Structural basis of kainate subtype glutamate receptor desensitization

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Glutamate receptors are ligand-gated tetrameric ion channels that mediate synaptic transmission in the central nervous system. They are instrumental in vertebrate cognition and their dysfunction underlies diverse diseases1,2. In both the resting and desensitized states of AMPA and kainate receptor subtypes, the ion channels are closed, whereas the ligand-binding domains, which are physically coupled to the channels, adopt markedly different conformations3-6. Without an atomic model for the desensitized state, it is not possible to address a central problem in receptor gating: how the resting and desensitized receptor states both display closed ion channels, although they have major differences in the quaternary structure of the ligand-binding domain. Here, by determining the structure of the kainate receptor GluK2 subtype in its desensitized state by cryo-electron microscopy (cryo-EM) at 3.8 Å resolution, we show that desensitization is characterized by the establishment of a ring-like structure in the ligand-binding domain layer of the receptor. Formation of this 'desensitization ring' is mediated by staggered helix contacts between adjacent subunits, which leads to a pseudo-four-fold symmetric arrangement of the ligand-binding domains, illustrating subtle changes in symmetry that are important for the gating mechanism. Disruption of the desensitization ring is probably the key switch that enables restoration of the receptor to its resting state, thereby completing the gating cycle.

Ionotropic glutamate receptors are tetrameric ligand-gated ion channels that mediate excitatory synaptic transmission in the central nervous system7. They have been classified into large families of AMPA, kainate and NMDA receptors that have distinct functional, pharmacological and structural properties8,9. Understanding the structural basis of glutamate receptor function is vital to understanding their roles in learning and memory, and by extension their roles in neuropsychopathology. Previous crystallographic and cryo-EM studies of AMPA and kainate receptors3-6,10-15 revealed large conformational changes between states, including notable changes in the arrangement of the ligand-binding domain (LBD) in the desensitized state in both receptor types. Although near-atomic resolution structures have been reported for the AMPA receptor GluA2 subtype in apo3, antagonist-bound6,14 and pre-activated states3,10, a high-resolution structure of the desensitized state of any glutamate receptor subtype has remained elusive. Thus, the central question of how a closed ion channel can be accommodated in both resting and desensitized states in the context of different LBD layer structures has remained unanswered. The structure we present here of the desensitized state of GluK2 reveals unexpected aspects of how conformational changes are coupled to symmetry mismatches across the length of the receptor, and thereby allows delineation of a complete model for the glutamate receptor gating cycle.

We used GluK2EM, a construct that binds GluK1-selective antagonists with nanomolar affinity, and trapped the desensitized state using (2S,4R)-4-methylglutamate, a high-affinity agonist that promotes profound desensitization16,17. The GluK2EM desensitized state structure at 3.8 Å resolution (Fig. 1a, b, Extended Data Fig. 1) reveals a two-fold symmetric amino-terminal domain (ATD) layer with an intact ATD tetramer interface (Fig. 1c), and a reorganized LBD layer that resembles...
a pinwheel (Fig. 1d). In the transmembrane domain (TMD) layer, all three membrane-spanning helices, and the S1–M1 and M3–S2 linkers were resolved (Extended Data Fig. 2a, b), where S1 and S2 indicate LBD polypeptide sequences preceding (S1) and following (S2) ion channel alpha helices (M1 and M3), and the four subunits within the ion channel display an approximately four-fold symmetric relationship (Fig. 1e). Densities for complex glycans at positions Asn244, Asn347 and Asn399 are evident (Extended Data Fig. 2c), whereas the M2 pore helices were not resolved and the ATD–LBD linker for the B and D subunits and the carboxy-terminal domain appear only at low density map contours. High sequence identity (>85%) between the M3 helices of AMPA and kainate receptors suggests that the general architecture of the central pore of the GluK2 channel will be relevant to other receptor subtypes from both families. In order to validate the use of two-fold computational symmetry in the reconstruction, the data was reprocessed entirely without symmetry (Extended Data Fig. 3). The resulting reconstruction yielded a structure with the same architecture and domain placement as that obtained using two-fold symmetry, but at slightly lowered resolution. This result, therefore, supports the use of two-fold symmetry in processing the data, and shows the presence of two-fold symmetry in the desensitized state.

The competitive antagonist LY466195 has nanomolar affinity for GluK2EM (Extended Data Fig. 4). We used this ligand to trap and determine the structure of the antagonist-bound resting state to ~12 Å and fit crystal structures of GluK2 ATD dimers and GluK2EM LY466195-bound LBD dimers to the cryo-EM map (Extended Data Fig. 5). The GluK2EM resting state structure is consistent with that observed previously using cryo-electron tomography, and its structural profile matches that of the cryo-EM and crystal structures of the antagonist-bound resting state of GluA2 (refs 4, 6). The lower resolution of the antagonist-bound resting state probably reflects greater conformational heterogeneity compared to the desensitized state, which potentially arises from variability in the relative orientations of the ATD and LBD layers.

Using the cryo-EM density maps, we built a de novo atomic model of full-length desensitized state GluK2EM, and a molecular model of resting-state GluK2 from crystal structures of ATD (PDB code, 3H6G) and LBD (PDB code, 5CMK) dimers. In the description below, we use A, B, C and D to refer to the four subunits of the tetrameric receptor, and AC and BD rather than the terms ‘proximal’ and ‘distal’ chains to clarify discussion of connectivity between the three (ATD, LBD and TMD) layers of the receptor. Comparison of the resting and desensitized state models show that although the ATD tetrameric layers are essentially similar in both states, the LBDs splay outwards upon desensitization, tugging on the ATD assembly via the ATD–LBD linkers and pulling it downwards compared to the resting state. This creates a substantial 1,250 Å2 buried surface area interface between the ATD AC subunits and their underlying LBDs (Extended Data Fig. 6). This interface, which probably explains how the ATD contributes to stabilization of the desensitized state18, is mediated by van der Waals interactions combined with salt bridges and hydrogen bonds formed between the side chains of Lys191 and Asp476, Glu219 and Arg400, and Tyr220 and Asp480 of the ATD and LBD, respectively. Notably, linkers connecting the ATD and LBD layers adopt a helical structure that packs against the upper lobe of the LBD, a feature not seen in previous AMPA and kainate receptor structures. In the GluK2EM resting state, the ATD and LBD layers do not interact and are separated by ~15 Å, similar to the GluA2 antagonist-bound resting state solved by cryo-EM4, which shows a separation of ~12 Å. This is notably different from GluA2 resting-state crystal structures, which have 4–6-residue deletions in the ATD–LBD linkers (PDB codes, 3KG2, 4U2P and 4U4G), and closely approached ATD and LBD layers with a buried surface area of 400–530 Å2 per subunit, but without the helical structure found in the ATD–LBD linker of the GluK2 desensitized state5,6,14. Comparison of the GluK2EM resting state with the extended structure of the pre-activated GluA2 complex with con-ikot-ikot toxin10, in which the ATD–LBD linkers contain only 2 deletions, suggests that wild-type GluA2 ATD–LBD linkers can extend to sufficient length such that the ATD and LBD are not in contact in both receptor subtypes, as also suggested by computational experiments19. In GluA2 and GluK2 resting states solved by cryo-EM, no buried surface is observed for the ATD–LBD interface, whereas in the subset of GluA2 resting states solved by crystallography, these domains are in contact. We conclude that the collapsed arrangement of the ATD–LBD linkers in the X-ray structure is probably caused by the effects of truncation in the linker region in the GluA2 construct used for crystallography constructs, in addition to the dynamic structure of these domains19. However, although the GluA2 and GluK2 resting-state structures are similar overall, they are both different from the compact resting-state structure recently reported for GluA2/GluA3 heteromers11.

The organization of the LBD layer in the desensitized state is characterized by ~125° rotations of AC subunit LBDs and displays approximately four-fold in-plane rotational symmetry (Fig. 1d), in contrast with the two-fold symmetry of the ATD layer (Fig. 1c). Helices E and G from individual subunits abut onto the same two helices on neighbouring LBDs (Fig. 2a–c). Instead of the arrangement that would be expected if the LBD layer showed perfect four-fold symmetry (Fig. 2d), we observe instead a circular arrangement that proceeds around the interior surface of the LBD layer with the E/G helices in an alternating staggered pattern that we name the ‘desensitization ring’ (Fig. 2e). This ring-like structure effectively creates a local two-fold organization in the context of the global pseudo-four-fold arrangement of the overall LBD layer. As a result, there are asymmetric contacts at AB and AD subunit interfaces that have buried surfaces of 860 Å2 and 447 Å2, respectively. Hydrogen bonds are formed between the Lys645 main chain and the side chain of Thr670, and salt bridges between the side chains of Asp672 and Lys667, and Asp672 and Lys673 in the AB subunit interface, with hydrogen bonds connecting the side chains of Ser639 and Arg681, and Tyr671 and Ser680 in the AD subunit interface. A structural consequence of the staggered E/G helix organization is that the BD subunit LBDs have a different pitch than the AC domains, with the AC subunits tilting away from the central receptor axis by ~10° more than their counterparts (Fig. 2e). It is this tilting that permits the E/G helices of the AC subunits to occupy the more elevated position in the staggered ring. Taken together, we observe that the LBD tetramer thus displays elements of both four-fold and two-fold symmetry. The apparent four-fold symmetry manifests when considering the in-plane rotation of the LBD domains, as viewed down the central axis of the receptor. The two-fold symmetry, which ultimately defines the symmetry of the LBD layer, is apparent when accounting for the staggered helix E/G arrangement in the ‘desensitization ring’. This staggered ring structure requires a symmetry mismatch in AC versus BD subunit LBDs, which is accomplished by the mismatched pitches in the domains.

To test if the desensitization ring contributes to the stability of the desensitized state, we used the structure to guide placement of arginine mutations at positions that would be expected to disrupt inter-subunit interactions. We chose S669R and D672R, both on helix G of the LBD, and measured their effects on the extent and rates of onset and recovery from desensitization. The extent of desensitization did not decrease (Fig. 2g), but both mutations speed recovery from desensitization (Fig. 2h, i, Extended Data Fig. 7, and Extended Data Table 1). Additionally, the nature and magnitude of this effect is consistent with previous reports identifying A676T and S679R mutations20,21, both of which reside on helix G (Extended Data Fig. 7), like the residues chosen in our functional experiments. These results support the hypothesis that the desensitization ring contributes to the stability of the state. Moreover, because these mutations do not reduce the extent of desensitization, other structural features must contribute to the deep energy well of the desensitized state. The ion channel region of the structure appears to be such a candidate, with its high relative structural stability in the cryo-EM density map (Extended Data Fig. 1e).
The desensitized state structure also reveals disruption of allosteric ion-binding sites that facilitate receptor activation\(^{22-24}\) and suggests an attractive mechanism for the recovery to the resting state from the desensitized state. Superposition of an open-cleft GluK2\(_{\text{DM}}\) LBD crystal structure (PDB code, 5CMK) via domain-1 coordinates on the closed cleft LBDs within the desensitized structure reveals severe sterical clashes. This implies that the desensitization ring must start to reorganize before the LBD can fully open. The observation that the desensitization ring must begin reorganizing before the LBD can immediately adopt an open-cleft conformation, provides a structural explanation for the electrophysiological observation of channel activity during recovery from desensitization\(^{25}\).

Inspection of the transmembrane region in the desensitized state shows that the overall channel architecture is similar to that reported for AMPA receptor structures in the apo and antagonist-bound resting states (Fig. 3a–d), with residues M633, T629 and T621 forming constrictions in the pore that prevent ion permeation. High sequence similarity in the transmembrane regions of AMPA and kainate receptors, with the GluK2 desensitized structure reported here complementing previously reported GluA2 structures in apo, resting and pre-activated states, makes possible a comparative analysis of closed-channel glutamate receptor structures. This comparison makes clear that although the ion channel in these different states adopts a similar overall profile (Fig. 3e), there is a substantial difference at the entrance to the pore in the desensitized and agonist-bound pre-activated states, with a collapse to the resting state structure in the desensitized state compared to the expanded conformation observed in the pre-activated state\(^{10}\). That all apo, resting and desensitized channels show similar profiles suggests that rearrangement in the LBD layer upon desensitization is sufficient to allow the desensitized state channel to adopt a non-conducting conformation, nearly identical to that present before activation.

The desensitized state atomic model allows examination of how the M3–S2 linkers connecting the ion channel and LBD layers mediate the differences between resting, pre-activated and desensitized state LBD structures. One aspect of these large changes in structure is that the M3–S2 linkers that couple LBD cleft closure to channel opening must somehow accommodate the height and pitch differences between AC and BD LBDs (Fig. 2f). We first considered differences between AC and BD chains of the TMD, and their connectivity to the LBD layer. Notably, although the two pairs of chains have nearly identical conformations in the TMD, the linkers adopt different conformations as evidenced by phi–psi analysis, and the heights of the structures gradually diverge (Fig. 4a). Specifically, beginning at M633, the AC linkers rise at a faster rate than their BD counterparts, with the difference reaching a maximum of ~6 Å at K645 (Fig. 4a, c). Thus, it is this height discrepancy that allows for the difference in pitch of the AC versus BD LBDs (Fig. 2f), permitting the E/G helices of the AC LBDs to occupy the elevated and tilted position in the staggered desensitization ring, whereas the compact BD subunit linkers map to the lower height of the BD LBDs. Despite the vertical asymmetry of linkers, their in-plane rotational symmetry is overall four-fold, as evidenced by the
orientation of helix E, the site at which linkers couple to the LBD. This mirrors the in-plane rotational symmetry of the LBD tetramer. Comparison of differences between the M3–S2 regions of the AC and BD subunit enables identification of the position that serves as the hinge for the symmetry change that occurs with desensitization. As a structural reference for this analysis, we used the antagonist-bound resting state of GluA2 (ref. 6). Chains A and C have similar conformations, consistent with the observation of small movements of the corresponding LBDs between resting and desensitized states. Conversely, in the B and D chains, there are 135–180° changes in main-chain psi angles over a three-residue stretch, from E634–P636 in the GluK2 desensitized state. The trajectory of the changes deviates significantly from that observed in the GluA2 resting state, indicating that this region serves as a pivot point around which channel closure drives LBD layer rearrangement during desensitization3.

Our study shows that the GluK2 desensitized state displays two-fold, quasi-four-fold and four-fold in-plane rotational symmetries, for the ATD, LBD and transmembrane regions, respectively. However, surprising mismatches between axial and in-plane symmetry are found in the LBD layer. These features ultimately confer the receptor with an overall 2-2-4 symmetry, with the arrangement in the LBD layer providing a structural compromise that accommodates the different symmetries above and below the LBD layer. The two-fold symmetry of the LBD desensitization ring is defined by the need to accommodate the ATD layer, which effectively ‘rocks’ the AC LBDs backwards away from the central axis. The quasi-four-fold symmetry of the LBD layer arises from the need to match the symmetry of the ion channel, and thereby allows closing of the ion channel to relieve receptor ‘tension’ driven by LBD closure during activation3,4,10.

Figure 4 | LBD–TMD linkers mediate channel closing and LBD reorganization. a, Comparison of the regions encompassing the M3, M3–S2 linkers, and E helices for AC (magenta) and BD (cyan) chains of desensitized state GluK2 (EM). b, The vertical rise in Cα position as a function of residue number for chains AC (magenta) versus BD (cyan). c, Measurement of how Cα positions on desensitized state GluK2 chains A,C, B and D deviate from corresponding positions on resting state GluA2, which was used as a reference. Line colouring is the same as in b. Dotted lines indicate AB chains, with solid lines for CD chains. The magenta traces show low deviation, reflecting the similarity between these regions of the AC subunits of desensitized GluK2 and the resting state GluA2 structure used as a reference (PDB code, 3KG2). The cyan trace begins to deviate significantly at the circular markers corresponding to R632 (R628) GluK2 (GluA2). This reflects the difference in BD linker arrangements between the resting and desensitized states. d, The desensitized GluK2 channel with the region considered in c. M3 helices and M3–S2 linkers are coloured as in other panels, with pre-M1, M1 and M4 regions coloured in yellow. Spheres mark residue R632 on all four transmembrane chains. e, Schematic view of structural rearrangements that are involved in transition from resting to activated to desensitized states of glutamate receptors. Supplementary Video 1 shows an animation of the gating cycle.

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the staggered helix interactions in the desensitization ring probably serve collectively as a molecular switch that, upon agonist unbinding and opening of the jaws of individual LBDs, is disrupted and triggers destabilization of the desensitized state. This event would thereby permit a structural rearrangement back to the resting state with an intact LBD dimer of dimers assembly, thus completing the gating cycle.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Burnashev, N. & Szepetowski, P. NMDA receptor subunit mutations in neurodevelopmental disorders. Curr. Opin. Pharmacol. 20, 73–82 (2015).
2. Lesca, G. et al. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. Nat. Genet. 45, 1061–1066 (2013).
3. Dürr, K. L. et al. Structure and dynamics of AMPA receptor GluA2 in resting, pre-open, and desensitized states. Cell 158, 778–792 (2014).
4. Meyerson, J. R. et al. Structural mechanism of glutamate receptor activation and desensitization. Nature 514, 328–334 (2014).
5. Schauer, D. M. et al. Glutamate receptor desensitization is mediated by changes in quaternary structure of the ligand binding domain. Proc. Natl Acad. Sci. USA 110, 5921–5926 (2013).
6. Sobolevsky, A. I., Rosconi, M. P. & Gouaux, E. X-ray structure, symmetry and conformational states of native AMPA receptor complexes. Nature 462, 745–756 (2009).
7. Traynelis, S. F. et al. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol. Rev. 62, 405–496 (2010).
8. Mayer, M. L. Glutamate receptors at atomic resolution. Nature 440, 456–462 (2006).
9. Watkins, J. C. & Evans, R. H. Excitatory amino acid transmitters. Annu. Rev. Pharmacol. Toxicol. 21, 165–204 (1981).
10. Chen, L., Dürr, K. L. & Gouaux, E. X-ray structures of AMPA receptor-cone snail toxin complexes illuminate activation mechanism. Science 345, 1021–1026 (2014).
11. Herguedas, B. et al. Structure and organization of heteromeric AMPA-type glutamate receptors. Science 352, aad3873 (2016).
12. Nakagawa, T., Cheng, Y., Ramm, E., Sheng, M. & Walz, T. Structure and different conformations states of native AMPA receptor complexes. Nature 433, 545–549 (2005).
13. Twomey, E. C., Yelshanskaya, M. V., Grassucci, R. A., Frank, J. & Sobolevsky, A. I. Elucidation of AMPA receptor-stargazin complexes by cryo-electron microscopy. Science 353, 83–86 (2016).
14. Yelshanskaya, M. V., Li, M. & Sobolevsky, A. I. Structure of an agonist-bound ionotropic glutamate receptor. Science 345, 1070–1074 (2014).
15. Zhao, Y., Chen, S., Yoshioka, C., Macoucius, I. & Gouaux, E. Architecture of fully occupied GluA2 AMPA receptor–TARP complex elucidated by cryo-EM. Nat. Methods 536, 108–111 (2016).
16. Alushin, G. M., Jane, D. & Mayer, M. L. Binding site and ligand flexibility revealed by high resolution crystal structures of GluK1 competitive antagonists. Neuropharmacology 60, 126–134 (2011).
17. Jones, K. A., Wilding, T. J., Huetter, J. E. & Costa, A. M. Desensitization of kainate receptors by kainate, glutamate and diastereomers of 4-methylglutamate. Neuropharmacology 36, 853–863 (1997).
18. Möykynnen, T., Coleman, S. K., Semenov, A. & Keinanen, K. The N-terminal domain modulates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor desensitization. J. Biol. Chem. 289, 13197–13205 (2014).
19. Dutta, A. et al. Cooperative dynamics of intact AMPA and NMDA glutamate receptors: similarities and subfamily-specific differences. Structure 23, 1692–1704 (2015).
20. Carbone, A. L. & Pledsted, A. J. Coupled control of desensitization and gating by the ligand binding domain of glutamate receptors. Neuron 74, 845–857 (2012).
21. Fleck, M. W., Cornell, E. & Mah, S. J. Amino-acid residues involved in glutamate receptor 6 kainate receptor gating and desensitization. J. Neurosci. 23, 1219–1227 (2003).
22. Chaudhry, C., Pledsted, A. J., Schuck, P. & Mayer, M. L. Energetics of glutamate receptor ligand binding domain dimer assembly are modulated by allosteric ions. Proc. Natl Acad. Sci. USA 106, 12329–12334 (2009).
23. Dawe, G. B. et al. Distinct structural pathways coordinate the activation of AMPA receptor–auxiliary subunit complexes. Neuron 89, 1264–1276 (2016).
24. Pledsted, A. J., Vijayan, R., Biggin, P. C. & Mayer, M. L. Molecular basis of kainate receptor modulation by sodium. Neuron 58, 720–735 (2008).
25. Patneau, D. K., Mayer, M. L., Jane, D. E. & Watkins, J. C. Activation and desensitization of AMPA/kainate receptors by novel derivatives of willardiine. J. Neurosci. 12, 595–606 (1992).

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Author Contributions J.R.M., M.L.M. and S.S. were involved in all stages of design of experiments and interpretation of results; S.C. carried out protein expression and purification; S.C. and M.L.M. carried out X-ray crystallography; T.H., L.M. and M.L.M. performed electrophysiological experiments; J.R.M., A.M., N.R. and S.C. carried out cryo-EM data collection; J.R.M. carried out cryo-EM image processing; J.R.M. and M.L.M. carried out structural analysis; J.R.M., M.L.M. and S.S. integrated all of the data, analysis of the implications and mechanism, and wrote the manuscript, with help from S.C.

Author Information Cryo-EM density maps for GluK2EM with 2SAR-4-methylglutamate, and GluK2EM with LY466195, have been deposited in the Electron Microscopy Data Bank under accession codes EMD-B289 and EMD-B290, respectively. Atomic coordinates for GluK2EM with 2SAR-4-methylglutamate, GluK2EM with LY466195, GluK2EM LBD with 2SAR-4-methylglutamate, and GluK2EM LBD with LY466195, have been deposited in the Protein Data Bank under accession codes, 5KUF, 5KUH, 5CMM and 5CMK, respectively. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.S. (ss1@nih.gov), M.L.M. (meyerm@nih.gov) or J.R.M. (jmeyerson@brandeis.edu).

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METHODS

Data reporting. No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessments.

Protein expression and purification. The full-length rat GluK2 subunit cDNA sequence (UniProt code, P42260) was cloned into the pFastBac1 vector for protein expression in insect cells. GluK2α2μ was created by introducing four mutations in the LBD (A487T, A658S, N690S and F704L) which convert the sequence to that found in the dimer-competent side chain GluK2α1, whereas the N690S mutation relieved a bad steric clash that prevents binding of LY466195 to wild-type GluK2. A dimer with two open-clft protomers was created by least squares superposition using domain one coordinates (r.m.s.d., 0.23 Å) to position two GluK2α2μ protomers. The GluK2α2μ LBD complex with LY466195 crystalsized as a dimer, but surprisingly one protomer contained a bound glutamate molecule, whereas the second protomer bound LY466195. The glutamate-bound protomer adopted a open-clft conformation, whereas the LY466196-bound protomer adopted an open-clft conformation produced by a 27° rotation of domain 2. The structure revealed hydrogen bonds with the bound LY466195 ligand formed by the hydroxyl groups of the A487T mutation. Whereas the N690S mutation relieved a bad steric clash that prevents binding of LY466195 to wild-type GluK2. A dimer with two open-clft protomers was created by least squares superposition using domain one coordinates (r.m.s.d. 0.31 Å) to position a copy of the LY466195-bound protomer in place of the glutamate-bound protomer. The GluK2α2μ desensitized state atomic model was built using rigid body fitting in UCSF Chimera39 of two copies of a GluK2 A3D dimer crystal structure (PDB code, 3H6G) and four copies of a 25,4R-4-methylglutamate-bound GluK2α2μ LBD protomer crystal structure (PDB code, 3CMK) to the cryo-EM density map, followed by rebuilding in COOT. The ion channel and linkers to the LBD were also built in COOT using the GluK2 α2-matt state crystal structure (PDB code, 3KQ2) as a guide, followed by real space refinement of the complete model using PHENIX. The A3D-LBD linker regions spanning residues 385–389 were then modelled using RosettaCM39 with C2 symmetry and the real space refined GluK2α2μ model as input. The antagonist-bound resting state model of GluK2α2μ was built from rigid body fits of two copies of a GluK2 A3D dimer crystal structure (PDB ID: 3H6G) and two copies of the manually generated dimer corresponding to the LY466195-bound GluK2α2μ LBD (PDB code, 3CMK).

Electrophysiological experiments. Outside-out patch recordings from HEK cells transfected with wild-type and mutant GluK2 constructs, with fast solution exchange, were perfused using four-barrelled microelectrodes, with a DC electric field stack driven by a P-270 HVA amplifier (Physik Instrumente), were performed at room temperature using an Axopatch 200 A amplifier as described previously40. The external solution contained (in mM) 145 NaCl, 2.5 KCl, 1.8 CaCl2, 1 MgCl2, 5 HEPES (pH 7.3), and 10 glucose, with 10 mM l-glutamate used for activation. The internal solution contained (in mM) 105 NaCl, 20 NaF, 5 Na3ATP, 0.5 CaCl2, 5 HEPES (pH 7.3), and 10 mM Na2ATP. Two electrode voltage clamp recordings at a holding potential of ~60 mV, with 3 M KCl agarose tipped electrodes of resistance 0.1–0.8 MΩ, were performed using stage 5–6 Xenopus oocytes, 2–3 days after injection of complementary RNA for either GluK2α2μ or wild-type GluK2. The bath solution contained 100 mM NaCl, 1 M KCl, 5 mM HEPES (pH 7.5), 0.8 mM BaCl2 and 1 mM MgCl2, with concanavalin A (Sigma Type IV) 0.6 mg/ml−1 used to block desensitization. The initial response to 100 μM glutamate following preincubation with 1–500 mM LY466195 was recorded as a step response before the slow increase in current due to desensitization of antagonist fit with the Hill equation.

26. Mayer, M. L. Crystal structures of the GluR5 and GluR6 ligand binding cores: molecular mechanisms underlying kainate receptor selectivity. Neuron 45, 539–552 (2005).
27. Meyerson, J. R. et al. Self-assembled monolayers improve protein distribution on holey carbon cryo-EM supports. Sci. Rep. 4, 7084 (2014).
28. Li, X. et al. Electron counting and beam-induced molecule correction enable near-atomic-resolution single-particle cryo-EM. Nat. Methods 10, 584–590 (2013).
29. Tang, G. et al. EMAN2: an extensible image processing suite for electron microscopy. J. Struct. Biol. 157, 38–46 (2007).
30. Scheeres, S. H. RELION: implementation of a Bayesian approach to cryo-EM structure determination. J. Struct. Biol. 180, 519–530 (2012).
31. Mendall, J. A. & Grigorieff, N. Accurate determination of local defocus and specimen tilt in electron microscopy. J. Struct. Biol. 142, 334–347 (2003).
32. Scheeres, S. H. Beam-induced motion correction for sub-megadalton cryo-EM particles. J. Struct. Biol. 148, 1–12 (2004).
33. Pettersen, E. F. et al. UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612 (2004).
34. The PyMOL Molecular Graphics System. Version 1.8 Schrödinger, LLC. (DeLano Scientific, 2002).
35. Smart, O. S., Goodfellow, J. M. & Wallace, B. A. The pore dimensions of gramicidin A. Biochim. Biophys. Acta 65, 2455–2460 (1993).
36. Scheres, J. B. & Blott, I. Image processing in electron microscopy. J. Struct. Biol. 133, 156–169 (2001).
37. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D Biol. Crystallogr. 66, 213–221 (2010).
38. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. Acta Crystallogr. D Biol. Crystallogr. 66, 486–501 (2010).
39. Wang, R. Y. et al. De novo protein structure determination from near-atomic-resolution cryo-EM maps. Nat. Methods 12, 335–338 (2015).
40. Horning, M. S. & Mayer, M. L. Regulation of AMPA receptor gating by ligand binding core dimers. Neuron 41, 379–388 (2004).
Extended Data Figure 1 | Desensitized GluK2 imaging and structure determination. a, b, Representative cryo-EM image of GluK2<sub>EM</sub> solubilized in DDM–CHS and bound by 2S,4R-4-methylglutamate (a), with the corresponding image power spectrum and CTF estimate showing signal beyond 3 Å resolution (b; solid and dotted lines, respectively). The defocus value for the image is 1.5 μm. Particles in a are highlighted with circles, and image binning at 4 × and uniform level adjustments were used to make particles apparent. Scale bar, 500 Å. c, Subset of selected two-dimensional class averages. d, FSC curve with reported resolution of 3.8 Å at the 0.143 crossing. e, Structure of agonist-bound GluK2<sub>EM</sub>, coloured according to local resolution, shown at three progressively increasing contours.
Extended Data Figure 2 | Desensitized GluK2 transmembrane and glycosylation features. a, b, Cryo-EM density for resolved S1–M1 (a) and M3–S2 (b) linkers and transmembrane helices, displayed with Cα trace of the atomic model. c, Representative sites with densities for complex glycans at Asn244, Asn347 and Asn399.
Extended Data Figure 3 | Reconstruction of desensitized GluK2 without computational symmetry. **a**, Cryo-EM density map for the reconstruction of agonist-bound GluK2 without imposition of computational symmetry, and coloured according to local resolution. **b**, FSC curve with reported resolution of 4.4 Å at the 0.143 crossing. **c**, Segmentation of individual GluK2 chains of the asymmetric reconstruction. The density map segmentation is shown fitted with a trace representation of the model displayed in Fig. 1b.
Extended Data Figure 4 | Inhibition of GluK2<sub>EM</sub> by LY466195 and LBD crystal structures for agonist and antagonist complexes. 

**a**, Crystal structure for the GluK2<sub>EM</sub>-isolated LBD dimer assembly complex with 25,4R-4-methylglutamate. The upper and lower lobes for the two subunits are coloured orange and pale yellow, and teal and pale cyan, respectively; the dashed line indicates the separation of the lower lobes measured as the distance between the C<sub>α</sub> positions of Ile637. 

**b**, Crystal structure for the GluK2<sub>EM</sub>-isolated LBD dimer assembly complex with LY466195 illustrating the large decrease in separation of the lower lobes compared to the agonist complex. Colouring is the same as in **a**.

**c**, Responses to 100 μM glutamate recorded under two electrode voltage clamp for GluK2<sub>EM</sub> (top) and wild-type GluK2 (bottom); the initial response to glutamate recorded after prior application of 300 nM LY466195 showed nearly complete block for GluK2<sub>EM</sub>, with no change in amplitude for wild type. 

**d**, Concentration dependence for inhibition of GluK2<sub>EM</sub> by LY466195 yielded an IC<sub>50</sub> of 30 nM; data points show mean ± s.e.m. of 4–7 observations per concentration.
Extended Data Figure 5 | Imaging and structure of GluK2EM bound by antagonist LY466195. a, b. Representative cryo-EM image of GluK2EM solubilized in DDM-CHS and bound by LY466195 (a), with the corresponding image power spectrum and CTF estimate showing signal beyond 6 Å resolution (b, solid and dotted lines, respectively). The defocus value for the image is 2.7 μm. Particles in a are highlighted with circles, and image binning at 4× and uniform level adjustments were used to make particles apparent. Scale bar, 500 Å. c, Subset of selected two-dimensional class averages. d, Cryo-EM density map for GluA2 bound to ZK200775 (ref. 4) (left), density map for GluK2 EM bound to LY466195 (middle) and its corresponding molecular model built from ATD and LBD dimers (right). e, FSC curve with reported resolution of 11.6 Å at 0.143 crossing.
Extended Data Figure 6 | ATD–LBD interface of desensitized GluK2. 

a, Desensitized GluK2 shown in surface representation with ATD–LBD interfaces highlighted. b, Top-down view of LBD layer shown with perspective indicated by eye icon in a. c, Underside of the ATD layer as viewed after peeling away from LBD layer. Dashed lines in a highlight where the layers are separated. In all panels, interfaces on chain A and C are in green and blue, respectively. d, Table with residues that mediate ATD–LBD interaction. e, f, Cartoon representation of LBD and ATD layers from same views as in b and c, with interface residues coloured to correspond with table in d.
Extended Data Figure 7 | Desensitization ring residues that influence recovery kinetics. 

**a**–**d**, Rate of recovery from desensitization measured using twin pulse applications of 10 mM glutamate (data points show mean ± s.d.; fits are shown in red). 

- **a**, Wild-type GluK2 fit with a single exponential. 
- **b**, D672R fit with the sum of two exponentials with the response for wild type shown as a black line. 
- **c**, S669R D672R double mutant fit with the sum of two exponentials with the response for wild type shown as a black line. 
- **d**, Time to 50% recovery in seconds. 

**e**–**h**, Top views of the GluK2 desensitization ring with residues found to influence recovery kinetics when mutated. Each panel shows the wild-type residue Cα position as a sphere. Positions for the S669R and D672R mutations from the present study are shown in **e** and **f**, respectively. The A676T and S679R positions found in previous studies are in **g** and **h**, respectively.
### Extended Data Table 1 | Electrophysiological analysis

|            | Amp (nA)       | % Desens | Tau des | Amp fast % | k<sub>fast</sub> s<sup>−1</sup> | k<sub>slow</sub> s<sup>−1</sup> |
|------------|----------------|----------|---------|------------|------------------------------|-----------------------------|
| WT         | 1.41 ± 0.27 (n=13) | 99.3 ± 0.19 | 3.56 ± 0.40 | 100 | 0.79 ± 0.09 |                          |
| D672R      | 0.56 ± 0.10 (n=12)  | 99.0 ± 0.21 | 2.06 ± 0.11 | 85.3 ± 3.4 | 2.48 ± 0.27 | 0.14 ± 0.04 |
| S669R/D672R| 0.21 ± 0.06 (n=10)  | 99.4 ± 0.17 | 2.08 ± 0.15 | 74.8 ± 3.7 | 3.25 ± 0.31 | 0.20 ± 0.06 |
| S679R      | NR                  | 99.5      | 4.8      | 100        | 1.3                          |                             |
| A676T      | NR                  | NR        | 3.0      | 100        | 1.4                          |                             |

For wild-type GluK2, the D672R mutant and the S669R/D672R double mutant values (mean ± s.e.m.) are reported for the peak amplitude of the response to 10 mM glutamate (Amp); the percentage of desensitization measured 50 ms after the start of the application of glutamate; the time constant of onset of desensitization determined using a single exponential fit (Tau des); the amplitude of the fast component of recovery from desensitization (Amp fast); and the rate constants of the fast (k<sub>fast</sub>) and slow (k<sub>slow</sub>) components of recovery from desensitization determined from double exponential fits. Values for S679R are from ref. 20; values for A676T are from ref. 21.
Extended Data Table 2 | Cryo-EM data collection and structural analysis

| Sample                      | Robot(s)       | Micrographs | Particles prior to depletion | Particles retained after 2D classification | Particles in 3D reconstruction | Resolution (Å)  |
|-----------------------------|----------------|-------------|------------------------------|------------------------------------------|--------------------------------|-----------------|
| Gluk2-(2S,4R)-4-methylglutamate | Leica EM GP    | 2,454       | 166,311                      | 166,284                                  | 62,244 (C2 symmetry)          | 3.78 (C2 symmetry) |
|                             | Vitrobot Mk IV |             |                              |                                          | 62,552 (C1 symmetry)          | 4.36 (C1 symmetry) |
| Gluk2-LY466195              | Vitrobot Mk IV | 1,774       | 93,583                       | 92,945                                   | 31,000                        | 11.60 (C2 symmetry) |

Imaging conditions and data processing. The table shows the robot or robots used to make samples; the number of micrographs used for image processing; the number of particles manually designated in the micrographs; the number of particles retained after two-dimensional (2D) classification; the number of particles retained after three-dimensional (3D) classification and used for structure refinement; and the resolution and symmetry.
Extended Data Table 3 | Crystallographic data collection and refinement statistics

| DATA COLLECTION | (2S,4R)-4-MethylGlu | LY466195 - Glu |
|----------------|---------------------|----------------|
| Space group    | P6₁                | P6₁,22         |
| Unit cell a, b, c (Å) | 52.3, 52.3, 170.9 | 102.8, 102.8, 282.0 |
| a, b, c       | 90, 90, 120        | 90, 90, 120    |
| Number per a.u.| 1                  | 2              |
| Wavelength (Å) | 1.0000             | 1.0000         |
| Resolution (Å) | 30 – 1.27 (1.29)   | 30 – 1.8 (1.83) |
| Unique observations | 68757            | 82330          |
| Mean redundancy | 7.0 (4.7)          | 14.5 (14.2)    |
| Completeness (%) | 99.4 (95.2)       | 100 (100)      |
| Rmerge          | 0.043 (0.335)      | 0.058 (> 1)    |
| Rpim           | 0.017 (0.166)      | 0.017 (0.326)  |
| I/s(I)         | 45.6 (3.8)         | 48.4 (2.8)     |

### REFINEMENT

| Resolution (Å) | 27.29 – 1.27 | 29.86 – 1.80 |
| Protein atoms (AC) | 2127 (80) | 4125 (95) |
| Ligand atoms | 11 | 34 |
| Li / Cl / SO₄ ions | - / - / - | 2 / 10 / 2 |
| Water atoms | 410 | 533 |
| Rwork / Rfree (%) | 14.3 / 16.3 | 16.4 / 18.7 |

### rms deviations

| Bond lengths (Å) | 0.009 | 0.014 |
| Bond angles °   | 1.24  | 1.40  |

### Mean B-Values (Å²)

| Protein overall | 20.9  | 27.5  |
| MC / SC °       | 18.6 / 23.2 | 23.4 / 31.7 |
| Ligand          | 13.2   | 19.8  |
| Li / Cl / SO₄ ions | - / - / - | 23.8 / 57.1 / 34.8 |
| Water           | 34.9   | 34.1  |

### Ramachandran %

| (°) Main chain or side chain | Preferred or disallowed conformations |
|-----------------------------|--------------------------------------|
| 97.8 / 0                    | 97.9 / 0                             |

Crystallographic data collection and refinement statistics for LBD structures. Superscripts a–h denote the following.

a Values in parentheses indicate the low resolution limit for the highest-resolution shell of data.

b Values in parentheses indicate statistics for the highest-resolution shell of data.

\[
R_{merge} = \frac{\sum |I_i - <I_i>|}{\sum |I_i|}, \quad \text{where} \quad <I_i> \text{ is the mean } I_i \text{ over symmetry-equivalent reflections.}
\]

\[
R_{pim} = \left(\frac{\sum |F_o - F_c|}{\sum |F_o|}\right)^{\frac{1}{2}}, \quad \text{where} \quad <I_i> \text{ is the mean } I_i \text{ over symmetry-equivalent reflections.}
\]

c Values in parentheses indicate statistics for the highest-resolution shell of data.

d Alternative conformations.

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