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Peer Review Information

Journal: Nature Structural and Molecular Biology
Manuscript Title: Tau Modification by the Noradrenaline Metabolite DOPEGAL Stimulates its Pathology and Propagation
Corresponding author name(s): Professor Keqiang Ye, Professor Zhentao Zhang

Editorial Notes:

EA delete any non-applicable rows, and then delete this instruction.

Redactions – unpublished data	Parts of this Peer Review File have been redacted as indicated to maintain the confidentiality of unpublished data.
Redactions – confidential patient information	Parts of this Peer Review File have been redacted as indicated to maintain patient confidentiality.
Redactions – published data	Parts of this Peer Review File have been redacted as indicated to remove third-party material.
Redactions – reviewer opt-out	Parts of this Peer Review File have been redacted as indicated as we could not obtain permission to publish the reports of reviewer no. XX.
Reviewer comments in marked-up manuscript	In their review of the [first/second/third/...] version of this manuscript, reviewer no. XX added their comments to the manuscript file. These comments, excluding minor textual revisions, have been copied into this Peer Review File.

Reviewer Comments & Decisions:

Decision Letter, initial version:

7th Oct 2021

Dear Dr. Ye,

Thank you again for submitting your manuscript "Tau Modification by the Noradrenaline Metabolite DOPEGAL Stimulates its Pathology and Propagation". I sincerely apologize for the delay in responding,

which resulted from the difficulty in obtaining suitable referee reports. Nevertheless, we now have comments (below) from the two reviewers, both experts on tau biochemistry and mouse models, who evaluated your paper. In light of those reports, we remain interested in your study and would like to see your response to the comments of the referees, in the form of a revised manuscript.

You will see that while the reviewers are positive about the interest and quality of the work, they both have several concerns that would need to be addressed prior to publication. Most importantly, reviewer #1 notes that trypsin digestion may not be optimal for detection of post-translational modification with DOPEGAL by mass spectroscopy and requests confirmation with additional proteases. The reviewer also suggests the addition of essential control experiments, e.g. for antibody specificity and lentiviral vectors. Reviewer #2 recommends that tau aggregation should be tested in the absence of seeds, as this is the more physiologically relevant setting. This reviewer also has various suggestions for additional controls and small explorative experiments into different directions that may strengthen the conclusions of the study. Both reviewers also have several helpful suggestions for improving the presentation and discussion of the findings. Please be sure to address/respond to all concerns of the referees in full in a point-by-point response and highlight all changes in the revised manuscript text file.

We appreciate the requested revisions are extensive. We thus expect to see your revised manuscript within 6 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision as long as nothing similar has been accepted for publication at NSMB or published elsewhere. Should your manuscript be substantially delayed without notifying us in advance and your article is eventually published, the received date would be that of the revised, not the original, version.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

As you already know, we put great emphasis on ensuring that the methods and statistics reported in our papers are correct and accurate. As such, if there are any changes that should be reported, please submit an updated version of the Reporting Summary along with your revision.

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Please note that the form is a dynamic ‘smart pdf’ and must therefore be downloaded and completed in Adobe Reader.

When submitting the revised version of your manuscript, please pay close attention to our
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Integrity Guidelines and to the following points below:

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Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

Please note that all key data shown in the main figures as cropped gels or blots should be presented in uncropped form, with molecular weight markers. These data can be aggregated into a single supplementary figure. While these data can be displayed in a relatively informal style, they must refer back to the relevant figures. These data should be submitted with the last revision, prior to acceptance, but you may want to start putting it together at this point.

SOURCE DATA: we urge authors to provide, in tabular form, the data underlying the graphical representations used in figures. This is to further increase transparency in data reporting, as detailed in this editorial (http://www.nature.com/nsmb/journal/v22/n10/full/nsmb.3110.html). Spreadsheets can be submitted in excel format. Only one (1) file per figure is permitted; thus, for multi-paneled figures, the source data for each panel should be clearly labeled in the Excel file; alternately the data can be provided as multiple, clearly labeled sheets in an Excel file. When submitting files, the title field should indicate which figure the source data pertains to. We encourage our authors to provide source data at the revision stage, so that they are part of the peer-review process. We also strongly encourage deposition of mass spectrometry datasets in the PRIDE database (https://www.ebi.ac.uk/pride/).

While we encourage the use of color in preparing figures, please note that this will incur a charge to partially defray the cost of printing. Information about color charges can be found at http://www.nature.com/nsmb/authors/submit/index.html#costs

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Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Kind regards,
Florian

Florian Ullrich, Ph.D.
Associate Editor
Nature Structural & Molecular Biology
ORCID 0000-0002-1153-2040

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
In this fascinating paper, Kang and colleagues show that DOPEGAL can interact directly with the tau protein and facilitate its aggregation, toxicity and spread. This is a follow-up to a JCI paper containing new data on tau.
DOPEGAL is a MAO-B metabolite of noradrenaline. The initiating role of the noradrenergic locus coeruleus in AD has been suggested in recent publications. Overall, these data may provide new insights into the mechanistic understanding underlying selective neuronal vulnerability in AD.
The study mainly used in vitro data and then the results were validated in mouse models and in humans. Although interesting, the study lacks controls and the number of independent experiments is still rather low.

The identification of Lys353 as a unique covalent binding site with DOPEGAL is surprising. Many Lys residues (n=44) are found on the 441 isoform of the tau protein. For example, Lys259 has a similar amino-acid environment. Further experiments need to be performed to assess that Lys353 is the only residue modified on the tau protein. Using trypsin to digest peptides in M/S may not be the best

approach since many Lys/Arg residues are found on tau proteins. In addition, Lys is the target amino acid for DOPEGAL binding. Other digestion enzymes should be tested.
As tau protein is known to undergo various post-translational modifications (PTM) such as acetylation and phosphorylation, M/S analysis of tau in a eukaryotic cell context should also be performed. Many Lys residues on the tau protein are also acetylated, these PTMs may alter the coupling of DOPEGAL to the Lys amino group.
Another question is the ability of DOPEGAL to link truncated tau since DOPEGAL also indirectly leads to truncation of tau at Asn368 after APE activation.
The next criticism is the lack of characterisation of the rabbit polyclonal antibody against K353-DOPEGAL. Its specificity is not assessed. Does the antibody recognise acetylated tau at K353? Is the signal clearly abolished with the epitope (Tau peptide-K353-DOPEGAL). Other antibodies or M/S (after anti-tau immunoprecipitation) can also be used.
In humans, there are six isoforms of tau. The electrophoretic profile of tau is not correct (Fig. 4). Please improve the quality of the blots and check the specificity. There is no information on human brain homogenates and the origin of the frozen tissue.
There is no information on the specificity of K353-DOPEGAL in young animals in areas lacking MAO-B and its metabolites.
Regarding AAV vectors, they can be transported along axons by neurons. Lentiviral vectors would have been a better choice to demonstrate spreading.
Overall, the study needs to be strengthened on several points.

Reviewer #2:
Remarks to the Author:
In Alzheimer’s disease, Tau pathology first emerges in the noradrenergic locus coeruleus (LC). Up to date, the mechanisms underlying the selective vulnerability of the LC to tau pathology in AD remains unclear. The manuscript by Kang et al. provides new insight into such mechanisms. The authors showed that DOPEGAL, a MAO-A metabolite of noradrenaline (NE) can selectively modify K353 residue of tau, which stimulates tau aggregation, toxicity, and propagation. The experiments and data are of good quality and mostly support the authors' conclusions.
Major point:
The authors demonstrated that K353 modification by DOPEGEL stimulates tau aggregation, but they did not provide sufficient evidence to support their conclusion. a. In vitro experiments revealed that K353 modification actually only mildly (about 10-15%, Fig. 1E) increases tau aggregation. b. In cells or mice, only the impact of K353 DOPEGEL on tau PFF-seeded tau aggregation, but not on tau aggregation without seeding, was examined. Since LC is the brain area where tau pathology starts in AD, it is better to test whether K353 DOPEGEL influences tau aggregation in the absence of seeds as well.
Minor points:

(1) Tau contains plenty of lysine residues and more importantly it is a natively unfolded protein, meaning most of the lysine residues may be exposed to modification by DOPEGAL. But here surprisingly, only K353 is modified by DOPEGAL at a concentration of 1uM. The authors need to comment why they selected to use 1uM DOPEGAL here. Have the authors tested higher concentrations of DOPEGAL and observed modifications of other lysine residues?
(2) On Page 8, the last sentence in Paragraph 1, the author stated " Hence, DOPEGAL enhances Tau aggregation and phosphorylation in cells". However, here only the overexpression of MAO-A was shown to induce the phosphorylation of tau and the amount of DOPEGAL was not measured at all. To draw this conclusion, the amount of DOPEGAL needs to be measured.
(3) On Page 7, the subtitle demonstrates "DOPEGAL enhances Tau aggregation and phosphorylation in cells". But, only PFF-induced tau aggregation was evaluated. It is worthy to test whether DOPEGAL can directly influence aggregation of tau in the absence of PFF, since in vitro experiments showed that DOPEGAL accelerates recombinant tau aggregation.
(4) As shown in Fig. 3A, DOPEGAL induced the generation of TauN368 in TauK353R overexpressed SY5Y cells. The author demonstrated "AEP activation and Tau N368 fragmentation, which were abrogated in K353R mutant transfected cells" (On Page 8, the last sentence). Then, a question is whether the generation of TauN368 was due to the truncation of the endogenous tau in SY5Y cells? To clarify this issue, an additional control---SY5Y cells without overexpression of tau treated with DOPEGAL is needed.
(5) Fig. 3D-H showed that DOPEGAL enhanced Tau PFF-induced AEP activation, Tau N368 truncation, aggregation and phosphorylation etc. It is of interest to test whether DOPEGAL can induce these changes in the absence of tau PFF. Such experiment would clarify whether DOPEGAL-induced changes are dependent on tau aggregation.
(6) As shown in Fig. 4J, the 3rd panel (from up to down), immunoprecipitation with anti-TauN368 antibody pulled down nearly an equal amount of K353-DOPEGEL TauN368 (< 50kD) in healthy controls and AD.
a. This is not consistent with Fig 4I, which showed no K353-DOPEGEL and TauN368 staining in healthy controls.
b. Notably, the amount of TauN368 in healthy controls is much lower than that in AD (Fig. 4J, 4th panel). Thus, it should be commented why the K353-DOPEGEL modified TauN368 in healthy controls is nearly equal to that in AD, despite of the much less amount of TauN368.
c. The K353-DOPEGEL modified intact tau (>50kD) in healthy controls is much less than that in AD (Fig. 4J, 1st panel), while K353-DOPEGEL modified tau N368 in healthy controls is equal to that in AD (Fig. 4J, 4th panel). The authors should comment how this occurs. In addition, the full image of the blot should be shown in 1st panel for better comparison of the amount of intact tau and TauN368.
(7) The authors did not describe how the MOA-A inhibitor was administrated in mice (Fig. 5A).
(8) The authors did not describe how they perform animal experiments to examine the propagation of tau pathology (Fig. 5E and 6E). They need to clearly state the age of the animals they treated and when they examined the tau pathology in LC, EC and HC, as (1) the age affects the spread of tau pathology,

and (2) the tau pathology does not appear in LC, EC and HC at the same time, if the spread of tau indeed occurs. In addition, the authors need to provide evidence showing that the occurrence of tau pathology in EC and HC is not due to the leaky expression of injected virus, instead of the spread of tau species from LC (Fig. 6E).
(9) In Fig. 5A, the DBH staining in vehicle treated P301S mice appears to be much weaker than staining in other mice. Is the expression of DBH reduced in these animals?
(10) The image resolution of Fig. 5F is too low. Such images can not be used for quantification.
(11) Inoculation of tauK353R PFF induced activation of MAO-A and increase of DOPEGEL (Fig. 7C and D). However, no obvious difference of staining of K353 DOPEGEL is observed between PBS and K353R PFF injected mice (Fig. 7A). Taken together, these results indicate that the increase of DOPEGEL did not cause K353 DOPEGEL of the overexpressed tau in K353R PFF injected MAPT mice. This is inconsistent with other results showing the modification of K353 by DOPEGEL. The quantification of K353 DOPEGEL should be shown here.

Author Rebuttal to Initial comments

Reviewer #1:

1. The referee notes "The identification of Lys353 as a unique covalent binding site with DOPEGAL is surprising. Many Lys residues (n=44) are found on the 441 isoform of the tau protein. For example, Lys259 has a similar amino-acid environment. Further experiments need to be performed to assess that Lys353 is the only residue modified on the tau protein. Using trypsin to digest peptides in M/S may not be the best approach since many Lys/Arg residues are found on tau proteins. In addition, Lys is the target amino acid for DOPEGAL binding. Other digestion enzymes should be tested".

A: We appreciate the reviewer's comments. As suggested, we used GluC enzyme to verify the result using trypsin and confirmed again that K353 is the only residue modified by DOPEGAL (Supplementary Fig 1C).

2. Th reviewer comments "As tau protein is known to undergo various post-translational modifications (PTM) such as acetylation and phosphorylation", s/he suggests "M/S analysis of tau in a eukaryotic cell context should also be performed". Many Lys residues on the tau protein are also acetylated, these PTMs may alter the coupling of DOPEGAL to the Lys amino group. Another question is the ability of DOPEGAL to link truncated tau since DOPEGAL also indirectly leads to truncation of tau at Asn368 after AEP activation.

A: Indeed, Tau contains multiple K residues that are post-translationally modified by various moieties. To explore whether these PTMs may alter the DOPEGAL modification, we performed M/S analysis of Tau in SH-SY5Y cell lysates. However, it was very challenging to detect the modified peptide in cells, even though we enriched Tau proteins via performing GST-Tau overexpression and pull-down assay. We barely detected this modified peptide at very low abundancy after deep fractionation of pull-down cell lysates.

In the supplementary Figure 1E, we compared the ability of DOPEGAL reacting with full-length and truncated Tau, and found that DOPEGAL covalently modified K353 primary amine in N368 truncated Tau via forming Schiff-base reaction more robustly as compared to FL Tau. As a matter of fact, DOPEGAL activates AEP (Fig 3A), which subsequently cleaves Tau, and the resultant Tau N368 fragment is more readily modified by DOPEGAL than full-length Tau (Supplementary Figure 1E). On the other hand, DOPEGAL modification facilitates its cleavage by AEP, resulting in Tau N368 upregulation (Fig 1F).

3. The next criticism is the lack of characterization of the rabbit polyclonal antibody against K353-DOPEGAL. Its specificity is not assessed. Does the antibody recognize acetylated tau at K353? Is the signal clearly abolished with the epitope (Tau peptide-K353-DOPEGAL). Other antibodies or M/S (after anti-tau immunoprecipitation) can also be used.

A: As requested, we performed additional experiments to validate the specificity of the antibody. Firstly, we pre-incubated the antibody with the epitope peptide of Tau K353-DOPEGAL, and found that this peptide abolished immune-reactivity. Secondly, we found that this antibody does not recognize acetylated-Tau at K353 (Fig 4C), underscoring its specificity.

4. In humans, there are six isoforms of tau. The electrophoretic profile of tau is not correct (Fig. 4). Please improve the quality of the blots and check the specificity. There is no information on human brain homogenates and the origin of the frozen tissue.

A: As requested, we provided blots with higher quality in the revised manuscript in order to visualize multiple bands of Tau isoforms (7th panel of Fig 4J). The human brain homogenates and frozen tissues were from the Emory Alzheimer's Disease Research Center (ADRC) Brain Bank. We included the information in the Method section on page 21-22.

5. There is no information on the specificity of K353-DOPEGAL in young animals in areas lacking MAO-B and its metabolites.

A: MAO-A is almost at adult levels at birth, whereas MAO-B increases several folds with aging. MAO-A and MAO-B are responsible for the generation of DOPEGAL and DOPAL, respectively. Nonetheless, we performed immunohistochemistry with the sections from the cerebellum of young wild-type mouse, which contains the lowest level of MAO-A and B in the brain (PMID, 23403377). We could not find any positive signals for K353-DOPEGAL in the cerebellar sections, further confirming the specificity of this antibody (Fig 4A).

6. Regarding AAV vectors, they can be transported along axons by neurons. Lentiviral vectors would have been a better choice to demonstrate spreading.

A: We appreciate the reviewer's recommendation. In the previous study (PMID, 31793911), we already confirmed that the spreading is almost due to Tau not AAV vectors using AAV-mCherry-Tau vectors. Tau pathologies spread from the LC to other brain regions in AAV-mCherry-Tau-injected group. However, we could not find any mCherry signals in other brain regions in the control groups of AAV-mCherry vectors-injected mice, supporting that it is the Tau pathology that spreads from the LC to other brain regions but not AAV vector itself. Moreover, we performed RT-PCR using the LC, EC, and HC samples to verify whether Tau protein or the virus was transported to the EC and HC. We confirmed that mRNA expression of hTau occurred only in the virus-injected LC region but not the EC or the HC (Supplementary Fig 6D).

Reviewer #2:

Major point:

The reviewer notes "The authors demonstrated that K353 modification by DOPEGEL stimulates tau aggregation, but they did not provide sufficient evidence to support their conclusion. a. In vitro experiments revealed that K353 modification actually only mildly (about 10-15%, Fig. 1E) increases tau aggregation. b. In cells or mice, only the impact of K353 DOPEGEL on tau PFF-seeded tau aggregation, but not on tau aggregation without seeding, was examined. Since LC is the brain area where tau pathology starts in AD, it is better to test whether K353 DOPEGEL influences tau aggregation in the absence of seeds as well".

A: We appreciate the reviewer's comments. In Fig 1E, DOPEGAL modification on Tau indeed mildly escalated Tau aggregation with Tau N368 truncate more robust than full-length. Nonetheless, this modification feeds back and strongly accelerates Tau N368 fragmentation (Fig 1F). Our previous study shows that Tau N368 potently facilitates Tau aggregations (Zhang et al., 2014, Nature Medicine). We further confirmed the effect of DOPEGAL on tau aggregation in the absence of seeds in Tau-transfected HEK 293 cells and updated this result in Supplementary Fig. 2. As expected, DOPEGAL elevates Tau aggregation in the absence of any seeds.

Minor points:

(1) Tau contains plenty of lysine residues and more importantly it is a natively unfolded protein, meaning most of the lysine residues may be exposed to modification by DOPEGAL. But here surprisingly, only K353 is modified by DOPEGAL at a concentration of 1uM. The authors need to comment why they selected to use 1uM DOPEGAL here. Have the authors tested higher concentrations of DOPEGAL and observed modifications of other lysine residues?

A: We used 500 μM of DOPEGAL in vitro assay as indicated in figure legend of Fig 1A and 60 μM in cellular studies (Fig 2&3). The [DOPEGAL] in normal postmortem LC region is around 1.4 μM, and it is elevated to 4-5 μM in AD patients (Burke WJ et al., Brain Res. 1999, 816, 633). 1 μM of DOPEGAL in the Result section on page 6 was a typo error and we fixed it. Even under higher concentration of DOPEGAL, only K353 modification in Tau was identified (please also see the response in reviewer #1's point #2).

(2) On Page 8, the last sentence in Paragraph 1, the author stated "Hence, DOPEGAL enhances Tau aggregation and phosphorylation in cells". However, here only the overexpression of MAO-A was shown to induce the phosphorylation of tau and the amount of DOPEGAL was not measured at all. To draw this conclusion, the amount of DOPEGAL needs to be measured.

A: As suggested, we performed HPLC analysis and measured the amount of DOPEGAL. We updated this result in Fig 2K.

(3) On Page 7, the subtitle demonstrates "DOPEGAL enhances Tau aggregation and phosphorylation in cells". But, only PFF-induced tau aggregation was evaluated. It is worthy to test whether DOPEGAL can directly influence aggregation of tau in the absence of PFF, since in vitro experiments showed that DOPEGAL accelerates recombinant tau aggregation.

A: As suggested, we tested the effect of DOPEGAL on Tau in cells in the absence of PFFs. In HEK293 cells stably transfected with tau, we confirmed that DOPEGAL induced Tau phosphorylation and aggregation in the absence of PFFs (Supplementary Fig. 2F).

(4) As shown in Fig. 3A, DOPEGAL induced the generation of TauN368 in TauK353R overexpressed SY5Y cells. The author demonstrated "AEP activation and Tau N368 fragmentation, which were abrogated in K353R mutant transfected cells" (On Page 8, the last sentence). Then, a question is whether the generation of TauN368 was due to the truncation of the endogenous tau in SY5Y cells? To clarify this issue, an additional control---SY5Y cells without overexpression of tau treated with DOPEGAL is needed.

A: As suggested, we used SH-SY5Y cells without overexpression of Tau to repeat the indicated experiment, and found that DOPEGAL still increased AEP activation and endogenous Tau N368 cleavage. However, the phosphorylation or oligomerization of endogenous Tau was barely observed, because endogenous Tau level in SH-SY5Y cells is very low. We updated this result in Fig. 3A. Further, DOPEGAL provokes much weaker AEP activation, Tau cleavage, and Tau phosphorylation in Tau K353R mutant than wild-type Tau transfected cells. These results demonstrate that K353R mutation diminishes AEP activation, Tau cleavage, and Tau phosphorylation. Conceivably, a portion of Tau N368 cleavage in K353R transfected cells may result from endogenous Tau (page 9).

(5) Fig. 3D-H showed that DOPEGAL enhanced Tau PFF-induced AEP activation, Tau N368 truncation, aggregation and phosphorylation etc. It is of interest to test whether DOPEGAL can induce these changes in the absence of tau PFF. Such experiment would clarify whether DOPEGAL-induced changes are dependent on tau aggregation.

A: As requested, we tested the effect of DOPEGAL on tau N368 truncation, phosphorylation and aggregation in the absence of tau PFF. We used the previously reported methods to stain the **insoluble Tau** in primary cultured neurons (PMID: 23827677), and found that DOPEGAL exhibits the same effect as with Tau PFFs on Tau phosphorylation and Tau N368 truncation (Fig. 3D & H). Our results indicate that DOPEGAL induces Tau phosphorylation, aggregation and N368 cleavage in the absence of tau PFF.

(6) As shown in Fig. 4J, the 3rd panel (from up to down), immunoprecipitation with anti-TauN368 antibody pulled down nearly an equal amount of K353-DOPEGEL Tau N368 (< 50kD) in healthy controls and AD.

a. This is not consistent with Fig 4I, which showed no K353-DOPEGEL and TauN368 staining in healthy controls.

b. Notably, the amount of TauN368 in healthy controls is much lower than that in AD (Fig. 4J, 4th panel). Thus, it should be commented why the K353-DOPEGEL modified TauN368 in healthy controls is nearly equal to that in AD, despite of the much less amount of TauN368.

c. The K353-DOPEGEL modified intact tau (>50kD) in healthy controls is much less than that in AD (Fig. 4J, 1st panel), while K353-DOPEGEL modified tau N368 in healthy controls is equal to that in AD (Fig. 4J, 4th panel). The authors should comment how this occurs. In addition, the full image of the blot should be shown in 1st panel for better comparison of the amount of intact tau and TauN368.

A: We apologize for the typo error. In Figure 4J 3rd panel, we used the antibody against Tau (but not anti-Tau N368) for immunoprecipitation, and the equal amount of bands in this panel is the heavy chains (around 50 kD) from the antibody but not Tau N368 proteins, and K353-DOPEGAL-modified Tau (the band above 50 and below 75 kD) in AD is much more abundant than control, in alignment with the expression patterns of TauK353-DOPEGAL (1st panel) and Tau (7th panel). We labeled K353-DOPEGAL (Tau) and heavy chain (H.C.) in the band of 3rd panel in the revised Fig. In order to clarify the issue, we presented the full images of the blotting in the revised Fig 4J. The top panel shows more extensive K353-DOPEGAL modification (1st panel) and much more abundant full-length Tau and Tau N368 fragments in AD patients versus healthy controls in 7th panel (please also see Reviewer #1 comment #4).

(7) The authors did not describe how the MOA-A inhibitor was administrated in mice (Fig. 5A).

A: The MAO-A inhibitor was administrated orally with drinking water. We included the information in the Method section on page 26.

(8) The authors did not describe how they perform animal experiments to examine the propagation of tau pathology (Fig. 5E and 6E). They need to clearly state the age of the animals they treated and when they examined the tau pathology in LC, EC and HC, as (1) the age affects the spread of tau pathology, and (2) the tau pathology does not appear in LC, EC and HC at the same time, if the spread of tau indeed occurs. In addition, the authors need to provide evidence showing that the occurrence of tau pathology

in EC and HC is not due to the leaky expression of injected virus, instead of the spread of tau species from LC (Fig. 6E).

A: We stated the age of the animals used in experiments and when we performed the experiments after drug administration or virus injection in the figure legend of Fig. 5 & 6. We tested tau pathology 1 and 3 months after the injection of virus and presented only 3 months' data, because we already verified that Tau spreads from the LC to other brain regions including EC, HC, and cortex in 3 months after AAV-Tau injection in the previous study (PMID, 31793911). However, as suggested, we included Tau K353-DOPEGAL staining of 1 month, which only appears in the LC but not EC or HC, to show the age-dependent spreading (Supplementary Fig. 6E). We also confirmed that the spreading of Tau pathology is due to Tau pathology but not AAV vectors in the previous study. We used AAV-mCherry Tau vectors for LC injection. Tau pathology spread from the LC to other brain regions in AAV-mCherry-Tau-injected group. However, we could not find any mCherry signals in other brain regions in the control groups of AAV-mCherry vectors-injected mice. This result supports that Tau pathology spreads from the LC to other brain regions, but not AAV vectors. To confirm again that the occurrence of Tau pathology in the EC and HC is due to propagation of aberrant Tau proteins from the LC rather than retrograde transport of the virus in this study, we performed RT-PCR and found that samples from EC and HC do not express human Tau mRNA (Supplementary Fig. 6D). Thus, the Tau pathology in those regions comes from the LC.

(9) In Fig. 5A, the DBH staining in vehicle treated P301S mice appears to be much weaker than staining in other mice. Is the expression of DBH reduced in these animals?

A: Although the fluorescent brightness (intensity) may not be always linearly linked to the protein expression levels, because this can be affected by many experimental conditions, we indeed quantified DBH-positive cells and confirmed that DBH-positive cell number was reduced in Tau P301S mice (Figure 5B). Therefore, the expression of DBH in the LC is decreased in Tau P301S mice based on the reduction of DBH-positive cells, which were reversed by MAO-A inhibitor.

(10) The image resolution of Fig. 5F is too low. Such images cannot be used for quantification.

A: As requested, we provided high-resolution image in the revised Figure 5F and quantified the images.

(11) Inoculation of tau K353R PFF induced activation of MAO-A and increase of DOPEGEL (Fig. 7D and E). However, no obvious difference of staining of K353 DOPEGAL is observed between PBS and K353R PFF injected mice (Fig. 7A). Taken together, these results indicate that the increase of DOPEGAL did not cause K353 DOPEGAL of the overexpressed tau in K353R PFF injected MAPT mice. This is inconsistent with other results showing the modification of K353 by DOPEGAL. The quantification of K353 DOPEGAL should be shown here.

A: DOPEGAL interacts Tau K353 residue and elicits covalent modification. Tau K353R is a mutant, in which lysine (K) is replaced with arginine (R) at 353 residue, blunting it from DOPEGAL covalent modification. Injection of Tau K353R PFFs into the LC region in MAPT mice increased both MAO-A activity and elevated DOPEGAL levels, though less robust than WT Tau PFFs, Tau K353-DOPEGAL staining induced by K353R PFFs in Fig 7A was more extensive than PBS control. As requested, we include the quantification of Tau K353-DOPEGAL-positive cells in revised Fig. 7C, revealing significantly more positive cells in K353R PFFs injected mice than PBS controls.

Decision Letter, first revision:

6th Jan 2022

Dear Dr. Ye,

Thank you for submitting your revised manuscript "Tau Modification by the Noradrenaline Metabolite DOPEGAL Stimulates its Pathology and Propagation" (NSMB-A45279A). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Structural & Molecular Biology, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

To facilitate our work at this stage, we would appreciate if you could send us the main text as a word file. Please make sure to copy the NSMB account (cc'ed above).

Thank you again for your interest in Nature Structural & Molecular Biology Please do not hesitate to

contact me if you have any questions.

Kind regards,
Florian

Florian Ullrich, Ph.D.
Associate Editor
Nature Structural & Molecular Biology
ORCID 0000-0002-1153-2040

Reviewer #1 (Remarks to the Author):

The authors have answered all of my comments. It is a very interesting study.

Reviewer #2 (Remarks to the Author):

All the questions have been well addressed. The manuscript is now ready for publication.

Final Decision Letter:

9th Feb 2022

Dear Dr. Ye,

We are now happy to accept your revised paper "Tau Modification by the Noradrenaline Metabolite DOPEGAL Stimulates its Pathology and Propagation" for publication as a Article in Nature Structural & Molecular Biology.

Acceptance is conditional on the manuscript's not being published elsewhere and on there being no announcement of this work to the newspapers, magazines, radio or television until the publication date in Nature Structural & Molecular Biology.

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