progress in neurobiology

Peer Review Overview

Manuscript Title: "Pathogenic tau accelerates aging-associated activation of transposable elements in the mouse central nervous system"

Received	09-Aug-2021
1st Decision	24-Sep-2021
Revision Submitted	05-Oct-2021
Accepted	12-Oct-2021

Decision Letter

Dear Bess,

Thank you for submitting your manuscript to Progress in Neurobiology. We have received comments from reviewers on your manuscript. Your paper should become acceptable for publication pending suitable minor revision and modification of the article in light of the appended reviewer comments.

When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, outline every change made point by point, and provide suitable rebuttals for any comments not addressed.

To submit your revised manuscript go to https://www.editorialmanager.com/proneu/ and log in as an Author where you will see a menu item called 'Submission Needing Revision'.

Please resubmit your manuscript by Nov 23, 2021.

We look forward to receiving your revised manuscript.

Kind regards,

Aimee Kao
Associate Editor
Progress in Neurobiology

Sabine Kastner
Editor-in-Chief
Progress in Neurobiology

Comments from the Editors and Reviewers:

Reviewer #1: This is a very well written paper on a highly topical area of research in aging, neurobiology and neurodegeneration. The potential role of somatic activation of retrotransposons during adult life, and especially in its role in driving the process of aging and its likely contribution to neurodegenerative diseases is highly relevant. The contributing author has already made seminal discoveries on the role of changes in heterochromatin and RTE activation in tau related neurodegeneration in flies, and also observation on RTEs in human Alzheimer's disease (AD). The present manuscript greatly extends these observations by demonstrating a clear association between tau and abeta42 related neurodegeneration in mouse models and the likely role of RTEs in such devastating neurodegeneration disorders as AD and "tauopathies". This paper provides critical new and important results on this highly topical area in neurobiology. The authors demonstrate the activation of endogenous retroviruses (ERVs) in the brains of mice expressing human tau as well as in mice expressing abeta42. These results are likely to be a

landmark contribution to the field and should be published in Progress in Neurobiology after addressing the following minor concerns.

1-It is noted in the beginning that:
"While the human and mouse genomes share a similar density of transposable elements, mice have retained a high level of transposon activity over the course of evolution, while most transposable elements in humans have become transpositionally inert1, with the exception of ~35-40 subfamilies of L1, Alu, and SVA9."
Based upon this, it appears that there may be a difference between humans and mice in the type of RTEs that are active and may contribute to neurodegeneration. In humans there do not appear to be very many active ERVs except perhaps in specific disorders such as ALS. Perhaps the authors could elaborate on this a bit more in the introduction or discussion.
2- Figure 1---"c"—could they explicitly indicate perhaps in the figure legend what the different categories apply to in the pie charts: Is "DNA"—DNA transposons as opposed to retrotransposons.
3- While the authors have provided the appropriate detailed information on the ERVs, it is not until the Results section of Fig. 1 that there is the first mention of L1. And there is little information on what exactly L1 is until the Results section of Fig. 5. Perhaps they could include such information in the same place as the other RTEs are being described.

4- In the results section they indicate that RNA levels of L1 increase in the mouse models of neurodegeneration. It is not clear to me whether they were able to also determine if L1 copy number is increased, as they find for ERVs. Would it be possible to determine copy number for L1 as well?

5-It is noted by the authors that dsRNAs that are encoded by RTEs can also affect cellular function—see introduction and Fig. 1a. Is it possible for the authors to comment on whether dsRNAs are also increased in either the aging brain or tau transgenic mice.

6- In Figure 2, the "phenotype" is missing from the PS19 mouse model.

7-It is known that one of the limitations of using RNA-seq to determine RTE expression is that fragments of RTEs may be contained within regions in which there is not a full length active RTE. For example, if fragments of RTEs are embedded within a gene that is activated, perhaps in response to either normal aging or neurodegeneration, it can appear to show an increase in RTE expression that does not accurately reflect RTE activation. It would be useful for the authors to discuss this issue in the manuscript.

8-Does the antibody that was used to detect the gag protein encoded by IAP only or does it also detect IAP-E elements. The IAP-E elements encode the envelope domain that could mediate a transfer between cells. Thus the specificity of the antibody is important to know in regard to the authors statement of a non-cell autonomous IAP activation as noted in Figs 3a and b.

9-The difficulty of using certain methodologies in determining changes in copy number of RTEs is well known. For both the digital PCR and NanoString approaches the authors suggest that there is an increase in those RTEs known to be active in the mouse genome—at the DNA level. Although the authors do discuss the limitations of these approaches—specifically the inability to discriminate between episomal elements versus those that have integrated into the genome—it may be useful for the them to also indicate other limitations of these approaches, including normalization to single copy genes for NanoString.

Reviewer #2: This is an interesting and timely manuscript entitled "Pathogenic tau accelerates aging-associated activation of transposable elements in the mouse central nervous system" by Ramirez et al. The present study strengthens the crucial link between transposable element activity, aging and tau pathology — a hallmark of several neurodegenerative diseases. (i) The authors analyzed RNA-seq datasets at different ages from three different mouse models of tauopathy: rTg4510, JNPL, and PS19. They found that retrotransposon transcripts particularly ERV, increases with age in the brain of all the three mouse models of tauopathy. (ii) Additionally, they found that gag capsid protein, a highly active mouse Class II ERV, is elevated in the brain of rTg4510 tau transgenic mice in an age-dependent manner. (iii) Further, dPCR and NanoString analysis revealed that DNA copy number of mobile retrotransposons with age in the brain of rTg4510 tau transgenic mice. This study offers a new groundwork for the link between tau-dependent pathologies and retrotransposon activation; however, further experimental data is required to validate the findings. Overall, this is a well-executed study with proper methods for a purpose; most results are convincing and support the author's conclusion.

Specific concerns: Few issues should be addressed to support the conclusions
* In Fig. 1, which strain or gender of mouse was used, please specify.
* Authors should clarify why they used spinal cord tissues as source for RNA-Seq instead of brain regions primarily involved in neurodegeneration on these mouse models.
* Why analysis has been performed in cortex of rTg4510 mice and spinal cord of JNPL3 and PS19? Why they did not compared the same brain regions (for example, cortices) in all the three mouse models of tauopathy?
* Perhaps the authors should consider using tau knock out animals to support tau-dependent activation of retrotransposons.
* Providing that retrotransposons activation is mediated pathological tau in an age-dependent manner in mouse models of tauopathy, does this observation relevant to human tauopathies? Does the retrotransposons activation correlates with clinical severity in AD patients or other primary tauopathies?
* How human tau (wild type and mutants) exacerbate activation of the transposable elements? Please provide the proposed model/hypothetical schematics that could be helpful for the next steps to investigate mechanistic insights.
* In Fig. 4A, can authors provide molecular weights in the schematics IAP-EPP? This could simplify their identification in the western blots.
* Authors should demonstrate that how retrotransposons activation results in neuronal/synaptic loss or gliosis in the tauopathy mice in an age-dependent manner? Further, authors should discuss the mechanistic insights of tau-dependent retrotransposons activation and subsequent neurodegeneration or cognitive decline.
* Evidences of direct Tau actions on regulatory sequence of retrotransposons need to be provided to establish its action on neurons transposable elements (Reporter Assay, DNA/RNA binding etc…) to give strength to the conclusions of the study.

Author Response Letter

RESPONSE TO REVIEWS

The reviewers' comments are reproduced in full in **bold**, followed by our responses in plain text.

REVIEWER 1

This is a very well written paper on a highly topical area of research in aging, neurobiology and neurodegeneration. The potential role of somatic activation of retrotransposons during adult life, and especially in its role in driving the process of aging and its likely contribution to neurodegenerative diseases is highly relevant. The contributing author has already made seminal discoveries on the role of changes in heterochromatin and RTE activation in tau related neurodegeneration in flies, and also observation on RTEs in human Alzheimer's disease (AD). The present manuscript greatly extends these observations by demonstrating a clear association between tau and abeta42 related neurodegeneration in mouse models and the likely role of RTEs in such devastating neurodegeneration disorders as AD and "tauopathies". This paper provides critical new and important results on this highly topical area in neurobiology. The authors demonstrate the activation of endogenous retroviruses (ERVs) in the brains of mice expressing human tau as well as in mice expressing abeta42. These results are likely to be a landmark contribution to the field and should be published in Progress in Neurobiology after addressing the following minor concerns.

1. **It is noted in the beginning that: "While the human and mouse genomes share a similar density of transposable elements, mice have retained a high level of transposon activity over the course of evolution, while most transposable elements in humans have become transpositionally inert1, with the exception of ~35-40 subfamilies of L1, Alu, and SVA9." Based upon this, it appears that there may be a difference between humans and mice in the type of RTEs that are active and may contribute to neurodegeneration. In humans there do**

not appear to be very many active ERVs except perhaps in specific disorders such as ALS. Perhaps the authors could elaborate on this a bit more in the introduction or discussion.

While much of the transposon field focuses on "active" elements with mobilization potential, we stress in the manuscript that retrotransposon-encoded products (RNAs, proteins, episomal DNA) can also impact cellular function. We are trying to get away from the idea that only "active" elements are relevant to cell biology. While specific active elements do indeed differ between mice and humans, mechanisms of retrotransposition, including transcription of transposable elements and production of retrotransposon-encoded products, is similar, suggesting that our studies in mice are relevant to human tauopathy.

The Introduction of the revised manuscript reads:

"In addition to mutations induced by transposition, proteins, single- and double-stranded RNAs, and episomal DNA produced from retrotransposons can also impact cellular function[1] (Fig. 1a). When considering potential toxicity of retrotransposon activation in a given system, retrotransposon-derived products must thus be considered in addition to consequences of retrotransposition to genomic DNA."

And the Discussion:

"Similar to human Alzheimer's disease and tau transgenic *Drosophila*, we find an overrepresentation of ERVs among the classes of transposable elements that are elevated at the transcript level in the context of aging and tauopathy in the mouse brain. Increased transcript levels of ERVs are also present in human disorders including but not limited to amyotrophic lateral sclerosis[2,3], multiple sclerosis[4], and various types of cancers[5]."

And:

"Much emphasis in human transposon biology is placed on young, active elements that retain full mobilization potential in the human genome, as they would produce novel insertions if they were to retrotranspose. L1 activation is also reported to induce DNA double-strand breaks[6] and somatic deletions[7]. In addition to consequences of complete and/or failed retrotransposition, the RNAs, double-stranded RNAs (dsRNAs), protein products, and episomal DNA generated from retrotransposons can also affect cellular function. For example, bidirectional transcription of retrotransposons and subsequent dsRNA formation can induce an interferon response through the RNA-sensing innate immune network[8-10], and ERV-encoded proteins can drive autoimmunity[10] and motor neuron disease[11]. We are currently investigating links between transposable activation and induction of the innate immune response in laboratory models of tauopathy and are analyzing de novo transposable element insertions in human tauopathy using long-read sequencing."

2. **Figure 1---"c"—could they explicitly indicate perhaps in the figure legend what the different categories apply to in the pie charts: Is "DNA"—DNA transposons as opposed to retrotransposons.**

The legend of Fig. 1c now reads:

"c. Pie charts representing the proportion of subfamily members within each retrotransposon (SINE, LINE, LTR) and DNA transposon (DNA) family."

3. **While the authors have provided the appropriate detailed information on the ERVs, it is not until the Results section of Fig. 1 that there is the first mention of L1. And there is little information on what exactly L1 is until the Results section of Fig. 5. Perhaps they could include such information in the same place as the other RTEs are being described.**

   We have added the following to the Introduction of the revised manuscript:

   "Intact LINE elements including some LINE-1 (L1) subfamily members are 6-8 kb and harbor two open reading frames encoding proteins with RNA binding, nucleic acid chaperone, endonuclease and reverse transcriptase activities[3,4]. LINE-encoded proteins function to reverse transcribe LINE and SINE RNA and subsequently insert the newly generated LINE or SINE DNA copy into genomic DNA."

4. **In the results section they indicate that RNA levels of L1 increase in the mouse models of neurodegeneration. It is not clear to me whether they were able to also determine if L1 copy number is increased, as they find for ERVs. Would it be possible to determine copy number for L1 as well?**

   L1 DNA copy number does indeed increase in brains of tau transgenic mice based on dPCR and NanoString. The revised manuscript reads:

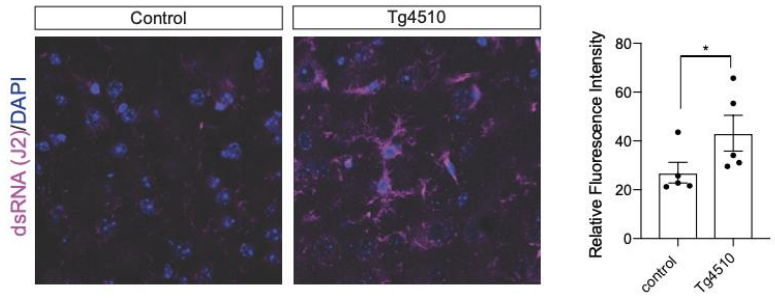
   dPCR: "While L1, IAP, ETn, and B2 DNA copy number are unchanged in brains of tau transgenic mice at two months of age (Fig. 5a), all of these elements with the exception of B2 are significantly increased in brains of tau transgenic mice compared to control by twelve months (Fig. 5b)."

   And:

   NanoString: "At two months of age, rTg4510 have a significant increase in L1 probeset A compared to controls based on NanoString (Fig. 5c, Supplemental Table 2). At twelve months of age, we detect a further three-fold elevation of elements recognized by L1 probeset A, as well as significant elevation of ERVK probesets A-E and ERV1 probeset A in brains of rTg4510 mice compared to controls (Fig. 5d, e, Supplemental Table 2)."

5. **It is noted by the authors that dsRNAs that are encoded by RTEs can also affect cellular function—see introduction and Fig. 1a. Is it possible for the authors to comment on whether dsRNAs are also increased in either the aging brain or tau transgenic mice.**

   Investigating links between pathogenic forms of tau, dsRNA and neuroinflammation is an active area of research in our laboratory. We detect robust elevation of dsRNAs in *Drosophila* and mouse models of tauopathy (see mouse data below) as well as in postmortem human Alzheimer's disease brain. As these data serve as the foundation for another manuscript focused on transposable element-induced neuroinflammation in tauopathy, we prefer not to include this line of investigation in the current manuscript.



6. **In Figure 2, the "phenotype" is missing from the PS19 mouse model.**

   We thank the reviewer for noticing this omission – it has been corrected in the revised manuscript.

7. **It is known that one of the limitations of using RNA-seq to determine RTE expression is that fragments of RTEs may be contained within regions in which there is not a full length active RTE. For example, if fragments of RTEs are embedded within a gene that is activated, perhaps in response to either normal aging or neurodegeneration, it can appear to show an increase in RTE expression that does not accurately reflect RTE activation. It would be useful for the authors to discuss this issue in the manuscript.**

   We have included this limitation in the Discussion of the revised manuscript:

   "Given that transposable element fragments are present within introns of many "normal" protein-coding genes, a caveat of transcriptomic analyses is that transposable elements embedded with introns of pre-mRNAs could be mistakenly attributed to independent transposable element transcripts. As we detect a rather high degree of similarity between transposable element families elevated at the transcript level in RNA-seq data from polyA-enriched and non-polyA-enriched datasets, we do not think that the bulk of the differentially expressed elements in these datasets derive from introns of "normal" genes. Nevertheless, this limitation could be resolved in future studies by long-read sequencing of RNA extracted from various tauopathy conditions."

8. **Does the antibody that was used to detect the gag protein encoded by IAP only or does it also detect IAP-E elements. The IAP-E elements encode the envelope domain that could mediate a transfer between cells. Thus the specificity of the antibody is important to know in regard to the authors statement of a non-cell autonomous IAP activation as noted in Figs 3a and b.**

   The epitope recognized by the IAP-gag antibody used in our studies is also present in IAPE elements.

   The associated Results of the revised manuscript reads:

   "Indeed, the IAP-gag antibody detects an epitope that is present within IAP elements as well as IAPE elements, which harbor an envelope gene that encodes a membrane glycoprotein that facilitates cell-to-cell spread of IAPE capsids[12,13]."

9. **The difficulty of using certain methodologies in determining changes in copy number of RTEs is well known. For both the digital PCR and NanoString approaches the authors suggest that there is an increase in those RTEs known to be active in the mouse genome—at the DNA level. Although the authors do discuss the limitations of these approaches—specifically the inability to discriminate between episomal elements versus those that have integrated into the genome—it may be useful for the them to also indicate other limitations of these approaches, including normalization to single copy genes for NanoString.**

   We have expanded the associated section of the Discussion to more fully discuss caveats of transposable element CNV analyses:

   "Having established that ERVs are elevated at the RNA and protein levels in tau transgenic mice, we next asked if brains of rTg4510 tau transgenic mice have increased retrotransposon DNA content using two complementary approaches. Both approaches identify LINE and ERV subfamily members that are elevated at the DNA level in brains of tau transgenic mice at 12 months of age. While use of both dPCR and NanoString to analyze retrotransposon DNA content contributes to the robustness of our study, each assay has its own inherent limitations. Given the high copy number of transposable elements in the mouse brain that would otherwise saturate dPCR and NanoString assays, the input DNA used was lower than what would be used to analyze a single copy gene. Low DNA input can negatively affect the precision of dPCR and can contribute to variation for NanoString. In addition, NanoString probesets recognize multiple members (active and inactive) of transposon subfamilies due to the high degree of sequence similarity between members of a subfamily. Differences in the absolute copy number of retrotransposons in dPCR versus NanoString-based CNV analyses likely result from the ability of dPCR probes to recognize specific active targets versus the redundant detection of subfamily members by NanoString. We also note that neither assay discriminates between genomic vs. episomal DNA. We thus do not currently know whether the extra retrotransposon DNA copies are integrated into the genome and/or exist in an episomal state. Determining the proportion of extra retrotransposon copies that are genomic versus episomal is an important next step, as genomic insertions generate novel mutations, while episomal DNA could drive a viral response as described in the context of aging, senescence and activation of LINE-1 elements in somatic tissues[14]."

REVIEWER 2

This is an interesting and timely manuscript entitled "Pathogenic tau accelerates aging-associated activation of transposable elements in the mouse central nervous system" by Ramirez et al. The present study strengthens the crucial link between transposable element activity, aging and tau pathology — a hallmark of several neurodegenerative diseases. (i) The authors analyzed RNA-seq datasets at different ages from three different mouse models of tauopathy: rTg4510, JNPL, and PS19. They found that retrotransposon transcripts particularly ERV, increases with age in the brain of all the three mouse models of tauopathy. (ii) Additionally, they found that gag capsid protein, a highly active mouse Class II ERV, is elevated in the brain of rTg4510 tau transgenic mice in an age-dependent manner. (iii) Further, dPCR and NanoString analysis revealed that DNA copy number of mobile retrotransposons with age in the brain of rTg4510 tau transgenic mice. This study offers new groundwork for the link between tau-dependent pathologies and retrotransposon activation; however, further experimental data is required to validate the findings. Overall, this is a well-executed study with proper methods for a purpose; most results are convincing and support the author's conclusion.

1. **In Fig. 1, which strain or gender of mouse was used, please specify.**

   The background of the mice is now noted in the associated text of the Results in the revised manuscript:

   "To determine if transposable elements are differentially expressed as a consequence of aging in the adult mouse brain, we analyzed transposable element transcript levels in forebrain lysates from B6C3HF1 mice aged to six, twelve and twenty months of age based on publicly available RNA-seq data available through the Accelerating Medicines Partnership – Alzheimer's Disease (AMP-AD)."

   Sex information has been added to the legend of Fig. 1. Genetic background and sex for aging analyses are also included in the Methods.

2. **Authors should clarify why they used spinal cord tissues as source for RNA-Seq instead of brain regions primarily involved in neurodegeneration on these mouse models. Why analysis has been performed in cortex of rTg4510 mice and spinal cord of JNPL3 and PS19? Why they did not compared the same brain regions (for example, cortices) in all the three mouse models of tauopathy?**

   The honest answer is that we used what our collaborators (Dr. Ray, Dr. Cao) had already generated or what was publicly available (AMP-AD). AMP-AD data exists for JNPL3 spinal cord because neuronal loss predominates in the spinal cord in that model. While brain is often used for PS19 analyses, Dr. Cao's colony has higher levels of pathology in the spinal cord compared to the brain, as others have reported, and thus spinal cord was used for RNA-seq. The text of the revised manuscript includes our rationale for using spinal cord versus brain for JNPL3 and PS19 RNA-seq analyses:

   "The JNPL3 model features *Prp*-driven expression of human tau^{P301L} on a C57Bl/6, DBA/2, SW mixed genetic background. JNPL3 mice produce human tau protein at a level similar to endogenous mouse tau. As neuronal loss in the spinal cord is a predominant feature of this model[19], we utilized publicly available RNA-seq data from spinal cord of homozygous JNPL3 mice at two, six, and twelve months of age."

   "The PS19 model features a five-fold *Prp*-driven overexpression of the familial tauopathy-associated tau^{P301S} mutation on a C57Bl/6, C3H genetic background. The original publication reports presence of neurofibrillary tangles in the brain and spinal cord by six months and neuronal loss by nine months[15]. As reported by others[16,17], our colony has delayed pathology compared to the original line, with higher levels of pathology in the spinal cord compared to the hippocampus."

3. **Perhaps the authors should consider using tau knock out animals to support tau-dependent activation of retrotransposons.**

   We agree that it would be worthwhile to analyze retrotransposons in tau knockout animals in order to determine what aspect of transposon biology is due to loss of tau function. In fact, studies in mice[18] report that tau knockout affects heterochromatin (similar to what we have reported in human tau transgenic *Drosophila*) that could impact transposable element activation. Unfortunately, there are no publicly available RNA-seq datasets from tau knock-

out mice, and neither we nor our collaborators have tau knock-out colonies. We are, however, well-poised to ask these sorts of questions in *Drosophila.* This would definitely be an interesting avenue of investigation in future studies.

4. **Providing that retrotransposons activation is mediated pathological tau in an age-dependent manner in mouse models of tauopathy, does this observation relevant to human tauopathies? Does the retrotransposons activation correlate with clinical severity in AD patients or other primary tauopathies?**

   In addition to our own work, in which we identify retrotransposons activated at the transcript level (predominantly ERVs and L1 elements) in brains of patients Alzheimer's disease at Braak V/VI and Progressive Supranuclear Palsy, Dr. Josh Shulman and colleagues have analyzed retrotransposon expression levels in the ROSMAP cohort. They find that activation of ERV1, 2, and 3 subfamilies are related to global cognitive performance in the year proximate to death.

   The Introduction of the revised manuscript includes the following text:

   "In human Alzheimer's disease, activation of HERV1, 2, and 3 and L1 retrotransposons are significantly associated with tau tangle burden, and HERV1, 2, and 3 activation is significantly associated with reduced cognitive performance[19]."

5. **How human tau (wild type and mutants) exacerbate activation of the transposable elements? Please provide the proposed model/hypothetical schematics that could be helpful for the next steps to investigate mechanistic insights.**

   The Discussion of the revised manuscript includes the following text in regard to the mechanism underlying tau- induced retrotransposon activation:

   "We have previously reported that pathogenic forms of tau disrupt heterochromatin- and piRNA-mediated silencing of retrotransposons in the *Drosophila* brain[20]. While our current study does not address mechanistic links between pathogenic forms of tau and retrotransposon activation in the mouse brain, depletion of heterochromatin protein 1 in motor neurons of the spinal cord in the JNPL3 mouse model of tauopathy[21] is consistent with our overall hypothesis that pathogenic tau-induced heterochromatin decondensation drives retrotransposon activation. In addition, studies in tau knockout mice suggest that maintaining the integrity of pericentromeric heterochromatin is a physiological function of tau[22]. Investigation into heterochromatin- and piRNA-mediated control over retrotransposons in the aging mouse brain and in mouse models of tauopathies will be the subject of future studies."

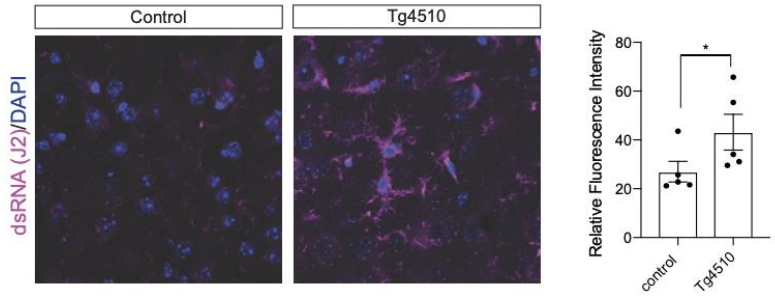
6. **In Fig. 4A, can authors provide molecular weights in the schematics IAP-EPP? This could simplify their identification in the western blots.**

   We completely agree with the reviewer that inclusion of molecular weights for IAP-encoded protein would help readers interpret the data. We had previously used Uniprot and two publications[23,24] that provide estimates for IAP-encoded protein products to create a version of Fig. 4A that includes molecular weights. In general, the size estimation varies widely among Uniprot entries. In addition, IAP and IAPE, both of which are recognized by the IAP-gag antibody, differ in the sizes of proteins that they encode. There are even slight differences in protein sizes among IAP subfamily members. As we do not want to include molecular weights

that are not 100% correct in all cases, we opted to present the figure without molecular weights.

7. **Authors should demonstrate that how retrotransposons activation results in neuronal/synaptic loss or gliosis in the tauopathy mice in an age-dependent manner? Further, authors should discuss the mechanistic insights of tau-dependent retrotransposons activation and subsequent neurodegeneration or cognitive decline.**

We detect robust elevation of dsRNAs in *Drosophila* and mouse models of tauopathy (see mouse data below) as well as in postmortem human Alzheimer's disease brain. As these data serve as the foundation for another stand- alone manuscript focused on transposable element-induced neuroinflammation in tauopathy, we prefer not to include this line of investigation in the current manuscript.



We have added the following paragraph to the Discussion of the revised manuscript:

"Much emphasis in human transposon biology is placed on young, active elements that retain full mobilization potential in the human genome, as they would produce novel insertions if they were to retrotranspose. L1 activation is also reported to induce DNA double-strand breaks[6] and somatic deletions[7]. In addition to consequences of complete and/or failed retrotransposition, the RNAs, dsRNAs, protein products, and episomal DNA generated from retrotransposons can also affect cellular function. For example, bidirectional transcription of retrotransposons and subsequent dsRNA formation can induce an interferon response through the RNA-sensing innate immune network[8-10], and ERV-encoded proteins can drive autoimmunity[10] and motor neuron disease[11]. We are currently investigating links between transposable activation and induction of the innate immune response in laboratory models of tauopathy and are analyzing *de novo* transposable element insertions in human tauopathy using long-read sequencing."

8. **Evidences of direct Tau actions on regulatory sequence of retrotransposons need to be provided to establish its action on neurons transposable elements (Reporter Assay, DNA/RNA binding etc...) to give strength to the conclusions of the study**.

We do not believe that the effect of tau on retrotransposon expression is due to a direct interaction between tau and retrotransposon DNA or RNA. Rather, evidence in *Drosophila* models of tauopathy suggests that the effects of cytoplasmic pathogenic tau on heterochromatin (via the LINC complex and lamin) and piwi/piRNA levels drive transposable element activation[20]. Our new text describing the mechanism of tau-induced retrotransposon activation was included in response 5.

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