Review TRENDS in Neurosciences Vol.27 No.6 June 2004

Structure and gating of the glutamate receptor ion channel

Lonnie P. Wollmuth and Alexander I. Sobolevsky

Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, NY 11794-5230, USA

Ionotropic glutamate receptors are ligand-gated ion channels that mediate rapid information transfer at most excitatory synapses in the brain. Crystal structures are now available for the ligand-binding domain, but the structure of the ion channel itself remains unknown. The core of the ion channel shares structural features with an inverted K^+ channel. In detail, however, differences are emerging. Most notable in the glutamate receptor ion channel are distinct structural and functional properties of a major pore-lining domain, the requirement of an additional transmembrane segment (M4), and twofold rather than fourfold symmetry. Together with ligand-binding domain structures and kinetic experiments, these findings have started to define the basic principles of channel gating in glutamate receptors.

Excitatory neurotransmission in the brain is predominantly mediated by glutamate. Presynaptic release of glutamate activates various glutamate receptors that can be broadly divided into ionotropic (or ligand-gated) and metabotropic (or G-protein-coupled) receptors. Ionotropic glutamate receptors mediate basic information processing in the brain and underlie changes in synaptic efficacy, such as those that are thought to be necessary for learning and memory, developing and maintaining cellular connections, and pain perception [1,2]. Their dysfunction also contributes to the cell death associated with numerous neurological diseases [3,4]. Hence, defining the structure of glutamate receptors, including that of their ion channel, has great relevance to basic and clinical brain research.

Ionotropic glutamate receptors – NMDA, AMPA and kainate receptor subtypes – although sharing a common endogenous ligand, show numerous pharmacological, biochemical and modulatory differences [5]. At the biophysical extremes, AMPA receptors show fast gating kinetics, desensitize strongly, are typically poorly permeable to Ca^{2+} and are blocked by intracellular polyamines, whereas NMDA receptors gate much more slowly, desensitize only weakly, are highly Ca^{2+}-permeable and are blocked by extracellular Mg^{2+} in a strongly voltage-dependent manner. It is this versatility and flexibility of glutamate receptors that make them so useful physiologically but also so fascinating and puzzling structurally.

Glutamate receptors, like all ligand-gated ion channels, comprise a ligand-binding domain and an associated ion channel. They also have several additional, modulatory regions, including large N-terminal and highly regulated C-terminal domains (Figure 1a,b). All of these components of the glutamate receptor, as well as subunit diversity (Figure 1c), are essential to their versatility in synaptic physiology. This review, however, will focus on the structure of the ion channel. It will consider the ligand-binding domain only in terms of its direct relation to channel gating – the process whereby agonist-induced conformational changes in the ligand-binding domain are converted to channel opening/closure. Although no atomic resolution structures of a glutamate receptor ion channel are available at present, recent functional experiments have revealed structural similarities and differences between glutamate receptor and K^+ channels, and have delineated basic principles of glutamate receptor channel gating.

Core structure of the glutamate receptor ion channel: homology to K^+ channels

There is now wide consensus and good evidence that the glutamate receptor ion channel, or at least part of it, shares evolutionary and structural kinship with K^+ channels and their relatives. When glutamate receptors were first cloned more than a decade ago, such an association would have seemed preposterous. Indeed, hydropathy plots of glutamate receptor subunits identified four hydrophobic segments, M1–M4, as was found in the 'classical' ligand-gated ion channels, the nicotinic ACh and $GABA_A$ receptors [6]. Glutamate receptors were therefore thought to be part of a large family of ligand-gated ion channels sharing a common structure: pentameric proteins in which the individual subunits have four transmembrane (hydrophobic) domains with both the N and C termini located externally. Fueling this homology was the early discovery that the functionally crucial Q/R/N site – a key determinant of ion permeation – is located in M2 [7], the main pore-lining domain in nicotinic ACh and $GABA_A$ receptor channels.

Various lines of evidence led to the currently accepted view of the membrane topology of glutamate receptor subunits [5] (see also Ref. [8] for intermediate models of topology). Several findings were seminal in developing this view. The first was the identification in both ionotropic and metabotropic glutamate receptors of domains showing sequence similarity to bacterial periplasmic binding proteins [9,10]. At the time, these findings, especially for ionotropic glutamate receptors, were curiosities because in

Corresponding author: Lonnie P. Wollmuth (lwollmuth@notes1.cc.sunysb.edu).

 0166-2236/$ - see front matter doi:10.1016/j.tins.2004.04.005

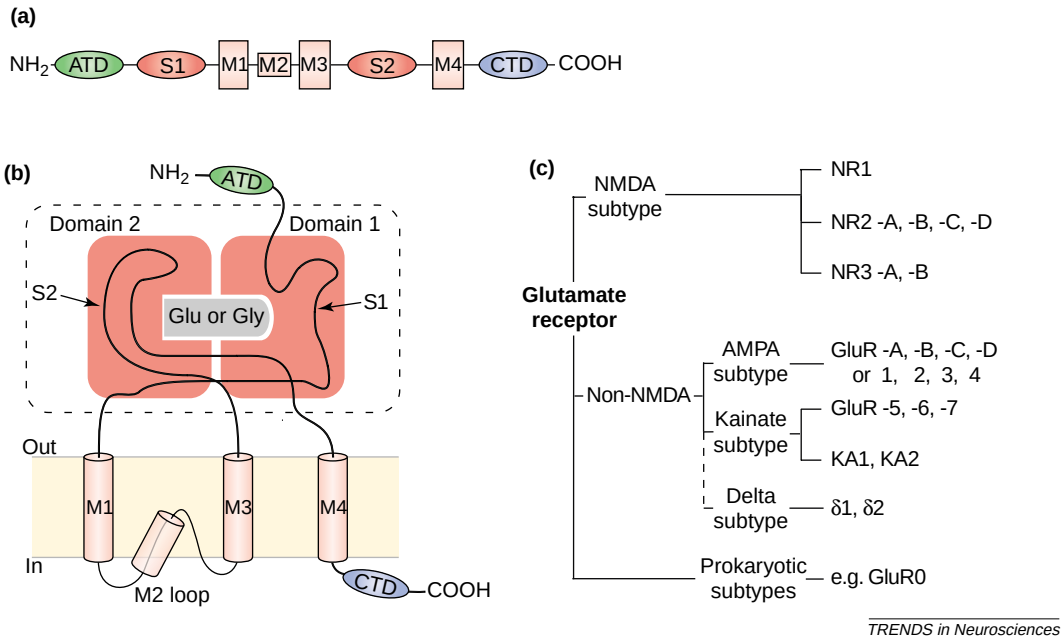

Figure 1. General structural features of ionotropic glutamate receptors. **(a)** A glutamate receptor subunit containing four hydrophobic domains (M1–M4) and several domains homologous to bacterial periplasmic binding proteins. One of them is located in the N-terminal domain (ATD) and shows homology to leucine, isoleucine, valine-binding protein (LIVBP). Two other domains, one N-terminal to M1 (S1) and the other between M3 and M4 (S2), show homology to lysine, arginine, ornithine-binding protein (LAOBP). The variable-sized C-terminal domain (CTD) contains numerous sites of phosphorylation and protein–protein interaction [55]. **(b)** Membrane topology of glutamate receptor subunits. Of the four hydrophobic segments, M1, M3 and M4 are membrane-spanning, whereas M2 forms a pore loop entering and exiting the membrane on its cytoplasmic side. The S1 and S2 lobes form the ligand-binding domain (S1S2 complex, indicated with broken lines), for which crystal structures are available [56]. For non-NMDA and NMDA receptor NR2 subunits, the S1S2 complex forms the glutamate-binding site, whereas for NMDA receptor NR1, it forms the glycine-binding site [5]. The large ATD and CTD domains are not shown to scale. **(c)** Family of ionotropic glutamate receptor subunits. Each ionotropic glutamate receptor subtype has different isoforms (subunits) arising from different gene products. There is no known mixing of subunits between subtypes. NMDA receptor channels are obligate heteromultimers requiring NR1 and NR2 subunits to form functional channels. NR3 might also form functional channels with NR1 [57], but it mainly co-assembles with NR1 and NR2 to form complexes with unique properties [58]. The AMPA and kainate receptor subunits can form either functional homomeric or heteromeric channels. KA1 and KA2 form functional channels only with other kainate receptor subunits. Further subunit diversity arises because of RNA editing and alternative splicing [5]. The delta subtype is an orphan receptor and functions as an ionotropic channel only under pathological conditions [46]. Prokaryotic glutamate receptor subunits, either identified (GluR0 [11]) or proposed to exist [19], create a possible evolutionary link between K^+ and glutamate receptor channels.

the original topology some of these domains were on the intracellular side of the membrane. We now know that two of them, specifically the lysine, arginine, ornithine-binding protein (LAOBP)-like domains located N-terminal to M1 (S1) and between M3 and M4 (S2), are extracellular and together form the ligand-binding domain (Figure 1b).

Other, more direct evidence showed that the N and C termini are located on different sides of the membrane and that there are three rather than four transmembrane segments, with M2 forming a pore loop, leading ultimately to the proposal that the glutamate receptor ion channel shares common structural features with K^+ channels [5]. This idea was strongly supported by the discovery of a prokaryotic glutamate receptor (GluR0) sharing features with both glutamate receptors and K^+ channels [11]. The finding that glutamate receptors, like K^+ channels, are apparently tetramers [12,13] (but see also Ref. [14]) and recently defined properties of pore-lining domains support the general kinship of glutamate receptors to K^+ channels.

Figure 2a (left) shows the crystal structure of two of the four subunits of the KcsA K^+ channel [15]. With the notable distinction of being inverted in the membrane (Figure 2a, right), this structure represents an approximate outline of the core of the glutamate receptor ion channel (M1–M3). Indeed, M2 in glutamate receptors not only forms a pore loop, but its secondary structure is highly reminiscent of the P loop in K^+ channels, being α-helical in its C-terminal part and making a transition near its tip to an extended region [16–19]. As in K^+ channels, the narrow constriction or selectivity filter in the glutamate receptor channel is positioned near the tip of the loop.

Similar to the inner helix (M2) of an inverted K^+ channel, the glutamate receptor M3 segment represents a major pore-lining domain that is extensively involved in channel gating [20–24]. In addition, M3 is α-helical, with the tip of the M2 loop, defined by the Q/R/N site, positioned about halfway across it [25] (Figure 3b,c). Although M1 in glutamate receptors contributes to the ion conduction pathway [20,24], like the homologous domain in K^+ channels, little is known about its structural or functional properties.

The structure of the K^+ channel represents a template for understanding that of glutamate receptors. However, the evolutionary history between these ion channel types is long and glutamate receptors have unique features to accommodate their specific and versatile role in synaptic physiology. For example, K^+ channels are highly selective for K^+ ions, whereas glutamate receptors are non-selective for monovalent cations and are also, in some instances, Ca^{2+} permeable. Such functional differences might arise because of local structural differences (i.e. close relatives of K^+ channels, such as cyclic-nucleotide-gated channels, are also cation non-selective). Recent evidence suggests, however, that glutamate receptor and K^+ channels have

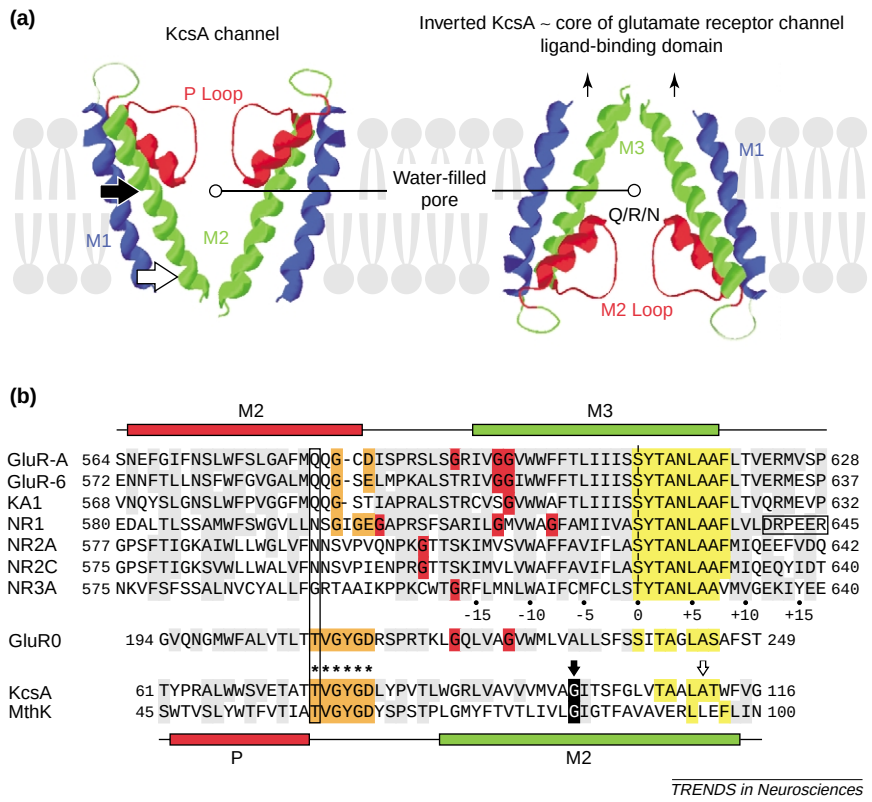

Figure 2. Structural kinship between the K^+ and glutamate receptor ion channels. **(a)** Crystal structure of the KcsA K^+ channel [15] in its proper orientation (left) and flipped upside down in the membrane (right). Only two of the four subunits are shown, with the front and back subunits removed for clarity. The inverted form corresponds to the approximate structure of the core of the glutamate receptor ion channel (M1–M3). Homologous domains in the K^+ and glutamate receptor channels are shown in the same color. On the left, the approximate location of the glycine gating hinge and the activation gate (bundle crossing of α-helices) in M2 are indicated by filled and open arrows, respectively [49]. In glutamate receptors, both the functionally crucial Q/R/N site and the narrow constriction of the channel are positioned at, or near, the tip of the M2 loop [16,18]. **(b)** Sequence alignment of the pore-forming domains in structurally related channels. Shown are amino acid sequences for AMPA receptor (GluR-A), kainate receptor (GluR-6 and KA1), NMDA receptor (NR1, NR2A, NR2C and NR3A), prokaryotic GluR0 and K^+ channel (KcsA and MthK) subunits. The M2 and M3 hydrophobic segments in the glutamate receptor subunits, and the pore helix (P) and inner helix (M2) in the K^+ channel subunits, are indicated. The absolute numbering corresponds to the mature protein. To aid comparison, amino acids in the M3 segment are also numbered relative to the first position (S) in the SYTANLAAF motif (yellow), which is the most highly conserved motif in glutamate receptors. The residues located at the tip of the re-entrant loops (the N site in NMDA receptors [16], the Q/R site in AMPA receptors [18] and the T site in K^+ channels) are boxed. The amino acids that form the selectivity filter in K^+ channels (asterisks) and homologous residues in glutamate receptor subunits are highlighted in orange. The gating hinge glycine residues and the approximate position of the activation gate in K^+ channels are indicated by filled and open arrows, respectively [50]. Glycine residues in M3 and the M2–M3 linker in glutamate receptor subunits are highlighted in red. The DRPEER motif (boxed) in the NR1 subunit is essential for Ca^{2+} permeability in NMDA receptor channels. Other positions occupied by similar residues are highlighted in gray. Reproduced from Ref. [25] © (2003) by the Society for Neuroscience.

more global structural differences including (i) a structural asymmetry between glutamate receptor subunits, (ii) an apparent requirement for an additional transmembrane segment (M4) in glutamate receptors, and (iii) differences in the structure of a major pore-lining domain involved in gating.

Structural asymmetry between NMDA receptor subunits

In most ligand- and voltage-gated ion channels, homologous positions in the pore-lining transmembrane segments are generally aligned in the vertical axis of the channel. Such an aligned positioning is most obvious in the homomeric KcsA channel [15], but it also exists in channels requiring heteromultimeric assemblies, such as nicotinic ACh and $GABA_A$ receptors [26,27]. NMDA receptors are obligate heteromultimers but their different subunits do not contribute equally to channel structure. The narrow constriction in NMDA receptors is formed by non-homologous asparagine residues: the N site of the NR1 subunit, and an adjacent one to the N site in NR2, the N + 1 asparagine [28]. Functionally, this asymmetry might provide a mechanism for the channel to interact with divalent cations in a fundamentally different way, allowing Ca^{2+} but not Mg^{2+} to permeate. Because the lack of Mg^{2+} permeability is essential to generating the strong voltage-dependent block [29], an asymmetric pore loop might be an efficient structural solution that enables the NMDA receptor to act as a coincidence detector. Nevertheless, the mechanism of Mg^{2+} block also depends crucially on the interaction of permeant monovalent ions (Na^+ and K^+) with the pore [30,31], and identifying the sites of monovalent ion interaction will be necessary to define in full the structural basis of the block.

The asymmetry between NMDA receptor subunits is more notable in the extracellular vestibule (Figure 3c), where the respective M3 segments, relative to the tip of the M2 loop (broken line), are offset by four amino acids comprising a single turn in an α-helix [32]. This vertical staggering of the NR1 and NR2 M3 segments presumably reflects a global rather than a local structural difference between subunits, because these segments share 70% identity (16 out of 23 residues) with SYTANLAAF

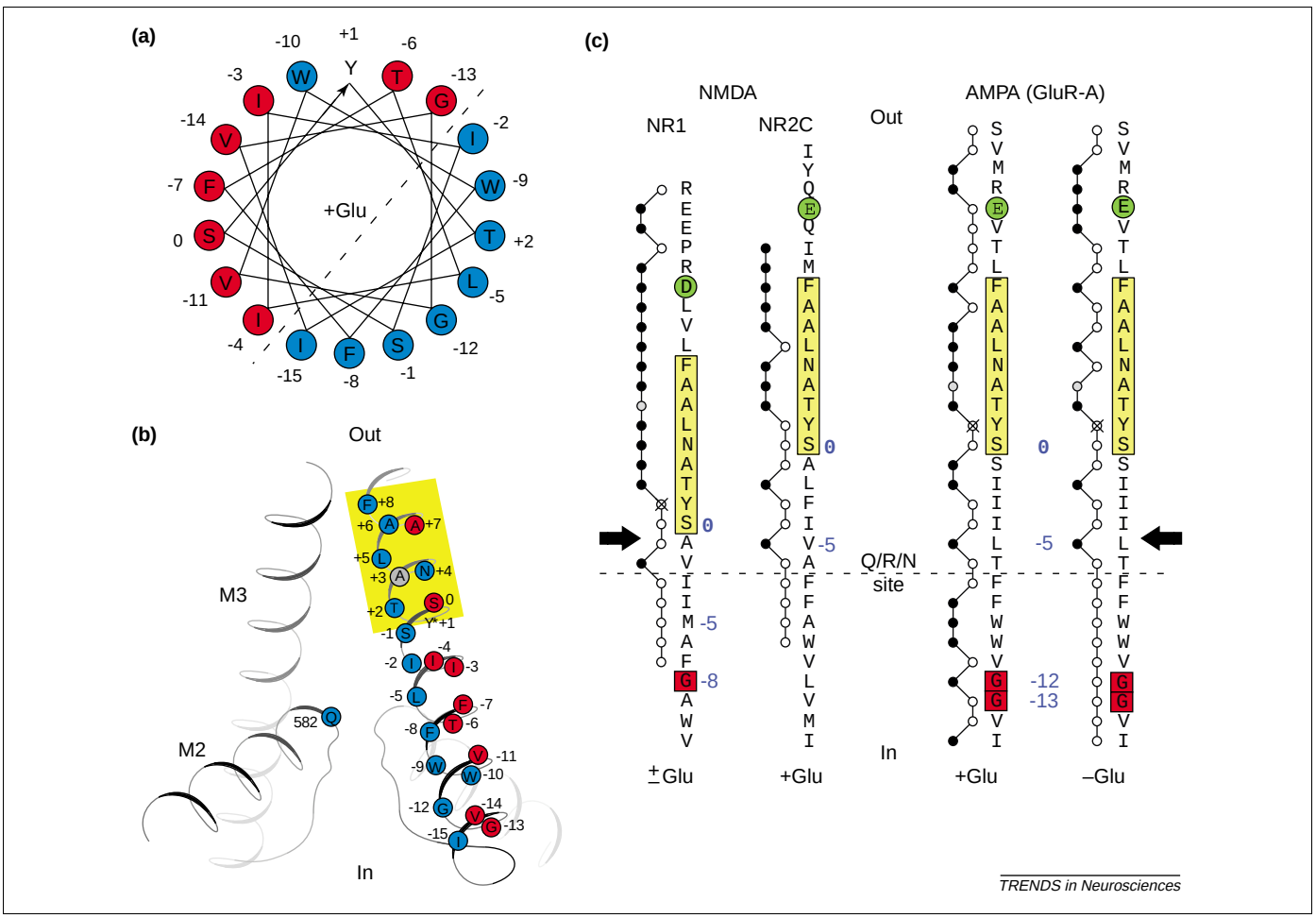

Figure 3. Structure and asymmetry of a major pore-lining and gating domain in glutamate receptor subunits. **(a)** Accessibility of substituted cysteine residues in the M3 segment suggests an α-helical secondary structure. Helical wheel analysis of cysteine residues substituted at positions −15 to +2 is shown for the AMPA receptor GluR-A subunit. Positions accessible to methanethiolsulfonate (MTS) reagents and thus presumably exposed to the water interface are in blue. Positions that are inaccessible and thus presumably buried in the protein or lipid interface are in red. Accessibility was tested in the presence of glutamate. All accessible positions, with the exception of W − 10, are on one side of the helical wheel, which strongly suggests that M3 is α-helical [25]. **(b)** The pore-lining M2 loop and M3 segment of two of the four AMPA receptor GluR-A subunits, with the back and front subunits removed for clarity. Presumed α-helical regions are shown as spirals. The left subunit shows the accessibility of the Q/R site (Q582 in the mature GluR-A protein), which is presumably located at the tip of the M2 loop. The right subunit shows the accessibility of positions in M3. Designations are the same as in (a). The SYTANLAAF motif is highlighted in yellow. In the presence of glutamate, numerous positions in SYTANLAAF are accessible; however, in the absence of glutamate, these positions show a more regular accessibility pattern consistent with an α-helical structure [25]. Some substitutions of A + 7 (the Lurcher position) [21] and A + 3 (gray circle) [25] yield channels that conduct under normal recording conditions. **(c)** Vertical alignment and staggering of glutamate receptor subunits. Binary representation of the accessibility of substituted cysteines to MTS reagents in the NR1, NR2C and GluR-A M3 segments in the presence (+Glu) or absence (−Glu) of glutamate. Filled circles indicate accessible positions, open circles nonaccessible positions. Results are derived from NR1 [20,35], NR2C [32] and GluR-A [25]. Y + 1C in NR1 and GluR-A does not produce functional channels (crossed circles). GluR-A(A + 3C) and NR1(A + 6C)–NR2C channels show a large leak current and a strong and irreversible inward current after MTS exposure, respectively (gray circles). Broken line indicates the approximate location of the Q/R/N site or the tip of the M2 loop relative to M3. The location of the SYTANLAAF motif (yellow boxes) relative to the broken line illustrates the vertical staggering of the M3 segments in NMDA receptor channels. The first negative charge in the DRPEER motif in the NR1 subunit, a key determinant of the high Ca^{2+} influx in NMDA receptor channels [35], and homologous residues in NR2C and GluR-A are highlighted in green. Glycine residues are highlighted in red. Filled arrows indicate the approximate location of the glycine gating hinge in a K^+ channel (Figure 2a). Reproduced, with permission, from Ref. [25] © (2003) by the Society for Neuroscience.

representing the most highly conserved motif in glutamate receptor subunits. AMPA receptor subunits have a vertical alignment similar to that in the NR2 subunits of NMDA receptors (Figure 3c).

Subunit- and subtype-specific staggering of M3 might underlie both the different functional contributions of NR1 and NR2 to NMDA receptor activation [33] and the differential sensitivity of NMDA receptors and AMPA receptors to channel blockers [34], but any direct relationship remains, at present, speculative. This staggering seems, however, to be essential to the high Ca^{2+} influx mediated by NMDA receptors, a property that has notable physiological and pathophysiological significance.

Ca^{2+} permeability in NMDA receptor channels is defined partially by the M2 loop (specifically the region forming the narrow constriction) and also by a unique domain, the DRPEER motif, that is positioned C-terminal to the NR1 M3 segment [35] (Figure 2b). In part, the significance of the DRPEER motif is due to its net negativity: three negative charges (one aspartate and two glutamate residues) but only one positive charge (the first arginine residue) are exposed to the water interface and hence can influence ion conduction. Because of subunit staggering, however, the DRPEER motif is also positioned closer to the ion conduction pathway (i.e. to the tip of the M2 loop) than are negative charges occupying homologous positions in NMDA NR2, AMPA and kainate receptor subunits (Figure 3c, green circles). Indeed, these negative charges have no significant effect on Ca^{2+} permeation [35,36]. Therefore, the staggering of the M3

segments might contribute both to the functional significance of the DRPEER motif and, to some degree, to the subtype specificity of Ca^{2+} permeability among glutamate receptors.

AMPA and kainate (GluR-5 to GluR-7) subunits can form functional homomultimers. Subunit staggering within a homomeric AMPA receptor channel is not obvious [25], but the possibility that it arises in heteromeric non-NMDA receptor assemblies is unknown and intriguing.

That pesky and peculiar M4 segment

In contrast to K^+ channels and bacterial glutamate receptors, all mammalian glutamate receptor subunits have an additional transmembrane domain, the M4 segment, positioned at the C-terminal end of the core of the ion channel (Figure 1b). This additional domain is not simply an auxiliary element but rather has acquired some essential, albeit unknown, function. Shorge and Colquhoun [37] studied NMDA receptor subunits lacking an M4 segment and C terminus. They found that these truncated subunits, which include the core of the ion channel and the ligand-binding domain, are not functional unless coexpressed with an additional construct encoding the M4 segment and C terminus. A similar dependence on the presence of M4 is found in AMPA receptors (A.I.S. and L.P.W., unpublished).

The reason why M4 is required for channel function is unknown. It is possible that it could act as a chaperone, somehow facilitating the transport of the glutamate receptor core from the endoplasmic reticulum to the membrane. Alternatively, M4 might be an essential transmembrane segment required either for correct protein folding or for the proper function of other (e.g. M3) structural domains. Indeed, M4 might contribute to the channel pore [20], and mutations in it also affect channel gating [38]. Nevertheless, the structural and functional significance of M4 remains unknown.

Gating rearrangements of the glutamate receptor ion channel

Channel gating in glutamate receptors is initiated in the ligand-binding domain with the final step, from a functional perspective, ion channel opening. Combining electrophysiology with recombinant channels, refined ionic conditions, kinetic modeling and crystal structures of the ligand-binding domain, recent work has developed more realistic models of glutamate receptor channel gating [12,33,39–44]. Some of these studies have delineated detailed kinetic aspects of glutamate receptor activation (Figure 4). The following section, however, focuses mainly on experiments that have directly addressed the structure and dynamics of the pore-forming domains during gating.

For glutamate receptors, all three transmembrane segments, M1, M3 and M4, are directly coupled to the ligand-binding domain (Figure 1). Not surprisingly, then, point mutations in each of these domains, as well as in the M2 loop, can affect gating [38,45–48]. Nevertheless, the main gating domain in glutamate receptors seems to be the M3 segment [21–23,25].

The inner helix M2 in K^+ channels, the domain homologous to M3 in glutamate receptors, also defines gating [49]. In the closed state, M2 is nearly straight and forms a gate for K^+ ions at the intracellular mouth of the channel, specifically at the bundle crossing of helices (Figure 2a, open arrow). With channel opening, M2 bends away from the central axis of the pore at the point of a gating hinge formed by a highly conserved glycine [50]. This glycine residue is positioned just below the tip of the P loop (Figure 2a, filled arrow), permitting movement of M2 independent of the P loop and leaving the selectivity filter essentially motionless during gating. Finally, the structure of K^+ channels in both the open and the closed (and presumably in all intermediate) conformations maintains fourfold rotational symmetry. Each of these features of K^+ channels – the gate location, the flexibility of the major gating domain and channel rotational symmetry – might differ in glutamate receptor channels.

Location of the activation gate

Because multiple domains contribute to the gating process, the term 'activation gate' is used here to refer to the specific structure that occludes the ion conduction pathway in the closed state. At present, there are two general models for the location of the activation gate in glutamate receptor channels. In analogy to an inverted K^+ channel, the activation gate might be formed at the extracellular mouth of the channel, presumably by a bundle crossing of M3 helices (Figure 2a). This model is consistent with the general assumption that pore blockers that get locked in with channel closure, so-called 'trapping blockers', do so behind the activation gate [51]. Furthermore, the presumed bundle helical crossing in glutamate receptors is near the highly conserved SYTANLAAF motif (Figure 3b). Substitutions of residues in this motif strongly alter channel function [20,21,25,52], as might be expected for a domain that forms the activation gate.

By contrast, experiments with substituted cysteine residues suggest that the activation gate is located deep in the pore, at the level of the tip of the M2 loop or deeper [20,22,25]. Indeed, nearly all substituted cysteine residues in M3 external to the tip of the M2 loop are accessible in both the presence and the absence of glutamate (Figure 3c), as though there is no barrier for the entry of small reagents. Trapping of pore blockers might arise because the extracellular vestibule constricts, but does not close completely, with channel closure [22]. Finally, many of the residues in the SYTANLAAF motif that are associated with constitutively active channels are, on the basis of the accessibility of substituted cysteine residues, not water accessible, suggesting that substitutions of them might disrupt contact interfaces of the protein domains.

A caveat must always be placed on substituted cysteine experiments, especially in respect to gate location, because one can get false-positives. In any case, the exact location of the activation gate in glutamate receptors, as well as the structural basis of concerted or subunit-specific activation gate opening (Figure 4), remains unresolved at present.

Glutamate receptor M3 segment is rigid during gating

Sequence alignments show that there are glycine residues in glutamate receptor M3 segments (Figure 2b), but none

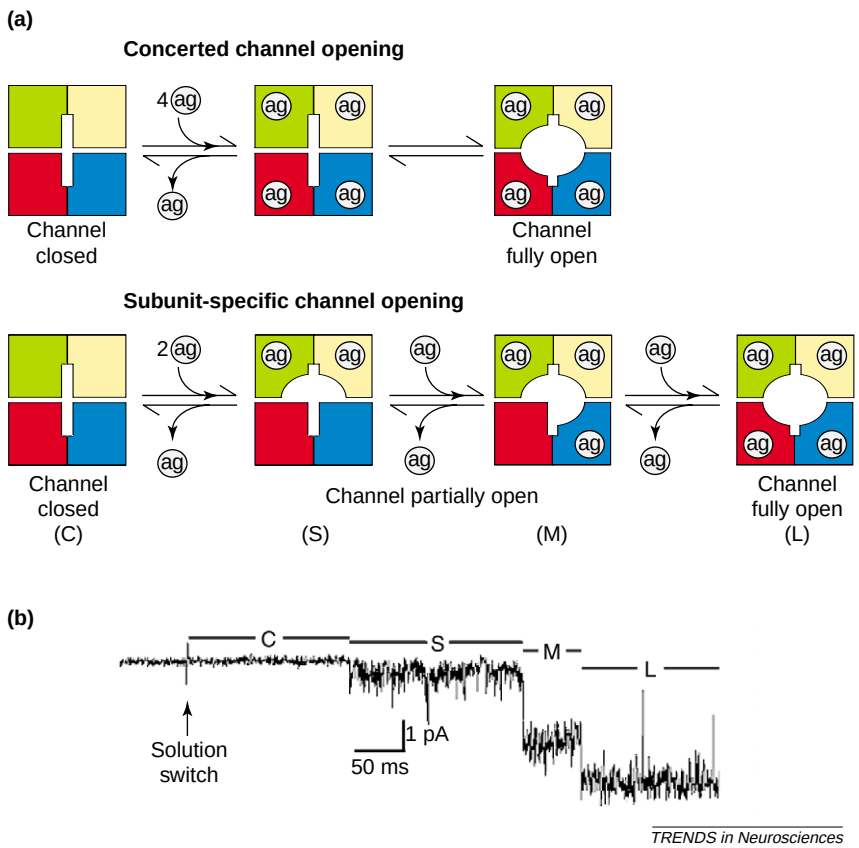

Figure 4. Gating in a multi-liganded channel. **(a)** Models of glutamate receptor activation. Each subunit in the glutamate receptor tetrameric complex, indicated by a different color, has a ligand-binding domain. Accordingly, two glutamate and two glycine molecules are necessary for full activation of NMDA receptors, whereas four glutamate molecules are necessary in non-NMDA receptors. Different models of this process can be envisioned, two of which are shown here. In the first, all four ligands or agonists ('ag') must be bound before producing a concerted opening of the activation gates ('concerted channel opening'). In the second, each subunit can make its own contribution to the activation process ('subunit-specific channel opening'); here, consecutive activation of subunit-associated gates gives rise to various degrees of channel pore opening and, correspondingly, different levels of single-channel conductance (S is small, M is medium, and L is large conductance). It seems that both mechanisms occur in glutamate receptor subtypes. Indeed, channel opening has been proposed to occur in a concerted manner in NMDA receptors [33], whereas there might be subunit-specific gates in AMPA receptors and most probably in kainate receptors. The subunit-specific gates in AMPA receptors was first suggested from single-channel studies in which AMPA receptors showed concentration-dependent subconductance states [12,39], and has gained recent support from a study combining single channels with crystal structures using partial agonists [44]. Because GluR-5–KA2 heteromers still show glutamate-activated currents in the presence of a selective GluR-5 neurotoxin (dysiherbaine) [59], a comparable subunit-specific gate mechanism might also occur in kainate receptors. **(b)** Current recorded from an outside-out patch containing a single AMPA receptor. Single-channel conductance has three different levels that correspond to different activation states of the subunit-specific gating model (a). The current record is reproduced, with permission, from Ref. [12] © (1998) American Association for the Advancement of Science (http://www.sciencemag.org).

of these is located at a position homologous to the gating hinge glycine residue in K^+ channels. Indeed, these glycine residues are positioned below the tip of the M2 loop (Figure 3c) and replacing them has no obvious effect on channel function [25]. By contrast, substitutions of the gating hinge glycine residue in K^+ channels typically yield non-functional constructs [53]. Because glycine and proline residues are the only known side chains that permit flexibility in an α-helix, the glutamate receptor M3 segment is presumably fairly rigid. Consistent with this idea, Cd^{2+} interacts with cysteine residues introduced both deep in the pore (L − 5) and more superficially (A + 6) in a manner that is clearly state dependent, suggesting that the whole of M3 moves during gating [54]. Thus, in contrast to M2 in K^+ channels, gating in glutamate receptors apparently involves a fairly rigid M3. The functional significance of this rigidity remains undefined, but it might cause the M2 loop to form the activation gate. Indeed, because M2 and M3 are in physical contact, movement of the whole of M3 will lead directly to movement of M2; furthermore, because M2 forms the narrowest part of the glutamate receptor channels, even subtle changes in its conformation will cause occlusion or opening of the channel pore during gating.

Symmetry of the ion channel

K^+ channels show fourfold rotational symmetry, indicating that each subunit is identical [49]. By contrast, the ligand-binding domain in glutamate receptors shows twofold symmetry, suggesting a possible symmetry mismatch between the ligand-binding domain and the ion channel [40]. Staggering of the M3 segments in heteromeric NMDA receptor channels suggests an unequal contribution of different subunits to channel structure. Recent evidence indicates, however, that even for homomeric AMPA receptors (GluR-A), the outer part of the pore shows twofold rather than fourfold rotational symmetry (Figure 5). Therefore, the symmetry of the ligand-binding domain might extend, at minimum, to the extracellular part of the pore, with a possible transition to fourfold rotational symmetry occurring deeper in the pore [54]. Nevertheless, how this symmetry contributes to the

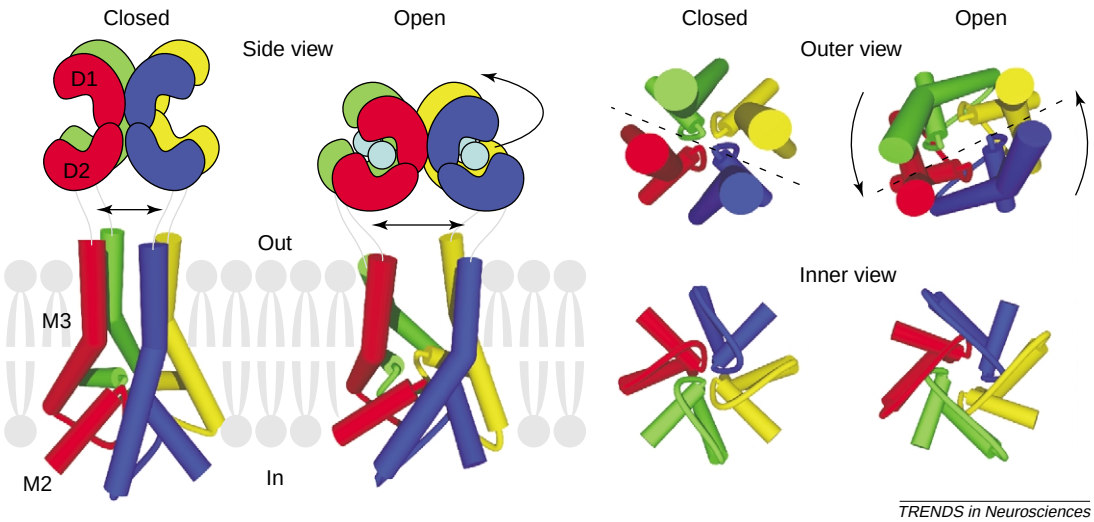

Figure 5. Rotational symmetry and gating rearrangements in the glutamate receptor ion channel. Shown are side, outer and inner views of the glutamate receptor ion channel in closed and open conformations. The side view also illustrates the ligand-binding domain that is located extracellularly to the channel and connected to it by linkers (gray). Each glutamate receptor subunit is shown in a different color. For the channel, only the M2 loops and M3 segments are shown. Cylinders represent α-helical regions. In terms of rotational symmetry, the ligand-binding domain is organized as a pair of dimers (red and blue for the first dimer and yellow and green for the second dimer), leading to a twofold rotational symmetry [56]. This symmetry extends down to at least the outer part of the pore (outer view) [54]. The inner part of the pore presumably shows fourfold symmetry (inner view). The transition from twofold to fourfold symmetry possibly arises because the extracellular third of the M3 segment in two neighboring subunits is kinked in the SYTANLAAF motif in opposite directions relative to the central axis of the pore (side view). In terms of gating, binding of agonist first induces closure of the ligand-binding domains (side view). With the interface holding the tops (D1 domains) of the dimers together, this closure leads to increase of ~8 Å in separation of the proximal portions (D2 domains) of the ligand-binding domain (horizontal arrows) [56]. This separation, applied via the linkers, pulls the tops of the M3 segments apart causing them to bend relative to the central axis of the pore. Movement of the M3 segments in turn induces movement of the M2 loops (which presumably form the activation gate), thereby opening the pore for passage of permeant ions. According to this model, the extracellular part of M3 (and correspondingly the ligand-binding domain) rotate relative to the intracellular part of the channel during gating (arrows in outer view). In addition, the receptor–channel complex (ion channel plus ligand-binding domain) compresses in the vertical dimension during channel opening (compare the closed and open conformation in side view). The structural model of glutamate receptor channel is modified, with permission, from Ref. [54].

multistep process of glutamate receptor activation remains unknown.

Future directions

Despite tremendous advances in our understanding of the structural dynamics of glutamate receptors, our image of these processes remains rudimentary. In the future, the use of traditional approaches to address these issues will hopefully be complemented by new – at least in terms of studying glutamate receptors – techniques, such as fluorescent resonance energy transfer, unnatural amino acid substitutions and NMR, among others. Clearly, the next quantum advance in understanding glutamate receptors will be crystal structures of the ion channel. By themselves, however, such structures will not be a panacea. Indeed, most of us who are interested in the structure of ion channels are physiologists; thus, it is not the structure *per se* that we are interested in but rather how it contributes to biological function. Combining crystal structures and other structural information with functional experiments (e.g. Refs [40,44]) will provide mechanistic insights that are necessary to appreciate in full the diversity and flexibility of glutamate receptors.

Acknowledgements

We thank C. Hewlet, M. Prieto, J. Watanabe, and M. Yelshansky for comments on the manuscript and Guy Crerend for technical assistance. This work was supported by NIH RO1 grants from NINDS and NIMH and a Sinsheimer Scholars Award (L.P.W.).

References

1 Bliss, T.V.P. and Collingridge, G.L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361, 31–39
2 Woolf, C.J. and Salter, M.W. (2000) Neuronal plasticity: increasing the gain in pain. *Science* 288, 1765–1769
3 Doble, A. (1999) The role of excitotoxicity in neurodegenerative disease: implications for therapy. *Pharmacol. Ther.* 81, 163–221
4 Lee, J.M. *et al.* (1999) The changing landscape of ischaemic brain injury mechanisms. *Nature* 399 (Suppl.), A7–A14
5 Dingledine, R. *et al.* (1999) The glutamate receptor ion channels. *Pharmacol. Rev.* 51, 7–61
6 Gasic, G.P. and Hollmann, M. (1992) Molecular neurobiology of glutamate receptors. *Annu. Rev. Physiol.* 54, 507–536
7 Burnashev, N. (1996) Calcium permeability of glutamate-gated channels in the central nervous system. *Curr. Opin. Neurobiol.* 6, 311–317
8 Hollmann, M. and Heinemann, S. (1994) Cloned glutamate receptors. *Annu. Rev. Neurosci.* 17, 31–108
9 Nakanishi, N. *et al.* (1990) A family of glutamate receptor genes: evidence for the formation of heteromultimeric receptors with distinct channel properties. *Neuron* 5, 569–581
10 O'Hara, P.J. *et al.* (1993) The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. *Neuron* 11, 41–52.
11 Chen, G.Q. *et al.* (1999) Functional characterization of a potassium-selective prokaryotic glutamate receptor. *Nature* 402, 817–821
12 Rosenmund, C. *et al.* (1998) The tetrameric structure of a glutamate receptor channel. *Science* 280, 1596–1599
13 Robert, A. *et al.* (2001) Subunit interactions and AMPA receptor desensitization. *J. Neurosci.* 21, 5574–5586
14 Premkumar, L.S. and Auerbach, A. (1997) Stoichiometry of recombinant *N*-methyl-D-aspartate receptor channels inferred from single-channel current patterns. *J. Gen. Physiol.* 110, 485–502
15 Doyle, D.A. *et al.* (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science* 280, 69–77
16 Kuner, T. *et al.* (1996) Structure of the NMDA receptor channel M2

segment inferred from the accessibility of substituted cysteines. *Neuron* 17, 343–352
17 Panchenko, V.A. *et al.* (2001) Structural similarities between glutamate receptor channels and K^+ channels examined by scanning mutagenesis. *J. Gen. Physiol.* 117, 345–360
18 Kuner, T. *et al.* (2001) Channel-lining residues of the AMPA receptor M2 segment: structural environment of the Q/R site and identification of the selectivity filter. *J. Neurosci.* 21, 4162–4172
19 Kuner, T. *et al.* (2003) A common architecture for K^+ channels and ionotropic glutamate receptors? *Trends Neurosci.* 26, 27–32
20 Beck, C. *et al.* (1999) NMDAR channel segments forming the extracellular vestibule inferred from the accessibility of substituted cysteines. *Neuron* 22, 559–570
21 Kohda, K. *et al.* (2000) Mutation of a glutamate receptor motif reveals its role in gating and δ2 receptor channel properties. *Nat. Neurosci.* 3, 315–322
22 Sobolevsky, A.I. *et al.* (2002) Molecular rearrangements of the extracellular vestibule in NMDAR channels during gating. *Neuron* 33, 75–85
23 Jones, K.S. *et al.* (2002) The NMDA receptor M3 segment is a conserved transduction element coupling ligand binding to channel opening. *J. Neurosci.* 22, 2044–2053
24 Kashiwagi, K. *et al.* (2002) Channel blockers acting at *N*-methyl-D-aspartate receptors: differential effects of mutations in the vestibule and ion channel pore. *Mol. Pharmacol.* 61, 533–545
25 Sobolevsky, A.I. *et al.* (2003) Different gating mechanisms in glutamate receptor and K^+ channels. *J. Neurosci.* 23, 7559–7568
26 Horenstein, J. *et al.* (2001) Protein mobility and GABA-induced conformational changes in $GABA_A$ receptor pore-lining M2 segment. *Nat. Neurosci.* 4, 477–485
27 Miyazawa, A. *et al.* (2003) Structure and gating mechanism of the acetylcholine receptor pore. *Nature* 424, 949–955
28 Wollmuth, L.P. *et al.* (1996) Differential contribution of the NR1- and NR2A-subunits to the selectivity filter of recombinant NMDA receptor channels. *J. Physiol.* 491, 779–797
29 Wollmuth, L.P. *et al.* (1998) Adjacent asparagines in the NR2-subunit of the NMDA receptor channel control the voltage dependent block by extracellular Mg^{2+}. *J. Physiol.* 506, 13–32
30 Antonov, S.M. and Johnson, J.W. (1999) Permeant ion regulation of *N*-methyl-D-aspartate receptor channel block by Mg^{2+}. *Proc. Natl. Acad. Sci. U. S. A.* 96, 14571–14576
31 Zhu, Y. and Auerbach, A. (2001) K^+ occupancy of the *N*-methyl-D-aspartate receptor channel probed by Mg^{2+} block. *J. Gen. Physiol.* 117, 287–298
32 Sobolevsky, A.I. *et al.* (2002) Staggering of subunits in NMDAR channels. *Biophys. J.* 83, 3304–3314
33 Banke, T.G. and Traynelis, S.F. (2003) Activation of NR1/NR2B NMDA receptors. *Nat. Neurosci.* 6, 144–152
34 Sobolevsky, A.I. (2003) Channel block of glutamate receptors. *Recent Research Dev. Physiol.* 1, 1–38
35 Watanabe, J. *et al.* (2002) DRPEER: A motif in the extracellular vestibule conferring high Ca^{2+} flux rates in NMDA receptor channels. *J. Neurosci.* 22, 10209–10216
36 Jatzke, C. *et al.* (2003) Extracellular vestibule determinants of Ca^{2+} influx in Ca^{2+}-permeable AMPA receptor channels. *J. Physiol.* 549, 439–452
37 Schorge, S. and Colquhoun, D. (2003) Studies of NMDA receptor function and stoichiometry with truncated and tandem subunits. *J. Neurosci.* 23, 1151–1158
38 Ren, H. *et al.* (2003) A site in the fourth membrane-associated domain of the *N*-methyl-D-aspartate receptor regulates desensitization and ion channel gating. *J. Biol. Chem.* 278, 276–283
39 Smith, T.C. and Howe, J.R. (2000) Concentration-dependent substate behavior of native AMPA receptors. *Nat. Neurosci.* 3, 992–997
40 Sun, Y. *et al.* (2002) Mechanism of glutamate receptor desensitization. *Nature* 417, 245–253
41 Bowie, D. and Lange, G.D. (2002) Functional stoichiometry of glutamate receptor desensitization. *J. Neurosci.* 22, 3392–3403
42 Popescu, G. and Auerbach, A. (2003) Modal gating of NMDA receptors and the shape of their synaptic response. *Nat. Neurosci.* 6, 476–483
43 Robert, A. and Howe, J.R. (2003) How AMPA receptor desensitization depends on receptor occupancy. *J. Neurosci.* 23, 847–858
44 Jin, R. *et al.* (2003) Structural basis for partial agonist action at ionotropic glutamate receptors. *Nat. Neurosci.* 6, 803–810
45 Schneggenburger, R. and Ascher, P. (1997) Coupling of permeation and gating in an NMDA-channel pore mutant. *Neuron* 18, 167–177
46 Zuo, J. *et al.* (1997) Neurodegeneration in *lurcher* mice caused by mutation in δ2 glutamate receptor gene. *Nature* 388, 769–773
47 Krupp, J.J. *et al.* (1998) N-terminal domains in the NR2 subunit control desensitization of NMDA receptors. *Neuron* 20, 317–327
48 Villarroel, A. *et al.* (1998) Glycine-independent NMDA receptor desensitization: localization of structural determinants. *Neuron* 20, 329–339
49 Yellen, G. (2002) The voltage-gated potassium channels and their relatives. *Nature* 419, 35–42
50 Jiang, Y. *et al.* (2002) The open pore conformation of potassium channels. *Nature* 417, 523–526
51 Qian, A. and Johnson, J.W. (2002) Channel gating of NMDA receptors. *Physiol. Behav.* 77, 577–582
52 Low, C.M. *et al.* (2003) Molecular determinants of proton-sensitive *N*-methyl-D-aspartate receptor gating. *Mol. Pharmacol.* 63, 1212–1222
53 Ding, S. and Horn, R. (2003) Glycine scan of Shaker's gating hinge. *Biophys. J.* 84, 73A.
54 Sobolevsky, A.I. *et al.* (2004) The outer pore of the glutamate receptor channel has 2-fold rotational symmetry. *Neuron* 41, 367–378
55 Song, I. and Huganir, R.L. (2002) Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci.* 25, 578–588
56 Gouaux, E. (2004) Structure and function of AMPA receptors. *J. Physiol.* 554, 249–253
57 Chatterton, J.E. *et al.* (2002) Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature* 415, 793–798
58 Perez-Otano, I. *et al.* (2001) Assembly with the NR1 subunit is required for surface expression of NR3A-containing NMDA receptors. *J. Neurosci.* 21, 1228–1237
59 Swanson, G.T. *et al.* (2002) Differential activation of individual subunits in heteromeric kainate receptors. *Neuron* 34, 589–598