

# Amyloid- $\beta$ -driven synaptic deficits are mediated by synaptic removal of GluA3-containing AMPA-receptors.

Niels Reinders, Sophie Spek, Remco Klaassen, Karin Koymans, Harold MacGillavry, August Smit, and Helmut Kessels

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## Review Timeline:

Submission Date:	28th Feb 2024
Editorial Decision:	13th Mar 2024
Revision Received:	11th Jun 2024
Editorial Decision:	27th Jun 2024
Revision Received:	4th Dec 2024
Editorial Decision:	10th Dec 2024
Revision Received:	18th Dec 2024
Accepted:	19th Dec 2024

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13th Mar 2024

Dear Dr. Kessels:

Thank you for submitting your manuscript to The Journal of Neuroscience.

We have received the reviews of your paper, "Amyloid- $\beta$ -driven synaptic depression is mediated by synaptic removal of GluA3-containing AMPA-receptors." (JN-RM-0393-24), which are appended to this email. Based on the reviewers' comments and our editorial assessment, we would like to reconsider your manuscript at The Journal of Neuroscience following major revisions. We hope that you will be able to address the reviewers' concerns in full and resubmit the manuscript, along with a point-by-point reply to the reviews that indicates your response to each concern. Before we make a decision about publication, we will have your revision reviewed by the reviewers and/or our editorial team.

Thank you and Reviewer 2 for participating in open peer review! Since Reviewer 1 did not wish to participate, we will also need a version of the rebuttal with the replies to Reviewer 1's critique redacted. This document will then enter the published open peer review record of your paper. Please send the redacted rebuttal in addition to the rebuttal letter containing replies to both reviewers' critiques.

Your revision must include the manuscript with new text highlighted, as well as a clean copy of the manuscript. Please carefully review your paper at this time for any corrections in style or substance. Please consult our Revised Submission Checklist for details on preparing your revision: [https://www.jneurosci.org/sites/default/files/files/JN\\_Revised\\_Submissions\\_Checklist.pdf](https://www.jneurosci.org/sites/default/files/files/JN_Revised_Submissions_Checklist.pdf).

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When uploading the revised manuscript, the corresponding author will need to complete the electronic License to Publish form; if not completed during submission, a link to the form will also be available on the author's home page at <https://jneurosci.msubmit.net>.

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Thank you for giving us the opportunity to consider your paper for publication in The Journal of Neuroscience. Please let us know if you have any questions or concerns.

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Yours sincerely,

Artur Kania  
Senior Editor

Erik Roberson  
Reviewing Editor

Sabine Kastner  
Editor-in-Chief  
The Journal of Neuroscience

Please consider that the Significance Statement is geared towards the general readership of the journal and as such, it could benefit from a definition of the acronyms used.

Reviewer #1 has declined to share their comments.

Reviewer #2 (Rationale for Significance Rating for Authors):

There are many studies indicating that synapses are the initial sites targeted by beta amyloid. It is thus crucial to understand how exactly beta amyloid negatively impacts synapses. This study provides important mechanistic information regarding the role of AMPA receptors in the damaging effects of beta amyloid on synapses. Most importantly, the data provided in this study indicate that GluA3 expression in neurons lacking GluA3 is sufficient to re-sensitize neurons to the damaging effects of A $\beta$  on the functional and structural properties of synapses. This is a rather remarkable finding. This finding speaks to two types of experiments that can be criticized: knock-out technology has developmental abnormalities; viruses can overexpress a protein leading to artifacts. This study can rescue a deficiency observed in tissue from knock-out animals by acute viral expression. This is effectively the type of 'wash' experiment that is critically important in pharmacological studies. These experiments definitively (as much as definitive can be in science) establish GluA3 as a necessary and sufficient protein for the synaptic effects of beta amyloid.

Reviewer #2 :

There are many studies indicating that synapses are the initial sites targeted by beta amyloid. It is thus crucial to understand how exactly beta amyloid negatively impacts synapses. This study provides important mechanistic information regarding the role of AMPA receptors in the damaging effects of beta amyloid on synapses. Most importantly, the data provided in this study indicate that GluA3 expression in neurons lacking GluA3 is sufficient to re-sensitize neurons to the damaging effects of A $\beta$  on the functional and structural properties of synapses. This is a rather remarkable finding. This finding speaks to two types of experiments that can be criticized: knock-out technology has developmental abnormalities; viruses can overexpress a protein leading to artifacts. This study can rescue a deficiency observed in tissue from knock-out animals by acute viral expression. This is effectively the type of 'wash' experiment that is critically important in pharmacological studies. These experiments definitively (as much as definitive can be in science) establish GluA3 as a necessary and sufficient protein for the synaptic effects of beta amyloid.

The study uses mutant receptor types to show that PDZ interactions by GluA3 are necessary for GluA3 to mediate the effects of beta amyloid. Given the multitude of previous studies of PDZ interactions at these AMPAR subtypes, an important model is proposed.

The use of subcellular immunolabeling provides important information regarding the distribution of GluA3 receptors under the influence of beta amyloid.

Use of proteomic analyses in wt and APP/PS1 animals provides important independent information regarding GluA3.

In conclusion, this study establishes a critical role for GluA3 in the mechanism by which beta amyloid negatively impacts synapses.

Specific comments below:

1. The use of GluA3 KO should probably be stated in the abstract
2. Fig 1A: why is \* (significance?) shown for this? Is it really significant? Fig legend says 'little difference' which looks about right. It would be important to state that expression of GluA3 in GluA3 KO rescues sensitivity of neurons to structural effects of A $\beta$ .
3. Recovery rate (Fig 1C right) seems relatively unimportant; can be put in supplementary figures
4. eEPSCs: state 'evoked EPSC' first time use.
5. Why are there multiple instances of 'uninfected' in bar graphs? Is it because each manipulation had its own control recordings? If so, pls state somewhere.
6. w/r to K887 mutant: 'did not change the fraction of immobile GluA3K887A (Fig. 2B)' replace with 'did not reduce the fraction of immobile GluA3K887A (Fig. 2B)'. This makes clear the important observation.
7. Should a reference be provided when this mutant is first mentioned (GluA3S885A, Fig. S4)? where was this mutant first described?
8. Are differences in eEPSC significant in Fig 3E? if so, indicate.
9. Replace:  
'These data suggest that A $\beta$  can only trigger synaptic depression when PDZ-protein interactions, likely with GRIP and PICK1, at GluA3-containing AMPARs are intact.'  
'These data suggest that A $\beta$  can only trigger synaptic depression when synapses contain GluA3 with competent PDZ-protein interactions.'
10. I don't follow the experiments with Rab7 labeling. What is the logic of looking at Rab7 labeling w/wo leupeptin? Is it that receptors are prevented from being degraded and thus display where they are being directed just prior to being degraded? Pls explain logic better.

#### Discussion

11. 'which explains our observation that GluA3 needs to bind GRIP to be stably expressed at dendrites and spines'; replace with 'which explains our observation that GluA3 885A, a mutant GluA3 that fails to bind GRIP, has low detection at dendrites and spines'.
12. Reference 57 appears to have extraneous text.

We thank the reviewers for their constructive and valuable comments on our manuscript. We addressed all these concerns in these revisions, which in our view have significantly improved the manuscript.

Below we present a point-by-point rebuttal (in blue) to the reviewer comments (in black).

Reviewer #1 has declined to share their comments.

Reviewer #2 (Rationale for Significance Rating for Authors):

There are many studies indicating that synapses are the initial sites targeted by beta amyloid. It is thus crucial to understand how exactly beta amyloid negatively impacts synapses. This study provides important mechanistic information regarding the role of AMPA receptors in the damaging effects of beta amyloid on synapses. Most importantly, the data provided in this study indicate that GluA3 expression in neurons lacking GluA3 is sufficient to re-sensitize neurons to the damaging effects of A $\beta$  on the functional and structural properties of synapses. This is a rather remarkable finding. This finding speaks to two types of experiments that can be criticized: knock-out technology has developmental abnormalities; viruses can overexpress a protein leading to artifacts. This study can rescue a deficiency observed in tissue from knock-out animals by acute viral expression. This is effectively the type of 'wash' experiment that is critically important in pharmacological studies. These experiments definitively (as much as definitive can be in science) establish GluA3 as a necessary and sufficient protein for the synaptic effects of beta amyloid.

The study uses mutant receptor types to show that PDZ interactions by GluA3 are necessary for GluA3 to mediate the effects of beta amyloid. Given the multitude of previous studies of PDZ interactions at these AMPAR subtypes, an important model is proposed.

The use of subcellular immunolabeling provides important information regarding the distribution of GluA3 receptors under the influence of beta amyloid.

Use of proteomic analyses in wt and APP/PS1 animals provides important independent information regarding GluA3.

In conclusion, this study establishes a critical role for GluA3 in the mechanism by which beta amyloid negatively impacts synapses.

Specific comments below:

1. The use of GluA3 KO should probably be stated in the abstract

We agree. In the revised manuscript we added the sentence to the abstract: *“Here, we used electrophysiology and AMPA-receptor imaging to demonstrate that GluA3 expression in neurons lacking GluA3 is sufficient to re-sensitize their synapses to the damaging effects of A $\beta$ , indicating that GluA3-containing AMPARs at synapses are necessary and sufficient for A $\beta$  to induce synaptotoxicity”*

2. Fig 1A: why is \* (significance?) shown for this? Is it really significant? Fig legend says 'little difference' which looks about right. It would be important to state that expression of GluA3 in GluA3 KO rescues sensitivity of neurons to structural effects of Abeta.

We apologize for this mistake. There is indeed no significant difference in Fig. 1A. The stray \* is omitted from Figure 1A in the revised manuscript.

3. Recovery rate (Fig 1C right) seems relatively unimportant; can be put in supplementary figures

We agree, and we have replaced the figures that display recovery rates after FRAP to Figure 1-3 of the revised manuscript.

4. eEPSCs: state 'evoked EPSC' first time use.

We have indicated evoked excitatory postsynaptic currents (eEPSCs) at page 10, line 253 of the revised manuscript.

5. Why are there multiple instances of 'uninfected' in bar graphs? Is it because each manipulation had its own control recordings? If so, pls state somewhere.

We made comparisons to nearby uninfected neurons from the same organotypic slice, which we stated in the revised manuscript on page 10:

*“...GFP-GluA3 expression did not change synaptic transmission compared with nearby uninfected neurons as measured by...”*

6. w/r to K887 mutant: 'did not change the fraction of immobile GluA3K887A (Fig. 2B)' replace with 'did not reduce the fraction of immobile GluA3K887A (Fig. 2B)'. This makes clear the important observation.

Thank you, we changed this accordingly in revised manuscript page 11.

7. Should a reference be provided when this mutant is first mentioned (GluA3S885A, Fig. S4)? where was this mutant first described?

This mutant of GluA3 has not been used before. However, the same mutation in GluA2 (GluA2-S880A) was first described by the Ziff and Huganir labs (Osten et al, Neuron 2000; Chung et al, JN 2000). The use of this mutation in GluA2 in these papers is acknowledged in the revised manuscript at page 11.

8. Are differences in eEPSC significant in Fig 3E? if so, indicate.

Statistical analysis (ANOVA) indicated that difference in eEPSCs in figure 4E did not reach statistical significance.

9. Replace: 'These data suggest that A $\beta$  can only trigger synaptic depression when PDZ-protein interactions, likely with GRIP and PICK1, at GluA3-containing AMPARs are intact.' 'These data suggest that A $\beta$  can only trigger synaptic depression when synapses contain GluA3 with competent PDZ-protein interactions.'

Thank you for this suggestion. We changed this sentence accordingly at page 11 of the revised manuscript.

10. I don't follow the experiments with Rab7 labeling. What is the logic of looking at Rab7 labeling w/wo leupeptin? Is it that receptors are prevented from being degraded and thus display where they are being directed just prior to being degraded? Pls explain logic better.

We agree this needs better explanation. It is correct that "*we inhibited lysosomal protease activity with leupeptin to make lysosomal ALFA-GluA3 accumulate instead of being degraded.*" This is now stated as such in the result section at page 13. The additional purpose of mapping Rab7 puncta is that changes in endo-lysosomal organelle size is reported in AD and AD models (Nixon, et. al. 2017, Lauritzen, et. al., 2016). Rab7 staining allowed us to detect changes in the size and number of endo-lysosomes and rule out that our observed increase in GluA3 degradation was due to an overall increase in endo-lysosomal organelles, rather than GluA3 being re-directed towards lysosomes. This is now better addressed in the results on page 13 and in the discussion on page 16.

Discussion

11. 'which explains our observation that GluA3 needs to bind GRIP to be stably expressed at dendrites and spines'; replace with 'which explains our observation that GluA3 885A, a mutant GluA3 that fails to bind GRIP, has low detection at dendrites and spines'.

Thank you for this suggestion, we changed this sentence accordingly at page 15 of the revised manuscript.

12. Reference 57 appears to have extraneous text.

Thank you, we removed this extraneous text.

27th Jun 2024

Dear Dr. Kessels:

Thank you for submitting your manuscript to The Journal of Neuroscience.

We have received the reviews of your paper, "Amyloid- $\beta$ -driven synaptic depression is mediated by synaptic removal of GluA3-containing AMPA-receptors." (JN-RM-0393-24R1), which are appended to this email. Based on the reviewers' comments and our editorial assessment, we would like to reconsider your manuscript at The Journal of Neuroscience following major revisions. We hope that you will be able to address the reviewers' concerns in full and resubmit the manuscript, along with a point-by-point reply to the reviews that indicates your response to each concern. Before we make a decision about publication, we will have your revision reviewed by the reviewers and/or our editorial team.

Your revision must include the manuscript with new text highlighted, as well as a clean copy of the manuscript. Please carefully review your paper at this time for any corrections in style or substance. Please consult our Revised Submission Checklist for details on preparing your revision: [https://www.jneurosci.org/sites/default/files/files/JN\\_Revised\\_Submissions\\_Checklist.pdf](https://www.jneurosci.org/sites/default/files/files/JN_Revised_Submissions_Checklist.pdf).

Your revision must conform to the JNeurosci policies for reporting experimental design and statistical analyses and include the full results of statistical tests in the Results section (see Information for Authors: <https://www.jneurosci.org/content/information-authors#materials>).

Your submission must also include publication-quality figures, each in a separate EPS or TIFF (300 dpi) file. Please make sure your figures adhere to style requirements to avoid delays in manuscript processing. Detailed guidelines for figures are available here: <https://www.jneurosci.org/content/information-authors#figures>

When uploading the revised manuscript, the corresponding author will need to complete the electronic License to Publish form; if not completed during submission, a link to the form will also be available on the author's home page at <https://jneurosci.msubmit.net>.

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Yours sincerely,

Artur Kania  
Senior Editor

Erik Roberson  
Reviewing Editor

Sabine Kastner  
Editor-in-Chief  
The Journal of Neuroscience

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Manuscript Instructions

The significance statement is meant for the general readership of the journal. Please include a functional definition of the acronyms used. Furthermore, your extended data should be incorporated into the main figures.

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Reviewer #1 (Rationale for Significance Rating for Authors):

Some of the experimental results are very interesting and may uncover some novel mechanisms underlying synaptic dysfunction associated pathophysiological changes in Alzheimer's disease. However, the main conclusion is still not supported by direct experimental evidence. Without, the scientific foundation of the present paper is very weak (see Critique 1 and Critique 2).

Reviewer #1 :

In the revised manuscript, the authors have addressed some but not critical concerns raised by prior reviewers. Some major weaknesses persist:

1. Some explanations have been added to argue that the APPCT100 effects are likely caused by Abeta oligomers. These are not very strong arguments. The authors have not experimentally ruled out the indirect effects of autophagy. Therefore, more direct evidence in support of the "title" and main conclusion is still needed (see Item 2 below). Without it, the manuscript is not rigorous.
2. New data in Fig 5A only showed that Abeta oligomers could cause synaptic depression but did not directly demonstrate that the depression was caused by GluA3 internalization. It remains to be determined whether the Abeta-induced changes in mEPSCs will be blocked in neurons expressing GFP-tagged GluA3 variants that block the internalization of this subunit. Also, to be rigorous, results from control neurons expressing GFP alone and WT-GluA3 are needed. Without this critical set of experiments, the scientific foundation of the paper is very weak.
3. What is the K-S test result of the Fig 5A? The cumulative curve of the EPSC amplitude is clearly shifted to the left, suggesting that the mEPSC amplitude was decreased. Visually, this is a very big shift and the shifting is likely to be significant statistically. Please report the K-S test result. If the KS test is not significant, please explain the discrepancy because previous studies have shown that oligomerized Abeta decreased the amplitude of mEPSCs.
4. Many K-S test results of cumulative curves are missing. Please include them.
5. Responding to prior Comment 5, the authors stated that both GluA1 and GluA3 are internalized by Abeta-induced cellular changes. If this is true, it will be very hard to explain why mEPSC amplitude is not decreased by exposure to Abeta oligomers. In the classical quantal model by Katz, quantal size is determined by the amount of postsynaptic receptors. Please provide an alternative hypothesis to the classical model.
6. The cumulative curves of inter-event time intervals of all mEPSCs are still missing. Inclusion of these curves is the norm in the field, which is important comparative data for ruling out the thresholding errors. These are necessary controls for the analyses of mEPSC frequency.

Reviewer #1 (Stat Analysis Explanation):

All cumulative curves of inter-event time intervals are missing. Many K-S test results are also missing. The inclusion of these statistical analyses is the norm in the field. This statistical issue has been raised in prior critiques but has not been sufficiently addressed.

Reviewer #2 (Rationale for Significance Rating for Authors):

Identifies a critical part of the mechanism underlying the pathogenesis of AD.

Reviewer #2 :

Authors have satisfactorily addressed the issues raised by reviews.

Reviewer #1 (Rationale for Significance Rating for Authors):

Some of the experimental results are very interesting and may uncover some novel mechanisms underlying synaptic dysfunction associated pathophysiological changes in Alzheimer's disease. However, the main conclusion is still not supported by direct experimental evidence. Without, the scientific foundation of the present paper is very weak (see Critique 1 and Critique 2).

Reviewer #1 :

In the revised manuscript, the authors have addressed some but not critical concerns raised by prior reviewers. Some major weaknesses persist:

1. Some explanations have been added to argue that the APP<sub>CT100</sub> effects are likely caused by Abeta oligomers. These are not very strong arguments. The authors have not experimentally ruled out the indirect effects of autophagy. Therefore, more direct evidence in support of the "title" and main conclusion is still needed (see Item 2 below). Without it, the manuscript is not rigorous.

We apologize for not having mentioned all evidence in our previous rebuttal that APP<sub>CT100</sub> effects on synapses are caused by amyloid-beta, most likely oligomeric. We would like to take this opportunity to more elaborately explain different lines of evidence that demonstrate that synaptic deficits caused by the expression of APP or APP<sub>CT100</sub> depend on the production of amyloid-beta and is therefore not an effect of autophagy independent of Abeta production:

1. Synaptic deficits are absent in control experiments using the overexpression of the alpha-secretase product of APP (APP<sub>CT84</sub>), or incubation of APP/APP<sub>CT100</sub>-expressing cells with a gamma-secretase inhibitor, or incubation of APP<sub>CT100</sub>-expressing cells with a drug that inhibits amyloid-beta aggregation (scyllo-inositol) (Kessels et al, PNAS 2013; Kamenetz et al, Neuron 2003). These findings indicate that expression of APP/APP<sub>CT100</sub> causes synaptic deficits because of the production of amyloid-beta and no other effects of APP/APP<sub>CT100</sub> expression.
2. Cells that express APP in our model system cause spine loss to nearby dendrites of neurons that do not express APP (Wei et al, Nat Neurosc. 2010). Similarly, when expressing APP in the majority of neurons in organotypic slices, rather than in a few, simultaneous recordings from infected and non-infected neurons showed comparable synaptic responses (Kamenetz et al, Neuron 2003). Presumably amyloid-beta was produced by all the infected cells that affected synaptic transmission onto non-infected cells. These results demonstrate that neurons do not need to express APP or APP<sub>CT100</sub> themselves to have amyloid-beta driven synaptic deficits. These experiments underscore the absence of a significant contribution of autophagy to synapse loss in APP or APP<sub>CT100</sub> expressing neurons.

3. There are many examples that APP/APP<sub>CT100</sub> expression and exposure to Abeta oligomers have the same effects on synapses. Several studies have reported that the effects of Abeta-oligomers on synapses were mechanistically similar to those of APP<sub>CT100</sub> expression (e.g. Kessels et al, Nature 2010; Kessels et al, PNAS 2013 versus Tamburri et al, Plos One 2013; Alfonso et al. Eur J Neurosc. 2014). Notably, we have performed a new experiment in cultured neurons exposed to Abeta oligomers that resulted in similar results as observed upon APP<sub>CT100</sub> expression in organotypic slice (see point 2 in this rebuttal).

Combined, these arguments make, in our view, a compelling case that synaptic deficits as a consequence of APP<sub>CT100</sub> expression are caused by the production of amyloid-beta, most likely as a consequence of the formation of Abeta-oligomers. In addition, we have provided more direct experimental evidence as suggested, see item 2 below.

We agree with the reviewer that the term 'synaptic depression' may be interpreted as selectively referring to measurements of synaptic currents, as was first mentioned in the initial review. We have therefore changed the title of our manuscript into: *Amyloid-β-driven synaptic deficits are mediated by synaptic removal of GluA3-containing AMPA-receptors.*

2. New data in Fig 5A only showed that Abeta oligomers could cause synaptic depression but did not directly demonstrate that the depression was caused by GluA3 internalization. It remains to be determined whether the Abeta-induced changes in mEPSCs will be blocked in neurons expressing GFP-tagged GluA3 variants that block the internalization of this subunit. Also, to be rigorous, results from control neurons expressing GFP alone and WT-GluA3 are needed. Without this critical set of experiments, the scientific foundation of the paper is very weak.

Thank you for this suggestion. We investigated the effects of Abeta-oligomer exposure in cultures of dissociated neurons expressing GFP, GFP-GluA3 or GFP-GluA3<sub>K887A</sub>. Rather than measuring mEPSCs through electrophysiology, we quantified Abeta-mediated reduction in synapse density, which we believe equally fits the amended title.

With this experiment we confirmed results obtained in organotypic slices with neurons expressing APP<sub>CT100</sub>. Abeta-oligomers induced a loss of synapses in neurons that were transfected with GFP-GluA3, but not in neurons transfected with GFP-tagged GluA3<sub>K887A</sub> (i.e. the variant with the mutation that blocks the internalization of GluA3). The results of this new experiment are incorporated in fig 5 of the revised manuscript.

3. What is the K-S test result of the Fig 5A? The cumulative curve of the EPSC amplitude is clearly shifted to the left, suggesting that the mEPSC amplitude was decreased. Visually, this is a very big shift and the shifting is likely to be significant statistically. Please report the K-S test result. If the KS test is not significant, please explain the

discrepancy because previous studies have shown that oligomerized Abeta decreased the amplitude of mEPSCs.

Thank you for pointing out this error. The K-S test in Figure 5A was indeed significant. In the current version the p-values of all K-S tests have been provided.

4. Many K-S test results of cumulative curves are missing. Please include them.

The p-values of all K-S tests have been provided in the revised manuscript.

5. Responding to prior Comment 5, the authors stated that both GluA1 and GluA3 are internalized by Abeta-induced cellular changes. If this is true, it will be very hard to explain why mEPSC amplitude is not decreased by exposure to Abeta oligomers. In the classical quantal model by Katz, quantal size is determined by the amount of postsynaptic receptors. Please provide an alternative hypothesis to the classical model.

This is indeed a relevant and intriguing point raised by the reviewer.

We have previously predicted based on quantal analysis of evoked EPSC recordings that APP<sub>CT100</sub> expression leads to synaptic depression as a consequence of a loss on synapses and a small decrease in quantal size in the remaining synapses (Lumeij et al, *Frontiers Cell Neurosc.* 2023).

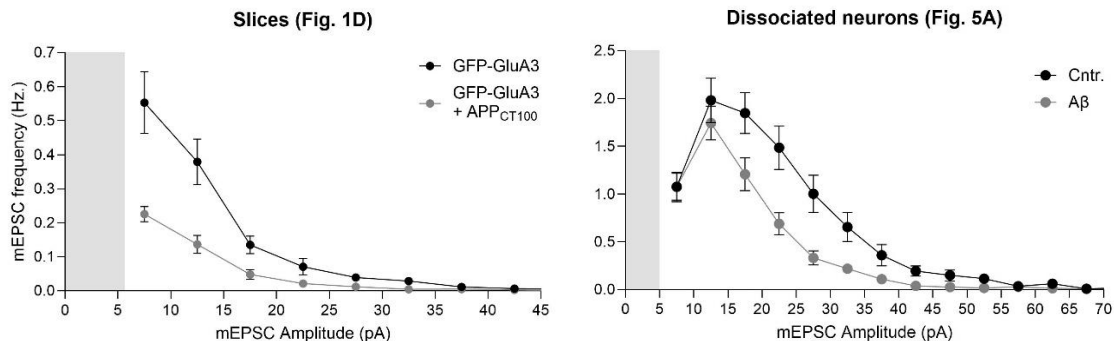
Then why is this not reflected in miniature EPSC recordings as both a decrease frequency and also a decrease in average amplitude? This seems a discrepancy but is caused by the fact that miniature events that fall below the detection limit of 5 pA are not detected in mEPSC recordings. This creates a misrepresentation of mini event distribution.

When all synapses are weakened, those that drop under the 5pA detection limit will not be detected anymore. This will exclude a subset of *small* mEPSCs from the analysis, effectively lowering the mean frequency, while increasing the mean amplitude. However, the weakening of all synapses still lowers the amplitude of the remaining mEPSCs that are still detected, which can negate the increase in mean amplitude, leading to no total change in mean amplitude. This is demonstrated in

<https://www.biorxiv.org/content/10.1101/2024.10.26.620084v1.full>.

To demonstrate that this applies to our own data as well, we plotted the mEPSC frequency per amplitude of our experiment in slices (fig. 1D) and dissociated neurons (fig. 5A) below. In recordings of organotypic slices, a large portion of mEPSCs have an amplitude close to the detection limit. APP<sub>CT100</sub>-expression weakens synapses, shifts the curve to the left to induce the effect described above. Hence, only mEPSC frequency is decreased. In recordings from cultured dissociated neurons, all mEPSC amplitudes are larger and a smaller portion of mEPSCs falls below the detection limit. According to the effect described above and in [the paper](#), this should make the weakening of synapses

more visible in the mean mEPSC amplitude. Indeed, we found a non-significant decrease in mean amplitude in cultured neurons ( $p=0.075$ ), while those in sliced remains unchanged ( $p=0.659$ ). We hope to have clarified this limitation which is inherent to the use of a 5pA threshold for mEPSC detection.



6. The cumulative curves of inter-event time intervals of all mEPSCs are still missing. Inclusion of these curves is the norm in the field, which is important comparative data for ruling out the thresholding errors. These are necessary controls for the analyses of mEPSC frequency.

Graphs for the cumulative distributions of inter-event intervals have been included in the figures S1-4, 2-2 and 3-2 of the revised manuscript.

### Special note:

We have made an extra amendment to our revised manuscript. We have removed 2 references from Prof. Eliezer Masliah due to the suspicion of him having manipulated more than 130 scientific publications. No further changes were made in relation to this.

The following 2 papers have been removed:

*Thorns, V., M. Mallory, L. Hansen and E. Masliah (1997). "Alterations in glutamate receptor 2/3 subunits and amyloid precursor protein expression during the course of Alzheimer's disease and Lewy body variant." Acta Neuropathologica 94: 539-548.*

*de Wilde, M. C., C. R. Overk, J. W. Sijben and E. Masliah (2016). "Meta-analysis of synaptic pathology in Alzheimer's disease reveals selective molecular vesicular machinery vulnerability." DADM 12: 633-644*

10th Dec 2024

Dear Dr. Kessels:

Thank you for submitting your manuscript to The Journal of Neuroscience.

We have received the reviews of your paper, "Amyloid- $\beta$ -driven synaptic deficits are mediated by synaptic removal of GluA3-containing AMPA-receptors." (JN-RM-0393-24R2), which are appended to this email. Based on the reviewers' comments and our editorial assessment, we would like to reconsider your manuscript at The Journal of Neuroscience following minor revisions. We hope that you will be able to address the reviewers' concerns in full and resubmit the manuscript, along with a point-by-point reply to the reviews that indicates your response to each concern. Before we make a decision about publication, we will have your revision reviewed by our editorial team.

Your revision must include the manuscript with new text highlighted, as well as a clean copy of the manuscript. Please carefully review your paper at this time for any corrections in style or substance. Please consult our Revised Submission Checklist for details on preparing your revision: [https://www.jneurosci.org/sites/default/files/files/JN\\_Revised\\_Submissions\\_Checklist.pdf](https://www.jneurosci.org/sites/default/files/files/JN_Revised_Submissions_Checklist.pdf).

Your revision must conform to the JNeurosci policies for reporting experimental design and statistical analyses and include the full results of statistical tests in the Results section (see Information for Authors: <https://www.jneurosci.org/content/information-authors#materials>).

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Please spell out the acronyms in the significance statement.

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Reviewer #1 has declined to share their comments.

## Manuscript Instructions

Please spell out the acronyms in the significance statement.

We spelled out  *$\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors* in the significance statement. After this change the word limit was exceeded which was corrected by changing the last sentence of the significance statement.

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Reviewer #1 has declined to share their comments.

