Synaptobrevin-2 dependent regulation of single synaptic vesicle endocytosis

Natali Chanaday and Ege Kavalali

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Review Timeline:

Submission Date:	2021-04-27
Editorial Decision:	2021-05-21
Revision Received:	2021-06-09
Accepted:	2021-06-21

Editor-in-Chief: Matthew Welch

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

RE: Manuscript #E21-04-0213
TITLE: Synaptobrevin-2 dependent regulation of single synaptic vesicle endocytosis

Dear Dr. Chanaday:

Thank you for submitting this interesting paper to MBoC. In your resubmission, please address all the reviewers' comments point by point. In particular, estimating the number of active synapses in the synaptobrevin-2 mutants should be feasible and important for comparison between bulk and single-vesicle measurements for the field. The alternative explanations proposed by the reviewers (eg reversibility of trans-SNARE complexes, calcium dependence of dwell times) as possible explanations for the observed phenotypes should be discussed.

Sincerely,

Avital Rodal
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Chanaday,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you haveopted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us at mboc@ascb.org.

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your

revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

In this Brief Report Chanaday and Kavalali expand on a previous report (elife 2018) where they reported on three different modes of synaptic vesicle endocytosis based on optical single vesicle fusion measurements. The previous work also indicated that loss of the synchronous Ca-sensor Syt1 leads to a relatively specific decrease of the slowest of the thee retrieval modes, while the fastest remained unaffected. In the present study, the same authors now provide complementary data for the main vesicle SNARE syb2/VAMP2. Surprisingly, syb2 mutants exhibit very similar phenotypes to syt1 - a reduction of the slow SV retrieval kinetics, but no change to the ultrafast. This is an interesting and important addition to the previous work that bolsters arguments by the Kavalali lab and others about the nature and Ca-dependency of different SV retrieval mechanisms. I consider this an excellent candidate for a MBoC Brief Report.

Strengths of the paper: It is very short and to the point. The important data are the dwell time measurements in Fig.3. The authors are world champions in the optical measurement of single CV exo- and endocytosis using the vGlut-phluorin reporter. The dwell time measurements allow to distinguish between fusion and retrieval and reacidification. and loss of syb2 very clearly only affects the slow pathway. The Ca-dependency of the slowdown is abolished. The similarity to syt1 is exciting.

Minor weaknesses of the paper: The dosage-dependence revealed in the heterozygotes is not prevalent across measurements - maybe a bit more careful discussion of what effects are dosage-dependent and which ones are less so would be helpful. It is in the nature of a brief report that there are no extensive discussion of caveats, but one issue might warrant a little more discussion: could loss of syb2 throughout development and function lead to slight developmental or compensatory effects that indirectly affect the SV retrieval properties?

All in all, I am happy to sign off on this manuscript without the need for a major revision.

Reviewer #2 (Remarks to the Author):

Summary: Accumulating evidence suggests that a major SNARE protein, VAMP2, plays a role in endocytosis and retrieval of synaptic vesicles. Past work in VAMP2 knockout synapses has collected data by visualizing the kinetics of multiple vesicles at once, but it remains unclear how VAMP2 works at the level of single synaptic vesicles. Here, Chanaday and Kavalali examine if VAMP2 modulates the endocytosis of single synaptic vesicles using pHluorin-based optical methods they previously established in a 2018 eLife paper. Using these methods, the authors demonstrate that:

1. VAMP2 null synapses have reduced exocytosis and slower endocytosis kinetics given high-frequency stimulation (40 Hz, 5 s).
2. VAMP2 null synapses show increased pHluorin amplitude readouts and increased multi-quantal fusion events following single action potentials-it is unclear if this result is due to fusion of larger vesicles, or increased incidence of multi-vesicular release.
3. Ultrafast retrieval is not impacted in VAMP2 null synapses given low-frequency stimulation.
4. VAMP2 null synapses are not affected by increases in extracellular calcium, suggesting that calcium sensitivity in single vesicle endocytosis is likely dependent on the presence of VAMP2.

I recommend this paper address several considerations:

Major:
-Compensation by Synaptobrevin 1. Imig et al. (2014) [PMID: 25374362] suggested that the absence of VAMP2 is partly compensated by Synaptobrevin 1 and that only a subset of synapses have a normal number of docked vesicles, and others do not, indicating some of these synapses may not be active. It would be interesting to know what fraction of the putative synapses actually responded to these stimuli.

-Number of v-SNAREs and reversibility of the trans-SNARE complex. Bao et al. (PMCID: PMC5808578) demonstrated that formation of the trans-SNARE complex is reversible in vitro and the number of the v-SNAREs determines this reversibility. Is it possible that the 'ultrafast retrieval events' seen here represent such reversible reactions (kiss-and-run?), but not ultrafast endocytosis? - this would be consistent with Dynamin-independence reported on BioRxiv by the author's group (https://www.biorxiv.org/content/10.1101/2020.06.12.147975v1.full).

-N. Please include the number of mouse litters used in the study (not just the number of animals).

-Dots on graphs. Please include individual data points in bar graphs.

- Gene dose dependence. Though it is clear in Figure 1B that there is a significant VAMP2 gene dependence for 40 Hz pHluorin amplitude, this reviewer hesitates to call Figure 1C another clear gene dose dependence, given the non-significant difference between WT and Het. If not statistically significant, isn't the observed effect more of a trend than a resolute dependence?

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times and retrieval of single vesicles in the assays, one cannot claim that this is due to VAMP2 alone. This can be a calmodulin-VAMP2 based interaction [PMCID: PMC16927] or through some other calcium-sensitive proteins. The way the authors situate this VAMP2 calcium dependence is like that of Syt1, which suggests direct calcium-protein interactions.

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Synaptobrevin-2 dependent regulation of single synaptic vesicle endocytosis

Natali L. Chanaday and Ege T. Kavalali

Responses to the Reviewers.

We want to thank the Reviewers for taking the time to evaluate our manuscript. The comments and suggestions were very constructive and insightful, and we believe that after careful addition of all the revisions requested our manuscript is now ready for publication.

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All in all, I am happy to sign off on this manuscript without the need for a major revision.

We are very thankful for the Reviewer's appreciation of our work. We have addressed the weaknesses mentioned by the Reviewer in our revised manuscript. Specifically, we wrote more clearly in the text what parameters showed gene-dose-dependency (retrieval) and which ones did not (fusion). We also discussed this point at the end of the manuscript. Regarding the putative compensation by other proteins, we added a brief discussion of possible SNAREs that may be mediating fusion and thus, endocytosis in the absence of syb2, namely VAMP4 and syb1/cellubrevin.

Reviewer #2 (Remarks to the Author):

Summary: Accumulating evidence suggests that a major SNARE protein, VAMP2, plays a role in endocytosis and retrieval of synaptic vesicles. Past work in VAMP2 knockout synapses has collected data by visualizing the kinetics of multiple vesicles at once, but it remains unclear how VAMP2 works at the level of single synaptic vesicles. Here, Chanaday and Kavalali examine if VAMP2 modulates the endocytosis of single synaptic vesicles using pHluorin-based optical methods they previously established in a 2018 eLife paper. Using these methods, the authors demonstrate that:

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3. Ultrafast retrieval is not impacted in VAMP2 null synapses given low-frequency stimulation.

4. VAMP2 null synapses are not affected by increases in extracellular calcium, suggesting that calcium sensitivity in single vesicle endocytosis is likely dependent on the presence of VAMP2.

We thank the Reviewer for the insightful comments and careful revision of our manuscript.

I recommend this paper address several considerations:

Major:

-Compensation by Synaptobrevin 1. Imig et al. (2014) [PMID: 25374362] suggested that the absence of VAMP2 is partly compensated by Synaptobrevin 1 and that only a subset of synapses have a normal number of docked vesicles, and others do not, indicating some of these synapses may not be active. It would be interesting to know what fraction of the putative synapses actually responded to these stimuli.

This is an interesting and important point. Zimmermann et al., 2014 used autaptic cultures and, as the Reviewer pointed out, found that syb1 can compensate for lack of syb2 in a subset of synapses. However, in our laboratory, using dissociated hippocampal cultures we were not able to detect syb1 or cellubrevin. We do not know if this is due to differences between autaptic and high density cultures, or if syb1 levels are below the limit of detection. Another vSNARE protein that is more abundant in these hippocampal neurons is VAMP4. We have previously shown that VAMP4 can partially rescue syb2 KO, so it is possible that VAMP4 is mediating fusion and driving endocytosis in the absence of syb2. These aspects are now discussed in the revised version of the manuscript.

-Number of v-SNAREs and reversibility of the trans-SNARE complex. Bao et al. (PMCID: PMC5808578) demonstrated that formation of the trans-SNARE complex is reversible in vitro and the number of the v-SNAREs determines this reversibility. Is it possible that the 'ultrafast retrieval events' seen here represent such reversible reactions (kiss-and-run?), but not ultrafast endocytosis? - this would be consistent with Dynamin-independence reported on BioRxiv by the author's group (https://www.biorxiv.org/content/10.1101/2020.06.12.147975v1.full).

This is indeed a very exciting interpretation of our results. Certainly the reversible closure of the fusion pore could be a mechanism explaining the ultrafast endocytic events detected via optical methods.

However, due to the limited resolution of these recordings, we cannot ascertain that this is the case. We have added this possibility and discussed how syb2 copy number in the vesicles can regulate fusion pore kinetics in the revised version of the manuscript.

-N. Please include the number of mouse litters used in the study (not just the number of animals).

We added this information in the Figure legends, together with the previous statistical data.

-Dots on graphs. Please include individual data points in bar graphs.

Bar graphs in Figure 1 were replaced by dot graph showing all individual data points (bar graphs are shown as insets to help visualize the results). For Figure 2 and 3, we show histograms which are constructed using all the data points, so these already represent the distribution of the whole population. Moreover, we analyzed hundreds of synapses, so showing dots on top of the bars is visually confusing (as you can appreciate in the new Figure 1). For this reason, histograms are better representations for Figures 2 and 3.

- Gene dose dependence. Though it is clear in Figure 1B that there is a significant VAMP2 gene dependence for 40 Hz pHluorin amplitude, this reviewer hesitates to call Figure 1C another clear gene dose dependence, given the non-significant difference between WT and Het. If not statistically significant, isn't the observed effect more of a trend than a resolute dependence?

Yes, the Reviewer is correct. Fusion is not significantly affected by 50% gene dose (heterozygous). We have corrected the text to clarify this issue.

-Percent or fold changes. The last half of the paper has great support for hypotheses by providing percent increases/decreases in the text, please do the same for the first half of the paper. Specifically, when discussing the reduction in mobilized vGLUT1-pHluorin for Figure 1B and reduced quantal events/increased multi-quantal fusions in Figure 2C-D.

We have added the percent changes in the first half of the manuscript. Regarding Figure 2, the number of Gaussians that best fit the data changes from group to group (from 1 to 2 or 3), so the percentages have no useful meaning in this case, the relevant value is the number of Gaussians (indicating the presence of multi-vesicle release or bigger vesicles, as we discuss).

-Calcium dependence. While clear that extracellular calcium concentrations are influencing the dwell times and retrieval of single vesicles in the assays, one cannot claim that this is due to VAMP2 alone. This can be a calmodulin-VAMP2 based interaction [PMCID: PMC16927] or through some other calcium-sensitive proteins. The way the authors situate this VAMP2 calcium dependence is like that of Syt1, which suggests direct calcium-protein interactions.

We apologize for the confusion. Our speculation is that syb2 and syt1 work together to modulate endocytosis, thus the calcium sensitivity is provided by syt1. We rephrased this part of the manuscript to make it more clear. We have also added the syb2-calmodulin interaction as another putative explanation.

-Figure 1E: why display decay rate this way as opposed to the typical pHluorin decay downward sloping plots which can also provide a Tau measurement? It is confusing to have negative time units, so consider reformatting this panel or provide a justification.

Fluorescence decay in syb2 KO is very slow to the limit that we cannot confidently fit it with an exponential in many cases. For this reason, we decided to calculate the rate of decay during the first 10 seconds post-stimulation. This is now explained in the revised manuscript.

-Figure 3G-H: not mentioned anywhere in the text so it is unclear how they contribute to the narrative.

The plots in Figure 3G-H are shown to aid direct comparison of the three experimental groups. This is now mentioned and referred to in the manuscript.

-Introduction, sentence 2-4: are these possible key functions for VAMP2 the authors' own hypotheses or supported by others' works? If supported, provide reference citations.

We thank the reviewer for this observation. These sentences reflected a combination of previous findings and our hypothesis. For clarification, we have rephrased this section and added the corresponding citations.

-Methods: Methods are too brief to evaluate the validity of some of the measurements performed.

In this report we only use one method, namely optical monitoring of pHluorin at individual synapses in dissociated hippocampal cultures. All the aspects of the process are explained in detail in the Methods section of the revised manuscript. Regarding the analysis of single synaptic vesicle fusion/endocytosis events, this is a more complex analysis that we have validated and explained in detail in our previous publication (Chanaday and Kavalali, eLife 2018) which is properly cited.

-Gaussian fitting: Please provide the table for the Gaussian Mixture Model and BIC values. Some of the data seem to better fit with two Gaussians, not three (Fig. 3 B, C).

This information is now available in Supplementary Table 1.

Minor:

-Figure 1A: all figures nicely compare KO, Het and WT synapses, so please show example Het pHluorin traces to be consistent throughout.

We have added example traces from syb2 Hets.

-Overall figure legends do a great job of listing p-values, include the asterisks that correspond to each p-value in the legends as well, to make their relative significance explicit at a glance.

We have added the asterisks.

-Low-frequency experiments. Though this paper relies on previously published methods, when switching into single action potential measurements on page 4 the stimulation method should be briefly listed like you did for high-frequency stim-i.e. (xx Hz, xx s).

We have added this information in the manuscript and the figure legend.

RE: Manuscript #E21-04-0213R
TITLE: "Synaptobrevin-2 dependent regulation of single synaptic vesicle endocytosis"

Dear Dr. Chanaday:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell. Thank you for thoroughly addressing the reviewer suggestions and for submitting this interesting work to MBoC.

Sincerely,
Avital Rodal
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Chanaday:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

Your paper is among those chosen by the Editorial Board for Highlights from MBoC. Hightlights from MBoC appears in the ASCB Newsletter and highlights the important articles from the most recent issue of MBoC.

All Highlights papers are also considered for the MBoC Paper of the Year. In order to be eligible for this award, however, the first author of the paper must be a student or postdoc. Please email me to indicate if this paper is eligible for Paper of the Year.

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We are pleased that you chose to publish your work in MBoC.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #2 (Remarks to the Author):

The authors have addressed all the concerns, and it should be ready for publication. There were 2 spelling errors that need to be corrected.

-rescue missing an "s" in the following sentence:
One candidate is VAMP4 which was previously shown to partially recue syb2 KO

-dwell misspelled in the Fig 3 G-H lengend:
Insets: Average dweel