

Structures of partially occupied hetero-tetramers provide insight into kainate receptor activation and desensitization

Corresponding Author: Professor Joshua Levitz

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

In this manuscript, the authors present high-resolution cryo-EM structures of GluK2/GluK5 hetero-tetrameric kainate receptors under different agonist-bound conditions (5-iodowillardiine and AMPA). These ligands confer different subunit-specific occupancies, thus enabling structural characterization of distinct ligand-binding domain (LBD) conformational states, ranging from intact to partially and fully ruptured dimers. The authors integrate structural, functional, and mutational data to propose a model for how subunit-specific ligand binding influences receptor gating, desensitization, and deactivation kinetics.

This work provides valuable mechanistic insight into occupancy-dependent gating of KARs and highlights how asymmetric subunit composition and occupancy drive specific LBD rearrangements. The data are technically impressive, and the combined use of cryo-EM and patch-clamp electrophysiology is a clear strength of the study. However, the current manuscript places disproportionate emphasis on structural detail at the expense of conveying the broader functional implications and mechanistic framework. Significant revision (writing) is required to improve the accessibility and impact of the findings, particularly for a broader readership.

Major comments:

While the structural biology is rigorous, the manuscript is currently tailored for a highly specialized audience. The broader relevance, particularly how these findings inform iGluR activation models more generally, should be emphasized.

A clearer mechanistic summary figure (Fig. 7E) should be presented and not buried as a subsection. Figure 7E needs to be modified to reflect the data. Specifically, the claim that all levels of occupancy can lead to the partially ruptured state is not directly supported by data and should either be revised or explicitly qualified.

The manuscript does not report the fraction of particles adopting each conformational class. This is critical to assess the relative stability and prevalence of intact, partially, and fully ruptured states under different ligand conditions. Correlation between particle classes and functional outcomes (e.g., degree of desensitization) should be explicitly discussed. If a quantitative correlation is not possible, this limitation should be acknowledged.

The discussion focuses primarily on comparison with other cryo-EM structures. A comparison to other techniques that probe glutamate receptor conformational states, such as smFRET on partial agonism of GluK2/GluK5, intra dimer cross linking study on AMPA receptors (Baranovic and Plested, eLife, 2018) needs to be added.

Minor comment:

Figures 1-6 are of the IW cryo-EM structures and can be combined into 3-4 figures.

Reviewer #2

(Remarks to the Author)

The manuscript by Khanra and colleagues reports the structure of partially occupied GluK2/GluK5 heteromeric kainate receptors, from which they propose a model of gating that is unlike other ionotropic glutamate receptors. This finding is interesting and informative because it is well established that partial occupancy of heteromeric KARs composed of subunits with large differences in agonist affinity will gate tonic currents rather than proceeding to near-complete desensitization. Here they take advantage of the differential affinity for two agonists, iodowillardiine and AMPA, for GluK2 versus GluK5 subunits to probe the molecular basis of this biophysical phenomenon. More broadly, these results provide insights into the

structure of activated states of an iGluR that do not rely on the use of desensitization-blocking compounds like PAMs or mutagenesis.

The data in the paper is of high quality and rigor and the conclusions derived from the structures are appropriate. The manuscript is well-written, carefully reasoned, and the discussion is informative and does a good job of putting the current data in context with the large number of other KA (and AMPA) receptor structures. A number of new insights result from the structures in addition to the pre-activate states, including identification of key cross-dimer residues that in part underlie the slow deactivation of GluK2/GluK5 heteromers and correlation of partially-occupied LBD conformations with gating linkers. The findings add to and complement the growing number of KA and AMPA receptor cryo-EM structures that each have illuminated aspects of receptor gating.

I have no major concerns.

Minor points

The first study to report gating by partial occupancy of heteromeric KARs was a study by Swanson et al., *Neuron*, 2002 (PMID: 12062042), using the agonist dysiherbaine, which differs in affinity for GluK2 and GluK5 by several orders of magnitude. That paper seems highly relevant to the current study and pre-dates the Fisher and Mott and other reports by at least 9 years.

Reviewer #3

(Remarks to the Author)

This study provides the structural basis for the activation and desensitization of a specific heterotetrameric GluK2/GluK5 kainate receptor (KAR), building on their previous work that reported the first structures of GluK2/GluK5 heteromers in resting (Apo), antagonist-bound (CNQX), and agonist-bound (L-Glu) states (Khanra et al., 2021, *eLife*). Using a pharmacological approach that combines a GluK5-selective agonist (5-IW and AMPA) with a GluK2-selective antagonist (kynurenic acid), the authors captured high-resolution cryo-EM structures of the GluK2/GluK5 receptor in both an intact ligand-binding domain (LBD) dimer-of-dimer state and a partially ruptured state with one dimer interface disrupted. These conformations—a “pre-active” state with intact dimer interfaces and a partially ruptured intermediate state—offer new atomic-level insights into the gating mechanism of heteromeric KARs, particularly regarding the transition from activation to desensitization. The structural findings are further supported by mutagenesis and electrophysiological recordings, which underscore the role of inter-subunit interactions and mobility in the slow deactivation process of GluK5-containing KARs. Overall, the manuscript is well-written, and the figures are clearly illustrated. The work establishes an occupancy-dependent framework for understanding KAR gating and provides a molecular basis for elucidating the gating mechanism of endogenous KARs in the brain.

Major points:

1. The electron density of the GluK5-LBD (particularly protomer C in Class 2, Fig.1C) appears very blurry. The authors also note that the ligand density was insufficient to model its binding pose. Did they attempt local refinement on the LBD tetramer to improve the local resolution of the LBD region?
2. It's interesting to note that both intra- and inter- dimer interface are involved in the deactivation properties of GluK2/GluK5 receptors. Could this conclusion be further supported by *in silico* simulations to probe additional intermediate conformations? A similar approach has been employed for NMDA receptors (Esmenjaud et al., *EMBO J* 2019).
3. The mutagenesis data are used to validate the structural findings. However, it remains unclear whether any of the mutations (e.g., Y683I) influence receptor surface expression or ligand binding affinity. Including such controls would strengthen the specificity of the conclusions.
4. The discussion could be further enriched by considering whether the identified “central cluster” and inter-dimer interfaces are unique to GluK4/GluK5-containing heteromers, or whether analogous—though mechanistically distinct—interaction networks might also exist in other iGluR subfamilies to fine-tune their gating kinetics. Also, it would be valuable to discuss the fact that a partially ruptured LBD dimer has also been observed in AMPARs (Fig. 4d, Zhang D et al., *Nature* 2023. doi: 10.1038/s41586-023-06528-0).

Minor Points :

1. “..., the intact class 1 structure shows rearrangement of the upper “D1” and lower “D2” subdomain interfaces with an overall loosening of both interfaces (Fig. 2B; Supplementary Video 1, 2)”.

The reference figure here is Fig. 2B or 2A? Please clarify. Similar inconsistencies appear elsewhere in the manuscript; the authors are advised to carefully check and correct all figure citations.

2. “Heteromers incorporating the GluK5(Q768K) variant in the A/C subunits opposing GluK2 Y521 in the B/D subunits showed only slightly accelerated desensitization kinetics compared to GluK2/GluK5(wt). In contrast, the GluK5(A504Y) substitution, a substitution that is homologous to GluA(L483Y), had no effect on desensitization, but showed a minor slowing of deactivation, supporting prior work indicating that the LBD dimer interface contributions may be different in KARs compared to AMPARs60–63. However the double substitution GluK5(N772E, N773G), which mimics the residues present in GluK2, resulted in 1.8-fold slower desensitization, revealing a subtype-specific contribution to KAR desensitization.”

In the paragraph describing the functional effects of the GluK5(Q768K), GluK5(A504Y), and GluK5(N772E/N773G) substitutions, no figure references appear to be provided. If this is correct, please include the relevant figure citation to guide the reader.

3. The residue numbering in Fig. 4C appears to be incorrect, and additional background file information are present in Fig. 4E–F. The authors are advised to carefully check all figure labels and correct these inconsistencies.

4. “The 2-fold symmetry of the LBD layer leads to the GluK2 (A/C) subunits being in poredistal M3 linker positions (Fig. 5D) while GluK5 (B/D subunits) occupy pore-proximal positions (Fig. 5G).”

GluK2 (A/C) should be GluK2(B/D subunits) and GluK5 (B/D subunits) should be GluK5 (A/C subunits).

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have addressed my concerns.

Reviewer #2

(Remarks to the Author)

I have no further concerns. This is a very interesting report of high quality.

Reviewer #3

(Remarks to the Author)

The author has addressed all my comments. I have no further suggestions.

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

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In this manuscript, the authors present high-resolution cryo-EM structures of GluK2/GluK5 heterotetrameric kainate receptors under different agonist-bound conditions (5-iodowillardiine and AMPA). These ligands confer different subunit-specific occupancies, thus enabling structural characterization of distinct ligand-binding domain (LBD) conformational states, ranging from intact to partially and fully ruptured dimers. The authors integrate structural, functional, and mutational data to propose a model for how subunit-specific ligand binding influences receptor gating, desensitization, and deactivation kinetics.

This work provides valuable mechanistic insight into occupancy-dependent gating of KARs and highlights how asymmetric subunit composition and occupancy drive specific LBD rearrangements. The data are technically impressive, and the combined use of cryo-EM and patch-clamp electrophysiology is a clear strength of the study. However, the current manuscript places disproportionate emphasis on structural detail at the expense of conveying the broader functional implications and mechanistic framework. Significant revision (writing) is required to improve the accessibility and impact of the findings, particularly for a broader readership.

We thank the reviewer for their very positive assessment of our study. However, we respectfully disagree with the assertion that the manuscript places disproportionate emphasis on structural details as there are many passages, including the bulk of the introduction and discussion, in which we relate our study to the complex, functional roles of iGluRs and a comparative analysis across the superfamily.

Major comments:

While the structural biology is rigorous, the manuscript is currently tailored for a highly specialized audience. The broader relevance, particularly how these findings inform iGluR activation models more generally, should be emphasized.

We appreciate this critique and agree that it is extremely important to engage a broad audience, yet, at the same time, we believe that the current manuscript is already quite accessible, especially in comparison to contemporary structural studies of iGluRs. Indeed, we took extra care to maintain a broad viewpoint in both the introduction and discussion to enable this paper to be understood by a wide-ranging audience of neuroscientists, biophysicists, and structural biologists. For example, our introduction is structured in the following way:

Paragraph 1: Broad description of iGluR superfamily, including molecular diversity and neurophysiological/pathophysiological roles

Paragraph 2: Synopsis of iGluR gating timescales (activation, desensitization, recovery)

Paragraph 3: Defining the relationship between ligand occupancy and gating

Paragraph 4: Overview of iGluR structure and key questions we aim to address

Paragraph 5: Summary of study

Our results and discussion are tailored in a similar way to balance a detailed analysis of our data and a comparative analysis across the iGluR family (e.g. Fig. 2C) with an eye toward physiology and pharmacology. We have made an effort in the revision to provide further comparison of gating across the iGluR subfamilies, particular in the context of inter-dimer LBD interfaces and the unique

large-scale rupture seen in KARs, which we now note may be shared by a subfamily of NMDARs and AMPARs. In the interest of further improving accessibility, we have also reduced technical terms throughout, added broader introductory/concluding sentences to some sections, expanded the discussion in a few areas and put more emphasis on the gating model, which is now its own figure (Fig. 8, see next point).

A clearer mechanistic summary figure (Fig. 7E) should be presented and not buried as a subsection. Figure 7E needs to be modified to reflect the data. Specifically, the claim that all levels of occupancy can lead to the partially ruptured state is not directly supported by data and should either be revised or explicitly qualified.

We very much appreciate this suggestion and have now separated this panel from main Fig. 7 to make a new main Fig. 8 with an expanded figure legend. We have also clarified the rationale for the decisions we have made in putting together this schematic (p. 13, lines 5-9). We would like to emphasize here, as we do in the discussion, that this is an oversimplified working model which is meant to both relate back to existing models in the field and guide future thinking on the relationship between agonist occupancy and KAR gating. In the interest of transparency about which states have been seen, we have clearly listed PDB numbers for existing KAR structures, including adding recently-published structures from *Gangwar et al (NSMB, 2025)* and *Zhou et al (Nat Comm, 2025)*, that support the existence of each state, including partially ruptured states with 0, 2, or 3/4 agonists bound. It is important to note that this model is based off of the existing, standard kinetic model in the iGluR field (see *Robert et al, J Neurosci, 2003*; *Perrais et al, J Neurosci, 2009*), which posited the existence of a shallowly and deeply-desensitized state purely based on functional studies. Other states are included for thermodynamic completeness of the kinetic models (e.g. D1), even if populated to a very low extent. We think our data, and data from other KAR structural studies, provide a compelling potential explanation whereby the shallow and deeply-desensitized states correspond to partially versus fully ruptured states.

The manuscript does not report the fraction of particles adopting each conformational class. This is critical to assess the relative stability and prevalence of intact, partially, and fully ruptured states under different ligand conditions. Correlation between particle classes and functional outcomes (e.g., degree of desensitization) should be explicitly discussed. If a quantitative correlation is not possible, this limitation should be acknowledged.

We appreciate this point. However, we would like to note that we also previously reported particle numbers in the cryo-EM processing pipeline figures (Fig. S2, Fig. S11). In addition, we now clearly state the fraction of particles contributing to each class in the text (p. 4, lines 30-31; p. 12, lines 14-19). We have purposely avoided a strong interpretation of these numbers as they represent the outcome from processed cryo-EM data and are not necessarily a direct report on the overall population in our sample. While the numbers generally match our expectations/interpretations with similar amounts of intact and partially ruptured states for the 5-IW condition and ~15% fully-ruptured particles for the AMPA condition, we have refrained from making strong quantitative correlations.

The discussion focuses primarily on comparison with other cryo-EM structures. A comparison to other techniques that probe glutamate receptor conformational states, such as smFRET on partial agonism of GluK2/GluK5, intra dimer cross linking study on AMPA receptors (Baranovic and Plested, eLife, 2018) needs to be added.

We thank the reviewer for this comment and suggestion. We have added further discussion putting our work in the context of smFRET (p. 12, lines 3-4). We had already included one very notable crosslinking study from a collaboration of Andrew Plested and Benoît Roux (Lau et al, Neuron 2013, now Ref. 71) and we have now also added the mentioned Baranovic and Plested paper (p. 14, lines 22-23).

Minor comment:

Figures 1-6 are of the IW cryo-EM structures and can be combined into 3-4 figures.

We appreciate this suggestion, but do not feel this is necessary or possible without sacrificing key information. These figures allow us to systemically work through the structural analysis, from the general scheme (Fig. 1) to LBD dimers (Fig. 2; including comparison across all KAR and AMPAR structures) to LBD tetramers (Fig. 3) to functional analysis of LBD interfaces (Fig. 4) to LBD/TMD coupling (Fig. 5) and to the unique partially ruptured state (Fig. 6). Also within individual figures we do not see major potential for shortening since comparing A/C and B/D subunit conformational changes is a key aspect of our study and contributes to the large number of panels needed in some places (e.g. Fig. 3, 5, 6). Ultimately, the aim of our study is to report our data with thoroughness and clarity, which we believe the current organization achieves.

Reviewer #2 (Remarks to the Author):

The manuscript by Khanra and colleagues reports the structure of partially occupied GluK2/GluK5 heteromeric kainate receptors, from which they propose a model of gating that is unlike other ionotropic glutamate receptors. This finding is interesting and informative because it is well established that partial occupancy of heteromeric KARs composed of subunits with large differences in agonist affinity will gate tonic currents rather than proceeding to near-complete desensitization. Here they take advantage of the differential affinity for two agonists, iodowillardiine and AMPA, for GluK2 versus GluK5 subunits to probe the molecular basis of this biophysical phenomenon. More broadly, these results provide insights into the structure of activated states of an iGluR that do not rely on the use of desensitization-blocking compounds like PAMs or mutagenesis.

The data in the paper is of high quality and rigor and the conclusions derived from the structures are appropriate. The manuscript is well-written, carefully reasoned, and the discussion is informative and does a good job of putting the current data in context with the large number of other KA (and AMPA) receptor structures. A number of new insights result from the structures in addition to the pre-activate states, including identification of key cross-dimer residues that in part underlie the slow deactivation of GluK2/GluK5 heteromers and correlation of partially-occupied LBD conformations with gating linkers. The findings add to and complement the growing number

of KA and AMPA receptor cryo-EM structures that each have illuminated aspects of receptor gating.

I have no major concerns.

We thank the reviewer for their very positive assessment of our study and our manuscript, including the discussion.

Minor points

The first study to report gating by partial occupancy of heteromeric KARs was a study by Swanson et al., *Neuron*, 2002 (PMID: 12062042), using the agonist dysiherbaine, which differs in affinity for GluK2 and GluK5 by several orders of magnitude. That paper seems highly relevant to the current study and pre-dates the Fisher and Mott and other reports by at least 9 years.

Thank you for referring to this study. Indeed, using dysiherbaine, a slowly dissociating GluK1 agonist, Swanson et al. already showed that partially occupied receptors can yield non-desensitizing currents, at least during recovery from full agonist occupancy. We have now slightly modified our text (p. 2, bottom) and added this reference to our introduction.

Reviewer #3 (Remarks to the Author):

This study provides the structural basis for the activation and desensitization of a specific heterotetrameric GluK2/GluK5 kainate receptor (KAR), building on their previous work that reported the first structures of GluK2/GluK5 heteromers in resting (Apo), antagonist-bound (CNQX), and agonist-bound (L-Glu) states (Khanra et al., 2021, *eLife*). Using a pharmacological approach that combines a GluK5-selective agonist (5-IW and AMPA) with a GluK2-selective antagonist (kynurenic acid), the authors captured high-resolution cryo-EM structures of the GluK2/GluK5 receptor in both an intact ligand-binding domain (LBD) dimer-of-dimer state and a partially ruptured state with one dimer interface disrupted. These conformations—a “pre-active” state with intact dimer interfaces and a partially ruptured intermediate state—offer new atomic-level insights into the gating mechanism of heteromeric KARs, particularly regarding the transition from activation to desensitization. The structural findings are further supported by mutagenesis and electrophysiological recordings, which underscore the role of inter-subunit interactions and mobility in the slow deactivation process of GluK5-containing KARs. Overall, the manuscript is well-written, and the figures are clearly illustrated. The work establishes an occupancy-dependent framework for understanding KAR gating and provides a molecular basis for elucidating the gating mechanism of endogenous KARs in the brain.

We thank the reviewer for their positive assessment of our study.

Major points:

1. The electron density of the GluK5-LBD (particularly protomer C in Class 2, Fig. 1C) appears very blurry. The authors also note that the ligand density was insufficient to model its binding pose. Did they attempt local refinement on the LBD tetramer to improve the local resolution of the LBD region?

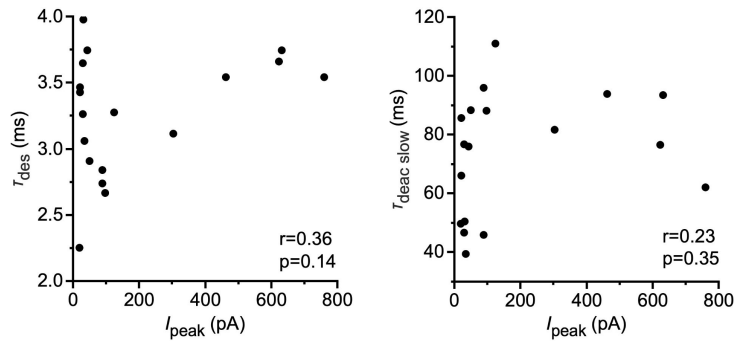
We thank the reviewer for this suggestion. Indeed, we have attempted different types of local refinements to improve the resolution of the LBD tetramer, and found that the reported strategy yields the best map. Specifically, when local refinement was performed only on the LBD tetramer, a resolution of ~ 4.05 Å was obtained (with similar, blob-like ligand density for protomer C but with an overall lower quality map), in comparison to the resolution of 3.8 Å we achieved when local refinement was performed on the combined LBD-TMD module. We note that this LBD-TMD combination is used for local refinement in almost all reported KAR and AMPAR cryo-EM structures (see for example recent studies: *Gangwar et al, Nature, 2024*; *Mondal et al, Nature, 2025*), suggesting that there is a general need for the TMD to guide particle alignment for these receptors.

2. It's interesting to note that both intra- and inter- dimer interface are involved in the deactivation properties of GluK2/GluK5 receptors. Could this conclusion be further supported by in silico simulations to probe additional intermediate conformations? A similar approach has been employed for NMDA receptors (Esmenjaud et al., EMBO J 2019).

We thank the reviewer for this helpful suggestion. We agree that future computational studies may be a very valuable way to probe these complex, dynamic interfaces. While clearly beyond the scope of this study, we have added a comment about this, along with a mention of such analysis in Esmenjaud et al, in the revised discussion (p. 14, lines 24-25).

3. The mutagenesis data are used to validate the structural findings. However, it remains unclear whether any of the mutations (e.g., Y683I) influence receptor surface expression or ligand binding affinity. Including such controls would strengthen the specificity of the conclusions.

We thank the reviewer for requesting important further details on the behavior of the tested heteromers. In our initial submission, we reported the peak current amplitudes that we obtained in our fast perfusion measurements for the different GluK5 variants (see Table S2). We observed somewhat different current amplitudes across variants, with some being higher and some being lower than the GluK2/GluK5(wt) combination. However, all reported variants gave reliable currents (see criteria for analysis in the methods section), were in a similar regime (mean peak currents of 60-440 pA), and formed comparable amounts of heteromers (~ 50 -80%). Most importantly, our analysis is focused on gating, namely desensitization and deactivation kinetics, which do not depend on current amplitude. To validate this, below we plot the time constants we report for GluK2/GluK5(wt) receptors against the peak current amplitude (x-axis), and, as expected, observe no correlation between current amplitude and gating kinetics.



To address the reviewer's question about surface expression, we additionally aimed at analyzing receptor cell surface levels by an independent method. We turned to immunostaining utilizing an N-terminal HA-tag on GluK5 which was labeled by an antibody without cell permeabilization (Fig. S7D,E). These stainings showed low GluK2/GluK5 heteromer expression levels compared to GluK2, but were clearly above the background signal obtained with untransfected cells or cells only expressing the GluK5 subunit, which does not traffic to the surface alone (see e.g. Ren et al, J Neurosci 2003, PMID 12878702; Nasu-Nishimura et al, J Neurosci 2006, PMID 16807331). This method allowed for a comparison of receptor surface levels which showed roughly similar heteromer expression levels for all GluK5 variants, with the exception of GluK2/GluK5(A504Y), which showed relatively weak surface expression.

Finally, as suggested, we also compared the apparent glutamate affinities of GluK5(Y683I) as well as GluK5(N772E,N773G) to GluK5(wt). For this we coexpressed GluK5 subunits with a binding-impaired GluK2 variant (GluK2(T690V)), which suppresses desensitization and prevents glutamate-induced background currents from GluK2 homotetramers. The corresponding dose-response curves from whole-cell recordings revealed no major effects on the EC₅₀ values (Fig. S7F,G), suggesting that the effects of these mutations are primarily on gating kinetics, rather than apparent ligand affinity.

4. The discussion could be further enriched by considering whether the identified “central cluster” and inter-dimer interfaces are unique to GluK4/GluK5-containing heteromers, or whether analogous—though mechanistically distinct—interaction networks might also exist in other iGluR subfamilies to fine-tune their gating kinetics. Also, it would be valuable to discuss the fact that a partially ruptured LBD dimer has also been observed in AMPARs (Fig. 4d, Zhang D et al., Nature 2023. doi: 10.1038/s41586-023-06528-0).

We thank the reviewer for raising this interesting point, which we now address in the discussion (p. 14-15). We presume that the “central cluster” as investigated here, is possibly unique to GluK5 and probably GluK4-containing receptors, which have unique residues at the start of helix G (Y683 and Q684; Fig. 4B), a uniquely dense LBD layer packing, and peculiar deactivation kinetics. With respect to other LBD interface differences, alignments show no strikingly different residues between GluK5 and GluK4 (see also Fig. 4C,D), but major differences to other GluKs and AMPARs. We very much appreciate the reference to the work from Zhang et al., which showed unusually large LBD rearrangements in certain AMPAR complexes with destabilized ATDs. We now included this in our discussion (p. 15, lines 22-27; new Ref. 87).

Minor Points:

1.“..., the intact class 1 structure shows rearrangement of the upper “D1” and lower “D2” subdomain interfaces with an overall loosening of both interfaces (Fig. 2B; Supplementary Video 1, 2)”.

The reference figure here is Fig. 2B or 2A? Please clarify. Similar inconsistencies appear elsewhere in the manuscript; the authors are advised to carefully check and correct all figure citations.

We thank the reviewer for catching this mistake and apologize for the numbering inconsistencies in the manuscript. We have carefully edited the text for the revision.

2.“Heteromers incorporating the GluK5(Q768K) variant in the A/C subunits opposing GluK2 Y521 in the B/D subunits showed only slightly accelerated desensitization kinetics compared to GluK2/GluK5(wt). In contrast, the GluK5(A504Y) substitution, a substitution that is homologous to GluA(L483Y), had no effect on desensitization, but showed a minor slowing of deactivation, supporting prior work indicating that the LBD dimer interface contributions may be different in KARs compared to AMPARs^{60–63}. However the double substitution GluK5(N772E, N773G), which mimics the residues present in GluK2, resulted in 1.8-fold slower desensitization, revealing a subtype-specific contribution to KAR desensitization.”

In the paragraph describing the functional effects of the GluK5(Q768K), GluK5(A504Y), and GluK5(N772E/N773G) substitutions, no figure references appear to be provided. If this is correct, please include the relevant figure citation to guide the reader.

We apologize for this oversight and have now added the appropriate figure references to the text.

3.The residue numbering in Fig. 4C appears to be incorrect, and additional background file information are present in Fig. 4E–F. The authors are advised to carefully check all figure labels and correct these inconsistencies.

We have now removed the file information from the figure, but we did not find any numbering inconsistencies in Fig. 4C (Q768, N772 and N773 do cluster together at the end of helix J).

4.“The 2-fold symmetry of the LBD layer leads to the GluK2 (A/C) subunits being in poredistal M3 linker positions (Fig. 5D) while GluK5 (B/D subunits) occupy pore-proximal positions (Fig. 5G).”

GluK2 (A/C) should be GluK2(B/D subunits) and GluK5 (B/D subunits) should be GluK5 (A/C subunits).

We thank the reviewer for pointing out this important mistake, which we have now fixed.