maps of the stargazer locus on mouse chromosome 15. *Genomics.* 43:62–68. http://dx.doi.org/10.1006/geno.1997.4780

Letts, V.A., R. Felix, G.H. Biddlecome, J. Arikkath, C.L. Mahaffey, A. Valenzuela, F.S. Bartlett II, Y. Mori, K.P. Campbell, and W.N. Frankel. 1998. The mouse stargazer gene encodes a neuronal Ca^{2+}-channel gamma subunit. *Nat. Genet.* 19:340–347. http://dx.doi.org/10.1038/1228

MacLean, D.M., S.S. Ramaswamy, M. Du, J.R. Howe, and V. Jayaraman. 2014. Stargazin promotes closure of the AMPA receptor ligand-binding domain. *J. Gen. Physiol.* 144:503–512. http://dx.doi.org/10.1085/jgp.201411287

Noebels, J.L., X. Qiao, R.T. Bronson, C. Spencer, and M.T. Davisson. 1990. Stargazer: a new neurological mutant on chromosome 15 in the mouse with prolonged cortical seizures. *Epilepsy Res.* 7:129–135. http://dx.doi.org/10.1016/0920-1211(90)90098-G

Priel, A., A. Kolleker, G. Ayalon, M. Gillor, P. Osten, and Y. Stern-Bach. 2005. Stargazin reduces desensitization and slows deactivation of the AMPA-type glutamate receptors. *J. Neurosci.* 25:2682–2686. http://dx.doi.org/10.1523/JNEUROSCI.4834-04.2005

Robert, A., N. Armstrong, J.E. Gouaux, and J.R. Howe. 2005. AMPA receptor binding cleft mutations that alter affinity, efficacy, and recovery from desensitization. *J. Neurosci.* 25:3752–3762. http://dx.doi.org/10.1523/JNEUROSCI.0188-05.2005

Sharp, A.H., J.L. Black III, S.J. Dubel, S. Sundarraj, J.P. Shen, A.M. Yunker, T.D. Copeland, and M.W. McEnery. 2001. Biochemical and anatomical evidence for specialized voltage-dependent calcium channel gamma isoform expression in the epileptic and ataxic mouse, stargazer. *Neuroscience.* 105:599–617. http://dx.doi.org/10.1016/S0306-4522(01)00220-2

Sobolevsky, A.I., M.P. Rosconi, and E. Gouaux. 2009. X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. *Nature.* 462:745–756. http://dx.doi.org/10.1038/nature08624

Tomita, S., M. Fukata, R.A. Nicoll, and D.S. Bredt. 2004. Dynamic interaction of stargazin-like TARPs with cycling AMPA receptors at synapses. *Science.* 303:1508–1511. http://dx.doi.org/10.1126/science.1090262

Tomita, S., H. Adesnik, M. Sekiguchi, W. Zhang, K. Wada, J.R. Howe, R.A. Nicoll, and D.S. Bredt. 2005. Stargazin modulates AMPA receptor gating and trafficking by distinct domains. *Nature.* 435:1052–1058. http://dx.doi.org/10.1038/nature03624

Traynelis, S.F., L.P. Wollmuth, C.J. McBain, F.S. Menniti, K.M. Vance, K.K. Ogden, K.B. Hansen, H. Yuan, S.J. Myers, and R. Dingledine. 2010. Glutamate receptor ion channels: Structure, regulation, and function. *Pharmacol. Rev.* 62:405–496. http://dx.doi.org/10.1124/pr.109.002451

Vandenberghe, W., R.A. Nicoll, and D.S. Bredt. 2005. Stargazin is an AMPA receptor auxiliary subunit. *Proc. Natl. Acad. Sci. USA.* 102:485–490. http://dx.doi.org/10.1073/pnas.0408269102

Yamazaki, M., T. Ohno-Shosaku, M. Fukaya, M. Kano, M. Watanabe, and K. Sakimura. 2004. A novel action of stargazin as an enhancer of AMPA receptor activity. *Neurosci. Res.* 50:369–374. http://dx.doi.org/10.1016/j.neures.2004.10.002

Zhang, W., Y. Cho, E. Lolis, and J.R. Howe. 2008. Structural and single-channel results indicate that the rates of ligand binding domain closing and opening directly impact AMPA receptor gating. *J. Neurosci.* 28:932–943. http://dx.doi.org/10.1523/JNEUROSCI.3309-07.2008