# Sigma-2 -Receptor Antagonists Rescue Neuronal Dysfunction Induced by Parkinson's Patient Brain-Derived α-Synuclein

Colleen S. Limegrover, Raymond Yurko, Nicholas J. Izzo, Kelsie M. LaBarbera, Courtney Rehak, Gary Look, Gilbert Rishton, Hank Safferstein, Susan M. Catalano

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1st Editorial Decision

Decision letter

Dear Dr Catalano:

Thank you for submitting your manuscript to the Journal of Neuroscience Research. We've now received the reviewer feedback and have appended those reviews below. As you will see, the reviewers find the question addressed to be of potential interest. Yet, they do not find the manuscript suitable for publication in its current form.

If you feel that you can adequately address the concerns of the reviewers, you may revise and resubmit your paper within 90 days. It will require further review. Please explain in your cover letter how you have changed the present version. If you require longer than 90 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). You can submit your revised manuscript directly by clicking on the following link: *** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. ***

https://mc.manuscriptcentral.com/jnr?URL_MASK=3d114c386d364bcfa83238a69ca162b0

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.

Best Wishes,

Dr Barrington Burnett
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Co-Editor-in-Chief, Journal of Neuroscience Research

Editor Comments to the Author:
A. The description of the data analyses and the statistical tests used should be thorough.
Please add a paragraph at the end of the methods section with a detailed description of the statistical methods used.
SEM should be substitute by SD considering the small sample size.
STATISTICAL TESTS
Articles containing statistical analyses should state the name of the statistical test, the n value for each statistical analysis, the comparisons of interest, and justification for the use of the test. It should be clear what statistical test was used to generate every P value. Moreover, the authors must include the values from the appropriate statistical test (e.g., $F(x,x) = xx$; $n = x$; $P = x.xxx$). If the tests violate any assumptions, the authors must provide this information.
This journal requests that p-values be shown using a consistent decimal exactness: values in this text are given to varying number of digits or they are expressed only as "P<0.05" and the like.  Kindly choose a decimal, say 3, and stay with it throughout, with "p<0.001" reserved as appropriate.


B. GRAPHICAL PRESENTATION
JNR does not support the use of bar graphs, please modify all the graphs following the journal guidelines. All continuous data plots should be depicted as scatterplots or box and whisker plots  to better visualize the distribution of data.
Please also be sure to review the following, to ensure accurate graphical visualization and transparent reporting: https://goo.gl/w5dnYa and https://onlinelibrary.wiley.com/doi/epdf/10.1002/jnr.24340

Please add separate figure legends after the references.


C. Please explain the following statement:
"There is no shared data associated with this study."
As also stated in the instructions to the authors:
DATA ACCESSIBILITY
To enable readers to locate archived data from Journal of Neuroscience Research papers, we require authors to include a 'Data Accessibility' section just before the References. This should list the database(s) and URL(s) or dataset DOIs for all data associated with the manuscript. Data deposit repositories might include unstructured repositories such as Dryad, FigShare, NeuroMorpho or centralized repositories from the institutions in which the research was conducted. We also strongly recommend depositing data in the Open Science Framework. JNR will also allow small data sets to be included as Supplementary Files with the article.


Reviewer: 1


Comments to the Author

In this work, Limegrover et al show that micromolar concentrations of sigma-2 receptor antagonists rescue alpha-synuclein-induced deficits in trafficking and autophagy in primary neurons. Previously, Catalano's group identified sigma-2 as a receptor of Alzheimer's Abeta and showed that anti-sigma-2 compounds were able to displace different Abeta oligomeric species from cultured neurons and had promising therapeutic effects in the mouse models of AD. Sigma-2 is probably not unique in acting as a hub for toxicity of different misfolded proteins. For instance, Corbett et al Acta Neuropathol 2020 showed that cellular prion protein acted as a toxicity receptor for different neurodegeneration-associated aggregates such as alpha-synuclein, Abeta and tau.  Other putative receptors of aggregated a-synuclein include Na-K ATPase, immune cells' LAG3 and neurexin-1b, and similar to AD with its many receptors of Abeta, all of these binding partners may play a role in a-synuclein toxicity or transmission.

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4. p.16 Legend to Fig.3: an incubation time with Tetrazolium salts is between 20 and 60 min, did the variable timing affect the assay sensitivity?


Minor remarks:

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p.10: what were dilutions of primary and secondary antibodies used for immunocytochemistry (e.g 1:100, 1:500)? Antibody concentrations expressed in mg/ml probably refer to the concentration of the stock solutions.


Reviewer: 2

Comments to the Author
The strength of this a manuscript is access to a proprietary library of CogRx CNS-drug-like small molecules, besides the NIH Clinical Collection (NCC) libraries, to examine their utility in rescuing

potential defects in trafficking and autophagy mediated by oligomeric forms of alpha-synuclein (alphaSyn). Identification of novel compounds that block negative cellular effects of alphaSyn proteoforms would advance the PD field.

Unfortunately there is a glaring weakness in the quality, relative amount, and homogeneity of the recombinant alphaSyn oligomer preparations and postmortem fractions from non-PD and PD brain samples used throughout the various assays and drug blocking screens. The central problem is that Western immunoblotting (Figure 2) detects relatively low amounts of oligomeric (maybe dimers at 30-37 kDa)? alphaSyn in both the case of recombinant preps and purified postmortem fractions from PD brain. Most of the recombinant prep is clearly monomeric (at 15 kDa) based on the predominance of immunoreactivity. Without more controls, it is also not clear if the 30-37 kDa species are indeed truly oligomeric, since the 37 kDa band in particular is often detected in monomeric recombinant preps likely due to random aggregation. On the other hand, Figure 1 suggests that monomers relative to the oligomer prep do not alter trafficking to the same extent but a side by side western of monomers vs oligomers is not shown. It was surprising that amyloid beta (Abeta) seeds were used to make alphaSyn "oligomeric" fractions? Although likely present in small relative amounts, it is important to know how much Abeta is present in a given alphaSyn prep. It would also be instructive to know which recombinant alphaSyn proteoform (monomer vs larger species) is actually taken up by the cell cultures .

There are similar problems with the PD postmortem fractions. Most of the fractions whether from PD or non-PD brain contain mostly very large immunoreactive > 100 kDa bands, while extremely low amounts of equally distributed dimeric/monomeric banding are detected mainly in the PD fractions. Disappointingly the same banding pattern, although diminished, was detected in non-PD samples raising concerns about the legitimacy of these bands as true oligomers related to disease. Having access to postmortem fractions is helpful, but require additional protein non-alphaSyn loading controls.

The trafficking and drug blocking expts with CogRx CNS-drug-like small molecules in Figures 3-6 are intriguing and show that one or more alphaSyn forms is having a reproducible effect. Minimally a subset of the drug blocking experiments with CogRx CNS-drug-like small molecules need to be repeated with more bonafide homogeneous oligomeric alphaSyn preps using both recombinant and PD brain sources

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Authors' Response

Dear Dr. Cristina Ghiani, Dr. Barrington Burnett, and Ponne Saravanaraman,
Thank you for reviewing our manuscript (Manuscript # jnr-2020-Jun-8864) for publication in *J Neurosci Res*. The editors and reviewers each had very constructive and insightful comments and suggestions. We were able to incorporate all of the suggestions (aside from those requiring extensive further experimentation, as already agreed to by the editors in an email dated 8/24/2020, see below). We feel that the revisions and additional explanations and figures significantly strengthen the manuscript and we are pleased to resubmit it for your further consideration for publication.
The changes, additions, and revisions made in accordance with each specific comment are detailed below.

Thank you,

Susan Catalano, PhD

Editor Comments to the Author:

A. STATISTICAL TESTS

1) The description of the data analyses and the statistical tests used should be thorough.

2) SEM should be substitute by SD considering the small sample size.

3) Kindly choose a decimal, say 3, and stay with it throughout, with "p<0.001" reserved as appropriate. It should be clear what statistical test was used to generate every P value.

4) Moreover, the authors must include the values from the appropriate statistical test (e.g., F(x,x) = xx; n = x; P = x.xxx).

5) Please add a paragraph at the end of the methods section with a detailed description of the statistical methods used.

**REPLY: Thank you for the specific instructions. We have made all the changes detailed in the comment. These changes are as outlined below:**

**1) Power analysis is detailed highlighted on page 17.**

**2) SEM changed to SD: pages 7, 13, 19, 20, 22**

**3) All P values carried to four decimal places and statistical test specified: pages 12, 17, 20, 22**

**4) F-test results detailed on page 12.**

**5) An additional paragraph was added explaining Statistical Tests highlighted on page 11.**

B. GRAPHICAL PRESENTATION

1) please modify all the graphs following the journal guidelines.

2) Please add separate figure legends after the references.

**REPLY: 1) All figures were brought into alignment with this comment. Figure 3 (Page 17) and Figure 6 (page 22) were changed to show individual data points. The graph added to Figure 2 (page 15) was created also to show individual data points.**

**2) Figure legends were added as requested (highlighted on page 51).**

C. Please explain the following statement: "There is no shared data associated with this study."

**REPLY: The statement was removed and a statement was added directing readers to the data sets available as Supplemental Files (Page 31).**

Reviewer: 1 Comments to the Author

1. Here, alpha-synuclein oligomers were prepared by seeding synuclein monomers with Abeta oligomers. **(A)** This method choice warrants some explanation, e.g. why alpha-synuclein fibrils weren't used instead of Abeta. **(B)** Also, a section about preparation of alpha-synuclein monomers (p.7 Methods) should go before the preparation on alpha-synuclein oligomers seeded with Abeta.

**REPLY: The Methods section "Oligomer Preparation" was rewritten in accordance with ALL of the several reviewer's comments about it. This revision clarifies, expands, and simplifies the description of these methods. Specific comments (A) and (B) above are incorporated as follows:**

**(A) The use of Abeta is explained in an added paragraph highlighted on page 7.**

**(B) The preparation of alpha-synuclein monomers is explained in an added highlighted statement on page 6.**

2. To compare effects of synthetic and patient-derived synuclein tested back-to-back in a cell assay (Fig. 3) it would be helpful to know protein concentration (or at least a densitometric quantification of Western blots) in patient-derived samples. Were synthetic oligomers present in excess compared to human material?

**REPLY: As suggested, the densitometric quantification of the Western blot among patient-derived samples was added as a new Figure 2c. (Page 15). Explanation was added in a highlighted statement**

**on page 14. It is not appropriate to directly compare densitometry of the synthetic versus patientderived**

**western blots.**

**While it was possible to compare protein density in the Western blots between PD patients and non-PD patient-derived material, it was not possible to determine the concentrations of proteins in the human brain material due to low quantities and the need to reserve material for experimental testing. A statement of explanation of this has been added to the text, highlighted on Page 8.**

**In addition, the amount of protein added was kept constant for comparisons between experimental conditions. A statement to this effect was added as highlighted on Page 15.**

**Furthermore, we have removed the synthetic preparation values from Figure 3 and do not compare them directly to human-brain derived material.**

4. (NOTE: There was no comment #3) p.16 Legend to Fig.3: an incubation time with Tetrazolium salts is between 20 and 60 min, did the variable timing affect the assay sensitivity?

**REPLY: This statement of methods was removed from the figure legend itself, but this comment was addressed in the statement of these methods in the text. The following statement was corrected and an explanation added, highlighted on page 7.**

Minor remarks:

1. p.6 line 17: cell density expression as "4.66104 cells per cm2" reads as less than 5 cells/cm2, this is probably incorrect

**REPLY: Thank you for seeing this formatting error: It has been corrected, highlighted on Page 6.**

2. p.6 line 38: evaporating HFIP not peptide (which will remain as a film)

**REPLY: This error has now been corrected in the rewriting of the Methods section highlighted on Page 6.**

3. p.8 line 16: post mortem intervals 5.42 h and 18.3 h should probably be expressed in hours and minutes.

**REPLY: This has been changed as suggested, highlighted on Page 8.**

4. p.10 lines 23 and 43: TritonX100 is used for permeabilization rather than blocking

**REPLY: This error has been corrected in two places, highlighted on Page 10.**

5. p.10: what were dilutions of primary and secondary antibodies used for immunocytochemistry (e.g 1:100, 1:500)? Antibody concentrations expressed in mg/ml probably refer to the concentration of the stock solutions.

**REPLY: This omission has now been rectified and the dilutions added in three places, highlighted on Page 9-11.**

Reviewer 2: Comments to the Author

**NOTE: Comments of reviewer #2 were truncated to answerable statements. All requests of reviewer #2 were incorporated into the manuscript (#1, #2, #3), except those that required extensive further experimentation (#4, #5, #6), as suggested by the editor. The comments suggesting specific changes were numbered for convenience. Changes to the manuscript are detailed below.**

**(#1)** a side by side western of monomers vs oligomers is not shown.

**REPLY: A side-by-side Western of monomers vs oligomers has now been added as Figure 2a (Page 15).**

**(#2)** It was surprising that amyloid beta (Abeta) seeds were used to make alphaSyn "oligomeric" fractions?

**REPLY: An explanation of this has now been added, as also requested by reviewer #1, highlighted on Page 7.**

**(#3)** (Although likely present in small relative amounts, it is important to know how much Abeta is present in a given alphaSyn prep.

**REPLY: A statement to this effect has now been added, highlighted on Page 7.**

**(#4)** It would also be instructive to know which recombinant alphaSyn proteoform (monomer vs larger species) is actually taken up by the cell cultures. **(#5)** additional protein non-alphaSyn loading controls. **(#6)** a subset of the drug blocking experiments with CogRx CNS-drug-like small molecules need to be repeated with more bonafide homogeneous oligomeric alphaSyn preps using both recombinant and PD brain sources.

**REPLY: These suggestions (#4, 5, 6) are recognized as good suggestions for future experiments, and we appreciate the interest shown by reviewer #2 in some next-steps in this line of research. The suggested experiments are beyond the scope of the present study, however, which focuses on 1) demonstrating that the newly developed methods of α-synuclein synthesis and derivation from human brain yield proteins that have a biological impact in functional assays and 2) a characterization of the small-molecule pharmacology that modulates this biological impact. Indeed, more detailed characterization of the protein preparations will be instructive, and useful, now that we have demonstrated their functional and human-disease relevance. We have added text discussing our rational for choosing the approach that we did perform, highlighted on Page 8.**

**In addition, as suggested by the editor to address these comments, we have expanded the discussion of the significance of the present results including a statement in the Discussion of the possible future studies characterizing the patient-derived material further, highlighted on Page 24.**

1
Susan Catalano
From: jnroffice <jnroffice@wiley.com>
Sent: Monday, August 24, 2020 6:26 AM
To: Jennifer Kahle; Susan Catalano
Cc: Nick Izzo
Subject: Re: Journal of Neuroscience Research - Decision on Manuscript # jnr-2020-Jun-8864
Dear Dr. Kahle and Dr. Catalano,
Thank you for your patience.
I have heard back from the handling Editor. Please see his comments below. I would suggest you follow the Editor's
advice and submit your revision at the earliest possible date.
------------------------------------------------------------
Most journals are making allowances for the pandemic since extensive experimental revisions are not practical at the
moment. In the case where experiments cannot be carried out, explaining the limits of the current data and potential
alternative interpretations would be sufficient. Of course, the explanation has to be scientifically sound and well
reasoned.
------------------------------------------------------------
Should I be of any further assistance, kindly contact me.
Best regards,
Ponne Saravanaraman
Editorial Assistant
Journal of Neuroscience Research
From: Jennifer Kahle <jkahle@ihsintl.com>
Sent: Wednesday, August 19, 2020 10:16 AM
To: jnroffice <jnroffice@wiley.com>; Susan Catalano <scatalano@CogRx.com>
Cc: barrington.burnett@usuhs.edu <barrington.burnett@usuhs.edu>; Nick Izzo <nizzo@cogrx.com>

Subject: Re: Journal of Neuroscience Research - Decision on Manuscript # jnr-2020-Jun-8864

⬜⬜⬜ This is an external email.

Dear Ponne,

I am writing for an update from the handling editors of our submitted manuscript regarding our request in the

email below. Can you tell me when to expect a reply from them?

Thank you,

Jennifer

From: jnroffice <jnroffice@wiley.com>
Sent: Thursday, August 13, 2020 7:05 AM
To: Susan Catalano <scatalano@CogRx.com>
Cc: barrington.burnett@usuhs.edu <barrington.burnett@usuhs.edu>; Nick Izzo <nizzo@cogrx.com>; Jennifer Kahle

2

<jkahle@ihsintl.com>

Subject: Re: Journal of Neuroscience Research - Decision on Manuscript # jnr-2020-Jun-8864

Dear Dr. Catalano,

Thank you for your email.

I have forwarded your concern to the handling Editors and I appreciate your patience until you hear back from either of

us.

If you have any other questions, just let me know!

Best regards,

Ponne Saravanaraman

Editorial Assistant

Journal of Neuroscience Research

From: Susan Catalano <scatalano@CogRx.com>
Sent: Wednesday, August 12, 2020 9:37 AM
To: jnroffice <jnroffice@wiley.com>
Cc: barrington.burnett@usuhs.edu <barrington.burnett@usuhs.edu>; Nick Izzo <nizzo@cogrx.com>; Jennifer Kahle

<jkahle@ihsintl.com>

Subject: RE: Journal of Neuroscience Research - Decision on Manuscript # jnr-2020-Jun-8864

⬜⬜⬜ This is an external email.

Dear Dr. Ghiani, and Dr. Burnett,

Thank you so much for your help in facilitating the completion of the peer-reviews of our manuscript (Manuscript ID: jnr-

2020-Jun-8864).

We can revise the manuscript and respond to all of the reviewer's comments and your associate editor's requests, with

the exception of the major request of reviewer #2 to redo all the experiments with further fractionated preparations.

The request is restrictively beyond the scope of the present study, and indeed beyond the state of the present methods of this field.

This field of study is unique in that we are still at the beginning stages of characterization of these oligomeric preparations, and while it is desirable that these requested experiments be done in the

future, the present research questions are at a higher level of detail, establishing functionality, non-toxicity, and similarity between laboratory preparations and PD patient brain-derived material.

I am writing to you with this information because if the request of reviewer #2 for extensive further experiments is a non-negotiable request, then we will have to withdraw the manuscript submission. I have appreciated your candid and rapid responses, and look forward to your response, so that we know the best way to proceed with this manuscript.

I understand that you are busy, and I truly appreciate the careful and thoughtful work that you do to administer this

highly regarded scientific journal.

Best regards,

Susan

3

From: Ponne Saravanaraman <onbehalfof@manuscriptcentral.com>

Sent: Saturday, August 8, 2020 9:20 PM

To: Susan Catalano <scatalano@CogRx.com>

Cc: barrington.burnett@usuhs.edu

Subject: Journal of Neuroscience Research - Decision on Manuscript # jnr-2020-Jun-8864

08-Aug-2020

Dear Dr Catalano:

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4

Please add separate figure legends after the references.

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As also stated in the instructions to the authors:

DATA ACCESSIBILITY

To enable readers to locate archived data from Journal of Neuroscience Research papers, we require authors to include

a 'Data Accessibility' section just before the References. This should list the database(s) and URL(s) or dataset DOIs for all data associated with the manuscript. Data deposit repositories might include unstructured repositories such as Dryad, FigShare, NeuroMorpho or centralized repositories from the institutions in which the research was conducted. We also strongly recommend depositing data in the Open Science Framework. JNR will also allow small data sets to be included as Supplementary Files with the article.
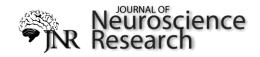
Reviewer: 1

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5

Reviewer: 2

Comments to the Author

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*********IMPORTANT: Instructions and checklists follow*********

When finalized, please upload your complete revised manuscript onto our website, preferably as a word document.

Please ensure to upload a highlighted version of your manuscript along with the clean version. The highlighted version should highlight the revised text or any other changes made to the manuscript. The clean version should have no highlighted sentences, strike-through words, or comments in margins. Kindly avoid submitting a document with tracked changes. Figures must be uploaded separately in .tif or eps format. Please review our submission checklist, which can be found in our author guidelines and also be sure to fill out the Transparent Science Questionnaire attached to this email.

JNR offers Open Science badges to qualifying authors. For more information please see the "Open Science initiatives" section of our author guidelines. If you would like to apply for one or more of the badges, please complete the included disclosure form and upload it as Supplemental Material Not for Review when submitting your final manuscript files.

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2nd Editorial Decision

Decision Letter
Dear Dr Catalano:

Thank you for submitting your manuscript to the Journal of Neuroscience Research. We've now received the reviewer feedback and have appended those reviews below. I'm glad to say that the reviewers are overall very enthusiastic and supportive of the study. They did raise some concerns and made some suggestions for clarification, but I expect that these points should be relatively straightforward to address. If there are any questions or points that are problematic, please feel free to contact me. I am glad to discuss.

We ask that you return your manuscript within 30 days. Please explain in your cover letter how you have changed the present version and submit a point by point response to the editors' and reviewers' comments. If you require longer than 30 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). To submit your revised manuscript: Log in by clicking on the link below

(If the above link space is blank, it is because you submitted your original manuscript through our old submission site. Therefore, to return your revision, please go to our new submission site here (submission.wiley.com/jnr) and submit your revision as a new manuscript; answer yes to the question "Are you returning a revision for a manuscript originally submitted to our former submission site (ScholarOne Manuscripts)? If you indicate yes, please enter your original manuscript's Manuscript ID

number in the space below" and including your original submission's Manuscript ID number (jnr-2020-Jun-8864.R1) where indicated. This will help us to link your revision to your original submission.)

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.

Best Wishes,

Dr Barrington Burnett
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research


Editor Comments to the Author:
1. The purity of the alpha-Syn oligomers needs to be further explained in the results section.
2. The interpretation of the bands in the Figure 2b needs more detailed explanations.
3. Can the authors explain what they mean with 'Hippocampal/cortical cultures'? At E18, it is possible to dissect/separate the hippocampus from the cortex, was this done on purpose?
4. There is no description of the characterisation of the primary cultures. The authors only state that "... mixed cultures of hippocampal plus cortical neurons and glia were used for all in vitro experiments described....." Please describe how the cultures were stained, the markers used and the percentage of each cell type present in the cultures. Have the authors stained for microglia or oligodendrocyte markers? Was any serum added to the cultures?
5. The authors are using the word 'glia' to refer to astrocytes, which is incorrect, since astrocytes are one type of glial cells. Thus, they should change the word 'glia' to 'astrocytes' throughout the manuscript, including the abstract (in cultured rat neurons and glia) since this seems to be the glial cell type present in the primary cultures used in this study, defined by the authors as glial fibrillary acidic protein (GFAP)-positive glia.


Reviewer: 2

Comments to the Author
The authors responded to most of the previous concerns and suggestions (preps, statistics etc) but questions remain regarding the quality of the synthetic recombinant alpha-Syn oligomers and the specificity of PD patient oligomers.

Unfortunately a side-by-side western comparison of recombinant alpha-Syn oligomers with the starting monomer preparation (Fig 2a) reveals an almost identical pattern of protein banding save for a higher density in the oligomeric prep. Both preps also show a similar amount of the predominant monomeric band at the bottom of each blot as well as ample amounts of aggregation at top. These observations raise questions about the quality of the alpha-Syn oligomeric preps used in so many of the additional experiments outlined in the manuscript.

Comparison of the PD vs non-PD patients' alpha-Syn protein profiles on westerns (Fig 2b) also remains problematic. While the doublet 35 kDa size band and multiple smaller 10-15 kDa bands, observed in the PD samples, are intriguing, they are also detected in the first non-PD sample (lane 2) and also in the

second non-PD sample (lane 9) although at reduced amount when viewed in the raw image. Without the appropriate random protein loading control such as actin or GPDH or other, it is uncertain if similar amounts of patient protein samples have been added to each lane or if there is enhanced degradation in the PD samples.

Reviewer: 1

Comments to the Author
The authors did a great job addressing all raised concerns and improving the manuscript. The findings are sound and will be of interest to PD community and beyond.
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Authors' Response
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 November 11, 2020
Dear Dr. Barrington Burnett and Dr. Cristina Ghiani,
We are pleased to resubmit the revised version of our manuscript (# jnr-2020-Jun-8864.R1). We incorporated all the reviewers' suggestions and revised in accordance with all of their comments. There were no questions or points that were problematic. We thank the reviewers for their time: their comments and suggestions were very helpful, and we feel the manuscript is stronger for their input. We look forward to hearing from you.
Our responses to the specific comments are detailed below.
Thank you,
Susan Catalano, PhD
Editor Comments to the Author:
1. The purity of the alpha-Syn oligomers needs to be further explained in the results section.

Please see our response to Reviewer 2 below.
2. The interpretation of the bands in the Figure 2b needs more detailed explanations.

Please see our response to Reviewer 2 below.
3. Can the authors explain what they mean with 'Hippocampal/cortical cultures'? At E18, it is possible to dissect/separate the hippocampus from the cortex, was this done on purpose?

Both hippocampus and neocortex were deliberately included in the dissections to obtain both neuronal populations. The Methods section has been revised to reflect this.
4. There is no description of the characterisation of the primary cultures. The authors only state that "... mixed cultures of hippocampal plus cortical neurons and glia were used for all in vitro experiments described....." Please describe how the cultures were stained, the markers used and the percentage of each cell type present in the cultures. Have the authors stained for microglia or oligodendrocyte markers? Was any serum added to the cultures?

We have further clarified the characterization of the cell types present in these cultures in the Methods section.
5. The authors are using the word 'glia' to refer to astrocytes, which is incorrect, since astrocytes are one type of glial cells. Thus, they should change the word 'glia' to 'astrocytes' throughout the manuscript,

including the abstract (in cultured rat neurons and glia) since this seems to be the glial cell type present in the primary cultures used in this study, defined by the authors as glial fibrillary acidic protein (GFAP)-positive glia.

Please see the answer to Question 4.

Reviewer: 2

Comments to the Author

The authors responded to most of the previous concerns and suggestions (preps, statistics etc) but questions remain regarding the quality of the synthetic recombinant alpha-Syn oligomers and the specificity of PD patient oligomers.

Unfortunately a side-by-side western comparison of recombinant alpha-Syn oligomers with the starting monomer preparation (Fig 2a) reveals an almost identical pattern of protein banding save for a higher density in the oligomeric prep. Both preps also show a similar amount of the predominant monomeric band at the bottom of each blot as well as ample amounts of aggregation at top. These observations raise questions about the quality of the alpha-Syn oligomeric preps used in so many of the additional experiments outlined in the manuscript.

We have amended the Results section to clarify the description of the quality of the α-synuclein preparations compared in Fig 2a. Only a small percentage of recombinant protein oligomerizes; most of it remains in the monomeric form. Similarly, for aggregation-prone proteins, freshly prepared monomer can also rapidly form low concentrations of oligomers under denaturing gel chromatography conditions. This is a widely recognized phenomenon that is also observed when oligomers are made from other synthetic or recombinant proteins, such as Aβ 1-42 (Izzo et al., 2014a). Despite this small increase in oligomer concentration, oligomer preparations are much more effective at inhibiting trafficking than monomer preparations, corresponding to a measurable four-fold difference in EC50 potency in the trafficking assay (Fig 1).

Comparison of the PD vs non-PD patients' alpha-Syn protein profiles on westerns (Fig 2b) also remains problematic. While the doublet 35 kDa size band and multiple smaller 10-15 kDa bands, observed in the PD samples, are intriguing, they are also detected in the first non-PD sample (lane 2) and also in the second non-PD sample (lane 9) although at reduced amount when viewed in the raw image. Without the appropriate random protein loading control such as actin or GPDH or other, it is uncertain if similar amounts of patient protein samples have been added to each lane or if there is enhanced degradation in the PD samples.

We have amended the Methods section to specify that the PD patient and control donor brain tissue starting volumes used to prepare material for immunoprecipitation were the same. Post-mortem intervals of PD and control brain donors were similar, as was the sizes of distinct bands observed on the Western blot, making it unlikely that there was selective degradation of the PD samples.

Reviewer: 1

Comments to the Author
The authors did a great job addressing all raised concerns and improving the manuscript. The findings are sound and will be of interest to PD community and beyond.

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3<sup>rd</sup> Editorial Decision

Decision Letter

Dear Dr Catalano:

Thank you for submitting your manuscript to the Journal of Neuroscience Research. I am glad to inform you that your manuscript has been accepted pending few minor concerns and changes. I expect that these points should be relatively straightforward to address. If there are any questions or points that are problematic, please feel free to contact me. I am glad to discuss.

We ask that you return your manuscript within 15 days. Please explain in your cover letter how you

have changed the present version. If you require longer than 15 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). To submit your revised manuscript: Log in by clicking on the link below https://rex-prod.resxchange.com/submissionBoard/1/e7581d01-2ba5-4966-a5a6-6494e5f8c3ef/current

If the above link space is blank, it is because you submitted your original manuscript through our old submission site. Therefore, to return your revision, please go to our new submission site here submission.wiley.com/jnr and submit your revision as a new manuscript; answer yes to the question "Are you returning a revision for a manuscript originally submitted to our former submission site (ScholarOne Manuscripts)? If you indicate yes, please enter your original manuscript's Manuscript ID number in the space below" and including your original submission's Manuscript ID number (jnr-2020-Nov-9209) where indicated. This will help us to link your revision to your original submission.)

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.

Best Wishes,


Dr Barrington Burnett
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research

Editors' Comments to the Author:
Thank you for reporting the characterisation of the cultures in the methods: "Approximately 36% ± 7% were OLIG2-positive oligodendrocytes (OLIG2, 1:500, 0.3 mg/mL, Sigma-Aldrich, St. Louis, MO, USA, catalog number ABN899, RRID: AB_2877641), and 7% ± 2% GFAP-positive astrocytes (GFAP, 1:500, 0.2 mg/mL, R&D Systems, Minneapolis, MN, USA, catalog number AF2594, RRID: AB_2109656), with the remainder likely microglia."

It is quite unusual to have neural cell cultures that contains more oligodendrocytes than astrocytes, do the authors really have only 7% astrocytes or is this a mistype?

At what time in vitro were the cultures assessed, at 21 DIV or earlier?

From the description, it seems that the authors have cultures of hippocampal and cortical neurons along with mixed glia, but in the results, it is stated that they "examined α-synuclein oligomer effects in rat primary neurons, grown for 21 DIV, which contain a mixture of both MAP2-positive neurons and glial fibrillary acidic protein (GFAP)-positive glia....." Please clarify or correct.

Regarding the request of using the word astrocyte and not glia:
Again, if as stated above, the authors only had MAP2+ and GFAP+ cells (astrocytes) in the cultures used in the experiments than they only had neurons and astrocytes, unless the authors used mixed glia and are referring to the mixed glia cultures described in the methods, but it does not seem like. Thus, the word 'glia' is used inappropriately in the discussion and other parts of the manuscript.

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Authors' Response

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 Manuscript # jnr-2020-Nov-9209

3-Dec-2020

Dear Drs Burnett and Ghiani:

Thank you for the good news regarding the acceptance of our manuscript (# jnr-2020-Nov-9209) for publication in *J Neurosci Res*!

We understand that this acceptance was pending a few minor concerns and changes, and we have incorporated all the suggestions and requested clarifications into the text without any problems. The details are outlined below.

Thank you for receiving our revised text.

Best Wishes,

Dr. Susan Catalano

Editors' Comments to the Author:

It is quite unusual to have neural cell cultures that contains more oligodendrocytes than astrocytes, do the authors really have only 7% astrocytes or is this a mistype?

RESPONSE: It was not a mistype; it was 7% astrocytes. We have added a small clarification into the Methods text on page 7 that may help readers more easily put this percentage into context.

At what time in vitro were the cultures assessed, at 21 DIV or earlier?

RESPONSE: The cultures were assessed at 21 DIV. This is clarified in the Methods on page 7.

From the description, it seems that the authors have cultures of hippocampal and cortical neurons along with mixed glia, but in the results, it is stated that they "examined α-synuclein oligomer effects in rat primary neurons, grown for 21 DIV, which contain a mixture of both MAP2-positive neurons and glial fibrillary acidic protein (GFAP)-positive glia....." Please clarify or correct.

RESPONSE: We agree and have revised the Results text (page 13) to reflect the editor's suggested correct description.

Regarding the request of using the word astrocyte and not glia:

Again, if as stated above, the authors only had MAP2+ and GFAP+ cells (astrocytes) in the cultures used in the experiments than they only had neurons and astrocytes, unless the authors used mixed glia and are referring to the mixed glia cultures described in the methods, but it does not seem like. Thus, the word 'glia' is used inappropriately in the discussion and other parts of the manuscript.

RESPONSE: We apologize for the misunderstanding. The revised Results text in the above response corrects this issue.

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4th editorial decision

Decision Letter

Dear Dr Catalano:

Thank you for submitting your manuscript "Sigma-2 -Receptor Antagonists Rescue Neuronal Dysfunction Induced by Parkinson's Patient Brain-Derived α-Synuclein" by Limegrover, Colleen S.; Yurko, Raymond; Izzo, Nicholas J.; LaBarbera, Kelsie M.; Rehak, Courtney; Look, Gary; Rishton, Gilbert; Safferstein, Hank;

Catalano, Susan M..

You will be pleased to know that your manuscript has been accepted for publication. Thank you for submitting this excellent work to our journal.

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Would you be interested in publishing your proven experimental method as a detailed step-by-step protocol? Current Protocols in Neuroscience welcomes proposals from prospective authors to disseminate their experimental methodology in the rapidly evolving field of neuroscience. Please submit your proposal here: https://currentprotocols.onlinelibrary.wiley.com/hub/submitaproposal

Congratulations on your results, and thank you for choosing the Journal of Neuroscience Research for publishing your work. I hope you will consider us for the publication of your future manuscripts.

Sincerely,

Dr Barrington Burnett
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research


Associate Editor: Burnett, Barrington
Comments to the Author:
(There are no comments.)

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