nature portfolio

Peer Review File

Bipolar and schizophrenia risk gene AKAP11 encodes an autophagy receptor coupling the regulation of PKA kinase network homeostasis to synaptic transmission

Corresponding Author: Professor Zhenyu Yue

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 by Lee et al., the authors investigate the neuronal role of AKAP11 in coupling PKA kinase signaling regulation to synaptic transmission. AKAP11 is a cAMP-dependent protein kinase (PKA) adaptor protein that has been implicated in the pathogenesis of bipolar disorder and schizophrenia. In prior work, the authors showed that AKAP11 promotes the degradation of PKA regulatory R1 subunits through selective autophagy, resulting in PKA activation. This AKAP11-mediated selective autophagy is critical for fueling mitochondrial metabolism and tumor cell growth in the context of glucose deprivation (Deng et al., 2021) and for controlling the homeostasis of the PKA-R1 complex and PKA activity in the soma and neurites of human and mouse neurons (Zhou et al, 2024).

In this manuscript, to assess how AKAP11-mediated autophagy is linked to the regulation of synaptic activity, the authors utilized a combination of multi-omics, cell biology, and electrophysiology approaches to assess the consequences of AKAP11 loss in mouse genetic models and human induced neurons. Using quantitative mass spectrometry-based proteomics, they found that loss of AKAP11 alters the level of numerous proteins involved in PKA signaling and synaptic function. The authors also analyzed phosphoproteomics data obtained from the brains of control and AKAP11 cKO mice and identified differentially abundant phosphoprotein sites, many of which are predicted PKA phosphorylation sites in proteins involved in synapse regulation and neurodevelopment disease. The authors also discovered compartment-specific alterations of PKA activities, with AKAP11 loss resulting in reduced PKA activity (and increased PKA-RI levels) in the cytosol and increased PKA activity (and PKA-C levels) in the synaptosome fraction. Moreover, the authors found decreased GSK3beta activity and increased AKT1 activity in the brains of AKAP11 KO mice. To further elucidate AKAP11 function, the authors investigated the interactome of AKAP11 in CNS neurons and identified SPHKAP and VAPA/B as AKAP11-binding proteins. They showed that AKAP11 colocalizes with PKA-RI subunits, SPHKAP, and VAPA/B in discrete puncta in mouse neurons, and that like PKA-RI subunits, AKAP11 mediates the selective degradation of SPHKAP through autophagy. Lastly, to investigate whether AKAP11-deficiency affects synaptic function, the authors performed electrophysiological recordings on the prefrontal cortex of control and AKAP11 cKO mice and observed decreased mEPSC frequency and amplitude, suggesting decreased excitatory synaptic neurotransmission. Decreased mEPSC frequency was also seen in AKAP11-KD human induced neurons, and synaptophysin staining was decreased in both mouse and human neurons. Based on these results, the authors conclude that AKAP11 plays a central role in coupling PKA regulation to synaptic transmission in human and mouse neurons. Moreover, the authors suggest that the AKAP11-SPHKAP-VAP complex regulates PKA homeostasis and neuronal activity by functioning as an assembly platform to recruit PKA-RI and autophagy machinery to the ER, resulting in the selective degradation of the PKA-RI complex.

Overall, this is a comprehensive timely study that investigates the neuronal function of an important protein implicated in both schizophrenia and bipolar disorder. For the most part, the figures are clear and thorough. The authors include an impressive amount of data in the manuscript, almost bordering on too much as the scope of this paper is broad and certain points (e.g. assessment of GSK3beta and AKT1 kinase activity) somewhat detract from the overall point of the manuscript. While the manuscript is generally strong, using multiple approaches to elucidate the neuronal role of AKAP11 in regulating PKA signaling homeostasis and synaptic transmission, I have some concerns outlined below, that if addressed would significantly strengthen the paper.

Specific Concerns:

1. The electrophysiology results presented in Figure 7 and the interpretation of this data are current weaknesses of this manuscript. The authors show a decrease in mEPSC frequency and amplitude when recording from L5/6 mPFC neurons in AKAP11 cKO (Fig. 7b,c) and a decrease in mEPSC frequency when recording from AKAP11-KD human iNeurons (Fig. 7h,i). How many neurons were recorded from and how many control and AKAP11 cKO mice were analyzed? I assume the dots on the graphs are from individual neurons, but both numbers of neurons and numbers of mice should be stated in the figure legend. Figure legend 7h,i states that mEPSC data was recorded from n>15 neurons, 2 independent replicates. At least 3 replicates are a standard practice in the field. The authors state that “Our recordings revealed a significant decrease in mEPSC frequency and amplitude in Akap11-cKO mice, indicating impaired neurotransmitter release (Fig. 7a-c).” This statement is not fully accurate. The decreased mEPSC frequency could reflect a reduced probability of neurotransmitter release from presynaptic terminals, but it could also indicate fewer excitatory synapses (especially given the decrease in the density of synaptophysin puncta). Moreover, the decreased mEPSC amplitude suggests a reduction in postsynaptic receptor function. The authors should discuss all these possibilities. The authors also state that “Our analysis revealed a large decrease (~3 fold) in the frequency of mEPSCs in AKAP11-KD human iNeurons, while the amplitude remained unaffected (Fig. 7e-i). These results indicated that KD of AKAP11 in human neurons reduces the strength of the excitatory transmission.” Here, since only mEPSC frequency was affected, the results suggest an impairment in presynaptic release and/or a reduction in excitatory synapses rather than synaptic strength alterations. Could the authors also comment as to why there is a difference in electrophysiology results between mouse and human iNeurons? Lastly, the representative images in Fig. 7d and Fig. 7j need to be improved. For Fig. 7d, the difference in Synaptophysin staining is difficult to see. Could the figure and the selected ROI be enlarged (or future cropped in)? Similarly, it would be helpful to include ROIs from the representative images in Fig. 7j (like Fig. 7d – and present the data in a similar manner). The authors could also costain neurons for both a pre- and post-synaptic marker to determine if there is indeed fewer excitatory synapses in neurons lacking AKAP11.

2. Another concern is that the connection between dysregulated PKA signaling networks and synaptic alterations caused by AKAP11 loss is somewhat correlative in the manuscript and potential mechanisms underlying this connection are only briefly discussed. The schematic presented in Fig. 7k is not helpful for understanding this connection since while it clearly depicts a model for how the AKAP11-SPHKAP-VAP interaction regulates PKA homeostasis through selective autophagy, it does not address the impact on excitatory synapses. As I understand it, the authors are arguing that AKAP11 loss results in excessive PKA activity at synapses, which dysregulates the functon(and/or level) of numerous synaptic proteins, resulting in altered excitatory synaptic transmission. If PKA activity is inhibited in the context of AKAP11 loss, are synaptic alterations ameliorated? This may have important therapeutic implications. Also, since GSK3beta and AKT1 activities are also dysregulated in the absence of AKAP11, is it possible that signaling by those kinases also impact synapse function?

Minor Concern

1. In Figure 1h, the representative western blot showing SYNJ2 staining is not ideal with a large smudge in last lane interfering with blot image. Can the authors provide a different blot or a lighter exposure?

2. Where appropriate, information about sample size and independent repeats should be included in the figure legends.

Reviewer #2

(Remarks to the Author)
In this manuscript titled “Bipolar and schizophrenia risk gene AKAP11 encodes an autophagy receptor coupling the regulation of PKA kinase network homeostasis to synaptic transmission”, Lee et al. applied various technologies to show the role of AKAP11 in autophagy as well as synaptic function and the possible underlying molecular mechanisms. The authors claim that AKAP11 is involved in autophagy through its interaction with SPHKAP and VAPA/B, and that abnormalities in neurotransmission resulting from AKAP11 knockout may be implicated in psychiatric disorders. However, there are substantial issues that should be addressed to support the claims made by the authors.

Major concerns:

1) In their previous study (Nat Commun, 2024), the authors demonstrated that AKAP11 is involved in autophagy. In the present study, the authors newly propose that AKAP11 regulates autophagy through its interaction with SPHKAP and VAPA/B, which represents a new aspect of this study. Given the authors’ previous data and established findings regarding VAPA/B and related proteins, this proposed mechanism appears plausible; however, it remains important to directly demonstrate the functional relevance of the interaction between AKAP11 and SPHKAP or VAPA/B, for example by using binding-deficient mutants.

2) The frequency of miniature excitatory postsynaptic currents (mEPSCs) can be affected by both the number of functional synapses and the presynaptic release probability. The observed reduction in Syn-positive puncta suggests a decrease in synapse number, which could plausibly contribute to the lower mEPSC frequency. If the authors intend to assert that altered neurotransmitter release is the primary cause, additional experiments—such as paired-pulse facilitation or other assays assessing presynaptic function—should be conducted to substantiate this claim.

3) Under the present experimental conditions, mEPSCs are considered to be AMPAR-mediated, and their amplitude is influenced by AMPAR activity. Figure 3f shows an increase in AMPAR phosphorylation, and since AMPAR phosphorylation

by PKA generally enhances AMPAR activity, the observed decrease in mEPSC amplitude appears inconsistent with this potential mechanism. This possible discrepancy should be addressed experimentally. In addition, since PKA phosphorylates a wide range of synaptic proteins beyond AMPARs, it would be important to examine changes in phosphorylation levels of other synaptic proteins as well.

4) The authors argue that AKAP11 knockout leads to abnormalities in neurotransmission that are associated with psychiatric disorders. However, if they intend to support this claim, mEPSC analysis alone is insufficient; more physiologically relevant forms of synaptic plasticity should also be examined.

5) As this has already been mentioned, if the authors intend to claim a link between AKAP11 and psychiatric disorders, it is necessary to perform behavioral assays relevant to psychiatric disorders, such as those assessing learning and memory, prepulse inhibition, and other behavioral tests.

Minor concerns

6) Neurons derived from iPSCs using NGN2 are mostly excitatory neurons, but based on the results from the mouse-based analysis, should we primarily focus on excitatory neurons? I'm also curious about the role of inhibitory neurons in this context.

7) In the immunostaining image of Figure 1g, the authors claim that RI is stained in a punctate pattern in the conditional KO, but the entire cell body appears brighter compared to the control. What could explain this?

8) Which dots in Figure 1b and 1c indicate AKAP?

9) Keratin contamination is a common issue in proteomics analysis, so it might be worth considering this possibility in Figure 5g.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I am satisfied that the authors have adequately addressed my specific concerns and that their revisions have strengthened an already interesting manuscript. In my opinion, it is now acceptable for publication.

Reviewer #2

(Remarks to the Author)
All of my concerns have been satisfactorily addressed.

Reviewer's Comments:

We are thankful to the constructive comments from the two reviewers. To address their critiques, we have performed new experiments, and the results were included in the revision. We have modified the figures and text, which were hightlighted with red bars at the left margin. We believe we have fully addressed their comments, and we look forward to further evaluation.

Reviewer #1 (Remarks to the Author)

In this manuscript by Lee et al., the authors investigate the neuronal role of AKAP11 in coupling PKA kinase signaling regulation to synaptic transmission. AKAP11 is a cAMP-dependent protein kinase (PKA) adaptor protein that has been implicated in the pathogenesis of bipolar disorder and schizophrenia. In prior work, the authors showed that AKAP11 promotes the degradation of PKA regulatory R1 subunits through selective autophagy, resulting in PKA activation. This AKAP11-mediated selective autophagy is critical for fueling mitochondrial metabolism and tumor cell growth in the context of glucose deprivation (Deng et al., 2021) and for controlling the homeostasis of the PKA-R1 complex and PKA activity in the soma and neurites of human and mouse neurons (Zhou et al, 2024).

In this manuscript, to assess how AKAP11-mediated autophagy is linked to the regulation of synaptic activity, the authors utilized a combination of multi-omics, cell biology, and electrophysiology approaches to assess the consequences of AKAP11 loss in mouse genetic models and human induced neurons. Using quantitative mass spectrometry-based proteomics, they found that loss of AKAP11 alters the level of numerous proteins involved in PKA signaling and synaptic function. The authors also analyzed phosphoproteomics data obtained from the brains of control and AKAP11 cKO mice and identified differentially abundant phosphoprotein sites, many of which are predicted PKA phosphorylation sites in proteins involved in synapse regulation and neurodevelopment disease. The authors also discovered compartment-specific alterations of PKA activities, with AKAP11 loss resulting in reduced PKA activity (and increased PKA-RI levels) in the cytosol and increased PKA activity (and PKA-C levels) in the synaptosome fraction. Moreover, the authors found decreased GSK3beta activity and increased AKT1 activity in the brains of AKAP11 KO mice. To further elucidate AKAP11 function, the authors investigated the interactome of AKAP11 in CNS neurons and identified SPHKAP and VAPA/B as AKAP11-binding proteins. They showed that AKAP11 colocalizes with PKA-RI subunits, SPHKAP, and VAPA/B in discrete puncta in mouse neurons, and that like PKA-RI subunits, AKAP11 mediates the selective degradation of SPHKAP through autophagy. Lastly, to investigate whether AKAP11-deficiency affects synaptic function, the authors performed electrophysiological recordings on the prefrontal cortex of control and AKAP11 cKO mice and observed decreased mEPSC frequency and amplitude, suggesting decreased excitatory synaptic neurotransmission. Decreased mEPSC frequency was also seen in AKAP11-KD human induced neurons, and synaptophysin staining was decreased in both mouse and human neurons. Based on these results, the authors conclude that AKAP11 plays a central role in coupling PKA regulation to synaptic transmission in human and mouse neurons. Moreover, the authors suggest that the AKAP11-SPHKAP-VAP complex regulates PKA homeostasis and neuronal activity by functioning as an assembly platform to recruit PKA-RI and autophagy machinery to the ER, resulting in the selective degradation of the PKA-RI complex.

Overall, this is a comprehensive timely study that investigates the neuronal function of an important protein implicated in both schizophrenia and bipolar disorder. For the most part, the figures are clear and thorough. The authors include an impressive amount of data in the manuscript, almost bordering on too much as the scope of this paper is broad and certain points (e.g. assessment of GSK3beta and AKT1 kinase activity) somewhat detract from the overall point of the manuscript. While the manuscript is generally strong, using multiple approaches to elucidate the neuronal role of AKAP11 in regulating PKA signaling homeostasis and synaptic transmission, I have some concerns outlined below, that if addressed would significantly strengthen the paper.

Specific Concerns:

1. The electrophysiology results presented in Figure 7 and the interpretation of this data are current weaknesses of this manuscript. The authors show a decrease in mEPSC frequency and amplitude when recording from L5/6 mPFC neurons in AKAP11 cKO (Fig. 7b,c) and a decrease in mEPSC frequency when recording from AKAP11-KD human iNeurons (Fig. 7h,i). How many neurons were recorded from and how many control and AKAP11 cKO mice were analyzed? I assume the dots on the graphs are from individual neurons, but both numbers of neurons and numbers of mice should be stated in the figure legend**.** Figure legend 7h,i states that mEPSC data was recorded from n>15 neurons, 2 independent replicates. At least 3 replicates are a standard practice in the field.

We appreciate the reviewer for the thoughtful and detailed comments regarding our data. We have carefully addressed all the points raised and revised the manuscript and figures accordingly. We apologize for that the figure legend for Figure 7 was not properly updated at the time of submission. The recordings were in fact performed across three biological replicates, but the detail was unfortunately not reflected in the original figure and has now been corrected in the revised version. mEPSCs were recorded from more than 20 neurons per group, across 3

independent biological replicates. We have also included the number of animals used per group (n = 3) to ensure full transparency (Fig 7h-k).

The authors state that “Our recordings revealed a significant decrease in mEPSC frequency and amplitude in Akap11-cKO mice, indicating impaired neurotransmitter release (Fig. 7a-c).” This statement is not fully accurate. The decreased mEPSC frequency could reflect a reduced probability of neurotransmitter release from presynaptic terminals, but it could also indicate fewer excitatory synapses (especially given the decrease in the density of synaptophysin puncta). Moreover, the decreased mEPSC amplitude suggests a reduction in postsynaptic receptor function. The authors should discuss all these possibilities.

We also appreciate the reviewer’s important point regarding the interpretation of the reduced mEPSC frequency and amplitude. We agree that the observed reduction in mEPSC frequency could result from decreased presynaptic release probability as well as a lower number of excitatory synapses. To test the idea, we have performed additional experiments which indicated a decrease in the number of excitatory synapse (Fig.7f), as evicenced by a reduction in the density of Synaptophysin+ puncta, PSD95+ puncta, and in co-localized puncta of Synaptophysin–PSD95, a well-established marker of mature excitatory synapses, in AKAP11-deficient neurons. In addition, we performed analysis of mEPSC kinetics and the results revealed no changes in rise time, half-width, or decay, but indicated a significant reduction in both peak amplitude and area under the curve (total charge per event), suggesting fewer AMPARs at each functional synapse (Fig.7d). While we cannot completely rule out the contribution of altered release probability, we believe it is unlikely to be the main driver of the observed effects and modified our conclusion.

The authors also state that “Our analysis revealed a large decrease (~3 fold) in the frequency of mEPSCs in AKAP11-KD human iNeurons, while the amplitude remained unaffected (Fig. 7e-i). These results indicated that KD of AKAP11 in human neurons reduces the strength of the excitatory transmission.” Here, since only mEPSC frequency was affected, the results suggest an impairment in presynaptic release and/or a reduction in excitatory synapses rather than synaptic strength alterations. Could the authors also comment as to why there is a difference in electrophysiology results between mouse and human iNeurons?

In the case of human iNeurons, mEPSC frequency was also decreased, while amplitude remained unchanged. This supports the idea that synapse number and/or presynaptic function are affected, rather than synaptic strength per se. We also have revised the Discussion section to briefly address potential species- or model-specific factors that may underlie the observed differences between mouse and human neurons.

Lastly, the representative images in Fig. 7d and Fig. 7j need to be improved. For Fig. 7d, the difference in Synaptophysin staining is difficult to see. Could the figure and the selected ROI be enlarged (or future cropped in)? Similarly, it would be helpful to include ROIs from the representative images in Fig. 7j (like Fig. 7d – and present the data in a similar manner). The authors could also co-stain neurons for both a pre- and post-synaptic marker to determine if there is indeed fewer excitatory synapses in neurons lacking AKAP11.

We appreciate the comments on the quality of the representative images in Figure 7d and 7j. As suggested, the selected ROIs were enlarged to better highlight the differences in Synaptophysin puncta. We also added the results from additional PSD95 staining and Synaptophysin–PSD95 co-staining, which confirm the reduction in excitatory synapse number in AKAP11-deficient neurons as shown above (Fig 7e,f).

2. Another concern is that the connection between dysregulated PKA signaling networks and synaptic alterations caused by AKAP11 loss is somewhat correlative in the manuscript and potential mechanisms underlying this connection are only briefly discussed. The schematic presented in Fig. 7k is not helpful for understanding this connection since while it clearly depicts a model for how the AKAP11-SPHKAP-VAP interaction regulates PKA homeostasis through selective autophagy, it does not address the impact on excitatory synapses. As I understand it, the authors are arguing that AKAP11 loss results in excessive PKA activity at synapses, which dysregulates the functon(and/or level) of numerous synaptic proteins, resulting in altered excitatory synaptic transmission. If PKA activity is inhibited in the context of AKAP11 loss, are synaptic alterations ameliorated? This may have important therapeutic implications. Also, since GSK3beta and AKT1 activities are also dysregulated in the absence of AKAP11, is it possible that signaling by those kinases also impact synapse function?

We thank the reviewer for the thoughtful feedback. We completely agree that it is important to establish a direct mechanistic link between dysregulated PKA signaling and the observed synaptic phenotypes in AKAP11-deficient neurons. Our manuscript is primarily focusd on the report of the changes of PKA activity and synaptic functions as a result of AKAP11 as well as AKAP11-SPHKAP-VAP complex in mediating PKA-RI complex degradation. We are currently investigating the mechanism by which deregulated PKA activity in synapse is causal to the dysfunctional synaptic transmission by manipulating PKA activity specifically at synapse. However, such an investigation will need additional time and effort and we hope to present the results in a separate manuscript. We also appreciate the

reviewer's point regarding the potential role of GSK3β and AKT1, whose activities are altered in the absence of AKAP11. While we did not explore their functional relevance in this study, we find this a highly interesting and important direction for future investigation. We have added a brief note in the Discussion to acknowledge the potential contribution of these kinase pathways to synaptic function.

Finally, we agree with the reviewer that the schematic in Figure 7k, while useful in illustrating the AKAP11–SPHKAP–VAPA complex and its role in regulating PKA homeostasis via autophagy, does not effectively convey the overall message of the report. To illustrate the main message of our findings, we have replaced it with a schematic figure (currently Fig. 8a).

Minor Concern

1. In Figure 1h, the representative western blot showing SYNJ2 staining is not ideal with a large smudge in last lane interfering with blot image. Can the authors provide a different blot or a lighter exposure?

We thank the reviewer for pointing this out. We agree that the quality of the SYNJ2 blot in Fig. 1h was not ideal. In response, we repeated the experiment with new samples and found that SYNJ2 levels were not significantly altered in AKAP11-deficient neurons. Given the lack of consistent changes and to maintain a clear presentation of the data, we have decided to remove the SYNJ2 blot from the revised figure and Results section, and the result affects little the conclusion.

2. Where appropriate, information about sample size and independent repeats should be included in the figure legends.

We have revised the figure legends throughout the manuscript to include detailed information on sample size (n) and the number of independent biological replicates where applicable.

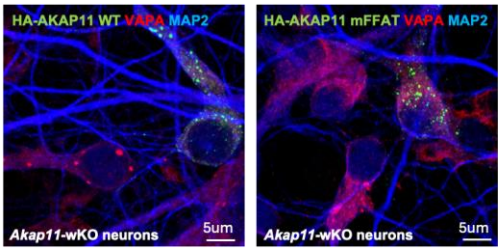
Reviewer #2 (Remarks to the Author)

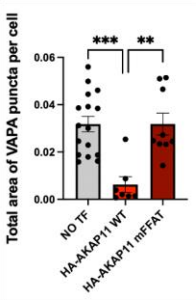
In this manuscript titled "Bipolar and schizophrenia risk gene AKAP11 encodes an autophagy receptor coupling the regulation of PKA kinase network homeostasis to synaptic transmission", Lee et al. applied various technologies to show the role of AKAP11 in autophagy as well as synaptic function and the possible underlying molecular mechanisms. The authors claim that AKAP11 is involved in autophagy through its interaction with SPHKAP and VAPA/B, and that abnormalities in neurotransmission resulting from AKAP11 knockout may be implicated in psychiatric disorders. However, there are substantial issues that should be addressed to support the claims made by the authors.

Major concerns:

1) In their previous study (Nat Commun, 2024), the authors demonstrated that AKAP11 is involved in autophagy. In the present study, the authors newly propose that AKAP11 regulates autophagy through its interaction with SPHKAP and VAPA/B, which represents a new aspect of this study. Given the authors' previous data and established findings regarding VAPA/B and related proteins, this proposed mechanism appears plausible; however, it remains important to directly demonstrate the functional relevance of the interaction between AKAP11 and SPHKAP or VAPA/B, for example by using binding-deficient mutants.

We thank the reviewer for this important comment. As suggested, we agree that functional validation of the AKAP11 interactions with SPHKAP or VAPA/B is critical to strengthen our proposed mechanism. To address this idea, we hypothesize that the FFAT motif in AKAP11 is required for VAP binding and therefore generated a mutant of AKAP11 by disrupting FFAT motif. Through immunoprecipitation (IP), we verified that the FFAT-mutant AKAP11 lost the interaction with VAPA/B, while AKAP11 WT robustly interacted with VAPA/B in transfected cells. Furthermore, AKAP11 WT showed colocalization with VAPA, while the FFAT mutant markedly reduced colocalization through cell imaging study. The similar results were observed in both HEK293T and primary neurons, confirming the lack of interaction of FFAT mutant AKAP11 with VAPA, a ER resident protein. Moreover, our results indicated AKAP11 KO led to a increase of VAPA puncta number/size due to lack of autophagy degradation (Figure 6). We observed the colocalization of reintroduced HA-AKAP11 and VAPA as well as reduced intensity of VAPA in the KO neurons; however, re-introduction of the FFAT mutant into KO neurons failed to colocalize with VAPA or normalize the VAPA levels, suggesting that binding of AKAP11 through FFAT to VAPA is essential for the recovery of selective autophagy activity. We have added these data to the revised manuscript (Figure 6h-j) and a figure below for your evaluation.



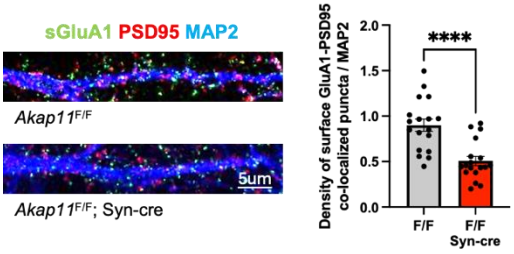


2) The frequency of miniature excitatory postsynaptic currents (mEPSCs) can be affected by both the number of functional synapses and the presynaptic release probability. The observed reduction in Syn-positive puncta suggests a decrease in synapse number, which could plausibly contribute to the lower mEPSC frequency. If the authors intend to assert that altered neurotransmitter release is the primary cause, additional experiments—such as paired-pulse facilitation or other assays assessing presynaptic function—should be conducted to substantiate this claim.

We thank the reviewer for this important point. We agree that mEPSC frequency reflects both synapse number and presynaptic release probability. In this revision, we have performed additional experiments and we found a significant decline in Synaptophysin-positive puncta, PSD-95 puncta, and colocalized Synaptophysin-PSD95 puncta, suggesting a reduced number of excitotary synapse in AKAP11 KO neurons (Fig 7). Thus, we attribute the observed decrease in mEPSC frequency (~45%) primarily to the reduction in synapse number. Thus, we made the changes accordingly in our text to clarify our interpretation.

3) Under the present experimental conditions, mEPSCs are considered to be AMPAR-mediated, and their amplitude is influenced by AMPAR activity. Figure 3f shows an increase in AMPAR phosphorylation, and since AMPAR phosphorylation by PKA generally enhances AMPAR activity, the observed decrease in mEPSC amplitude appears inconsistent with this potential mechanism. This possible discrepancy should be addressed experimentally. In addition, since PKA phosphorylates a wide range of synaptic proteins beyond AMPARs, it would be important to examine changes in phosphorylation levels of other synaptic proteins as well.

We appreciate this important point. To better understand this discrepancy, we reanalyzed our existing mEPSC recordings with a focus on the properties of individual synaptic events. While kinetic parameters such as rise time, half-width, and decay remained unchanged, we found a significant reduction in both peak amplitude and total charge (area under the curve) in AKAP11-cKO neurons. These results indicate that although the temporal dynamics of synaptic transmission are preserved, the amount of current transmitted per event is reduced, likely due to fewer AMPARs at individual synapses. We interpret the elevated GluA1 phosphorylation as a possible compensatory response, which appears insufficient to offset the reduction in receptor number. Supporting this interpretation, we also observed a significant decrease in both PSD95 puncta density and Synaptophysin–PSD95 co-localized puncta in AKAP11-deficient neurons as above described (Fig 7). Since PSD95 plays a key role in anchoring AMPARs at the postsynaptic density, these structural changes suggest impaired receptor stabilization and a reduction in functional excitatory synapse number. Furthermore, we found that the number of surface GluA1 puncta co-localized with PSD95 was also significantly reduced, providing direct evidence for impaired synaptic localization of AMPARs, as shown in the figure below for your evaluation.



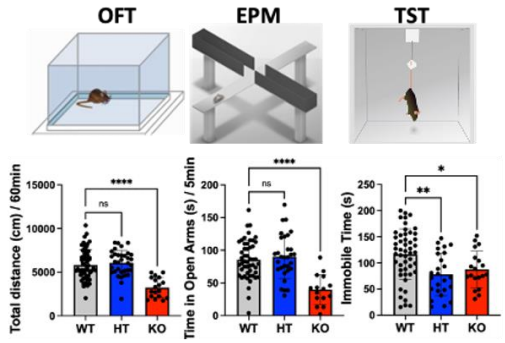
These findings raise the possibility that AMPAR trafficking to the synapse is disrupted in the absence of AKAP11, despite the increase of phosphorylation. The detailed mechanism awaits future investigation.

4) The authors argue that AKAP11 knockout leads to abnormalities in neurotransmission that are associated with psychiatric disorders. However, if they intend to support this claim, mEPSC analysis alone is insufficient; more physiologically relevant forms of synaptic plasticity should also be examined.

We appreciate the reviewer's suggestion regarding synaptic plasticity assays. We agree that synaptic plasticity assays are valuable and will explore them in subsequent studies; however, these are beyond the scope of the present manuscript. We now clarify the scope of the current study, justify our focus on molecular signaling and synapse organization, and outline how synaptic loss itself is a major contributor to psychiatric disease pathology. We also acknowledge future directions for plasticity studies beyond the present work.

5) As this has already been mentioned, if the authors intend to claim a link between AKAP11 and psychiatric disorders, it is necessary to perform behavioral assays relevant to psychiatric disorders, such as those assessing learning and memory, prepulse inhibition, and other behavioral tests.

We appreciate the reviewer's point regarding the importance of behavioral evidence in establishing a link between AKAP11 dysfunction and psychiatric disorders. While our current study focuses primarily on molecular and synaptic phenotypes, we fully agree that behavioral validation is essential to support disease relevance. We have begun to explore this in ongoing work using Akap11-cKO mice. Preliminary data have revealed alterations in specific behavioral domains, including anxiety- and depression-related behaviors (figure below). Although these data are still being collected and analyzed, the completion of the behavioral analysis will take months, considering breeding additional mice and testing them at multiple ages, thus it is beyond the scope of this study which mainly focused on molecular and cellular mechanism. In addition, two publications have examined the behavior in the Akap11-KO mice and showed SCZ-related phenotypes (36914641, 40408419).



Minor concerns

6) Neurons derived from iPSCs using NGN2 are mostly excitatory neurons, but based on the results from the mouse-based analysis, should we primarily focus on excitatory neurons? I'm also curious about the role of inhibitory neurons in this context.

As the reviewer correctly pointed out, neurons derived using NGN2 induction protocols are primarily excitatory, and our iNeuron experiments therefore reflect excitatory neuronal phenotypes. This aligns with our findings from the Akap11-cKO mouse model, where we observed alterations in excitatory synaptic transmission, including reduced mEPSC frequency and amplitude. That said, we also agree that the potential contribution of inhibitory neurons to the AKAP11-related phenotypes remains an important and open question. Although our current study focuses on excitatory neurons, we are actively considering strategies to examine how AKAP11 loss may impact inhibitory neuron function, including through the use of alternative differentiation protocols and mouse models. We have now included a brief note in the discussion section to acknowledge this important future direction.

7) In the immunostaining image of Figure 1g, the authors claim that RI is stained in a punctate pattern in the conditional KO, but the entire cell body appears brighter compared to the control. What could explain this?

In conditional KO neurons, we observed not only a more punctate distribution of RI but also an overall increase in RI signal intensity compared to wild-type neurons. Importantly, the images of WT and KO neurons were acquired under the same confocal microscope settings, indicating that the brighter signal in KO neurons reflects a true biological increase rather than an imaging artifact. This observation is consistent with our immunoblotting data (Figure 1e), which show elevated levels of PKA-RIα and RIβ proteins upon AKAP11 loss. We have adjusted the image contrast to avoid overexposure in KO neurons to better present the signals and contrast between the genotypes. The revised figure is now used in the manuscript. Together, these findings support the conclusion that AKAP11 deficiency leads to impaired degradation and accumulation of RI subunits, resulting in both increased overall signal intensity and altered subcellular distribution.

8) Which dots in Figure 1b and 1c indicate AKAP?
In Figure 1b and 1c, this dataset was generated by DIA platform, unfortunately, the AKAP11 peptide was not detected, which is not uncommon, as this platform usually covers 6000-8000 proteins.

9) Keratin contamination is a common issue in proteomics analysis, so it might be worth considering this possibility in Figure 5g.

Thanks for the helpful comments. We removed the Keratin from the PPI network (Figure 5g) .

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

I am satisfied that the authors have adequately addressed my specific concerns and that their revisions have strengthened an already interesting manuscript. In my opinion, it is now acceptable for publication.

Reviewer #2 (Remarks to the Author):

All of my concerns have been satisfactorily addressed.