EMBO
reports

Tau accumulation activates STAT1 triggering memory deficits via suppressing NMDA receptor expression

Xiao-Guang Li, Xiao-Yue Hong, Ya-li Wang, Shu-Juan Zhang, Jun-Fei Zhang, Xia-Chun Li, Yan-Chao Liu, Dong-Shen Sun, Qiong Feng, Jin-Wang Ye, Yuan Gao, Dan Ke, Qun Wang, Hong-lian Li, Keqiang Ye, Gong-Ping Liu, Jian-Zhi Wang

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

1st Editorial Decision 14th Nov 2018

Thank you for the submission of your research manuscript to EMBO reports. We have now received reports from the three referees that were asked to evaluate your study, which can be found at the end of this email.

As you will see, all referees think the manuscript is of interest, but requires a major revision to allow publication in EMBO reports. All three referees have a number of concerns and/or suggestions to improve the manuscript, which we ask you to address in a revised manuscript. As the reports are below, I will not detail them here, also as I think all of them need to be addressed.

Given the constructive referee comments, I would like to invite you to revise your manuscript with the understanding that all referee concerns must be addressed in the revised manuscript and/or in a detailed point-by-point response. Acceptance of your manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

REFEREE REPORTS:

Referee #1:

This paper identified JAK/STAT signaling with subsequent reduction in NMDAR as a contributor to synaptic and memory deficits in tau transgenic mouse models of neurodegenerative diseases. These findings are novel, timely and presented clearly. The experimental standard is very high. This

reviewer has only minor comments that should be addressed before publication of the manuscript in EMBO reports.

Line 83: Ref 25 should be cited correctly as physiological localization of tau to post-synapses.

Line 364: Since the data in Fig S1 is part of the supplementary, the main text should make clear that these experiments were done in transfected HEK295 cells.

Line 560: Please change the final sentence of the discussion from "...thus induces..." to "...thus contributing to..." to provide a more balanced summary that does not exclude other disease mechanisms.

Figure 4E,F: Please include the levels of control mice that have no hTau expression (but either eGFP or Cre) to compare the up-regulation of NMDAR in hTau+Cre to physiological levels and in the absence of STAT1 per se.

Referee #2:

Accumulation of Tau is possibly one of early steps of aggregates formation ultimately leading to neurofibrillary degeneration. A common neuropathological feature to many neurological disorders together knows as tauopathies. This early accumulation is supposed to have detrimental consequences on neuron functionality. However, early modification of gene expression or signaling pathway activation has not been determined.
In the present manuscript Li and collaborators address this question using multidisciplinary and complementary approach. The results are very interesting and suggest that accumulation of Tau is associated with a JAK2 / STAT1 activation process which is detrimental to neuronal plasticity and cognitive functions.

A major concern arises from this study. One hTau expressing system is used to demonstrate the mechanism of JAK2 / STAT1 early activation and consequent repression of NMDA receptor expression. Authors claims that this mechanism is specific to hTau. However, to demonstrate such a specificity additional experiments are needed.

If this mechanism is specific to hTau, what isoform was used in this study? What part of Tau is necessary to induce this mechanism. Either authors should use different isoforms of Tau or generate fragments of Tau to identify the minimal fragments that induce an increase STAT1 expression. How hTau is inducing STAT1 expression is also not addressed and therefore the mechanism is not fully addressed. Is STAT1 increased expression an epigenetic mechanism? To further demonstrate that STAT1 activation is specific to Tau, a non-related protein should be overexpressed in this system. Synuclein and TDP-43 are suggested.

In Figure 1 a significant increase of STAT1 is shown. This increase of STAT1 expression is not reflect by the western-blot of figure 3C. An increase phospho-STAT1 at tyrosine 701 is clearly observed as well as the enrichment of this activated form in the nucleus. Authors used a student-t test for the comparison of means and standard deviations. A non-parametric test such Mann-Whitney is preferred because of the low number of repeated experiments. This is even more important for the statistical analysis used in animal paradigms.

In figure 1G, the experimental control is eGFP. This is not sufficient to demonstrate that the activation of STAT1 is specific to hTau expression. Overexpression of proteins prone to form aggregates such as synuclein or TDP-43 should be used as a control of the specificity.

In figure 2, on would expect to see an increase nuclear staining of STAT1 and phospho-STAT1 in the nuclei of AD brain tissue. Could author comment? Pictures selected are showing a cytoplasmic staining. Western blot should also be performed to demonstrate that phospho-STAT1 is increase in AD brain tissue. If Western-blots are not possible a double-labelling of neurofibrillary degeneration and phospho-STAT1 should be performed to show an increase staining in degenerating neurons. A tau and amyloid staining would also suitable to show the presence of neurofibrillary tangles and

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amyloid plaques in the AD tissue and the absence in the ctrl brain tissue.

In figure 3 and 4, several control experiments are lacking. What is the basal expression of STAT1 in naïve animal as well as in CRE animals. In figure 3, eGFP expression was used as a control but this expression pattern of either hTau or eGFP is not shown (immunohistochemistry and western blots)

Importantly, in figure 4 a control of CRE expression alone should be used to demonstrate that NMDA receptor are under the control of STAT1 expression and that loss CRE expression also reduce the basal level of STAT1 expression when compared to eGFP or hTau overexpression.

Referee #3:

In this manuscript, Li et al. provide a mechanistic explanation of tau mediated alterations in synaptic deficit and memory impairment. They show that expression of human tau activates the neuronal Jak2/Stat1 pathway and further Stat1, by directly binding to GAS elements in the promoter regions of GluN1, GluN2A and GluN2B in the neurons, suppresses the activity of NMDAR. This leads to impaired fear memory, spatial memory and synaptic plasticity. Using a dominant negative mutant Stat1 construct that restores levels of GluN1, GluN2A and GluN2B, the authors show that overexpression of human tau in mouse hippocampus does not result in previously observed tau-associated memory deficit and LTP suppression. This establishes the mechanistic link.

In previous studies, amyloid beta has been shown to result in learning and memory impairment by regulating Stat1 and laminin beta1. The authors here show that laminin beta1 is not associated in the effects observed in this study. Previous studies have also shown that tau fragments (result of proteolysis) can bind active Stat1 and activating Stat1 mediated transcription. This is very important and has broad implications in terms of Alzheimer's pathogenesis. One pertinent question therefore is whether the authors in this present study observed anything similar - whether htau expression resulted in tau proteolysis and whether these tau fragments (not full length tau per se) contributed to the Stat1 mediated effects on NMDAR function.

More importantly, the authors have stated that htau accumulation is the trigger - there is no clear data presented to show this 'accumulation' - are these sarkosyl insoluble, silver positive tangles, pre-tangles or simply accumulation of phospho tau?

In the AAV-cre experiments, three crucial points stick out: 1) absence of AAV-cre as a control (not just eGFP as shown by authors), 2) absence of data showing that AAV-tau and AAV-cre are actually co-expressed in the same cells and 3) lack of data on ptau and conformationally altered tau (immunohistochemistry or western). In Fig. 3A, the demonstration of Stat1 knockdown by immunohistochemistry is not convincing as the panels have completely different background hues. On a similar note, in the shRNA experiments, the non-relevant shRNA control is missing.

Another point that needs clarification is whether Stat1 activation by tau results in secretion of soluble factors from the neurons that might result in non cell autonomous changes in glia? Could the authors show whether neuronal Stat1 activation results in increased levels of canonical products associated with generalized neuroinflammation?

Minor points:
1) please provide molecular weight markers on westerns.
2) please provide patient demographic details - what were the clinical diagnosis, age, Braak staging, etc.
3) please explain why htau is in the nuclear fraction (Fig 1E) - is there a precedence?
4) for fear conditioning test data, is this the result of context or tone fear memory?
5) is the tau tagged with GFP? What isoform?
6) is there a change in mouse tau?
7) in supplementary figures 6C and 10, housekeeping standard (actin) seems missing.
8) in Fig 2F, pStat1 is mostly in the cytoplasm - please explain the absence of nuclear pStat1
9) minor typos such as 'alternation' instead of 'alteration', etc. need to be fixed

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1st Revision - authors' response 24th Jan 2019

The point-by-point responses to reviewers' critiques are as follows.

Referee #1:

This paper identified JAK/STAT signaling with subsequent reduction in NMDAR as a contributor to synaptic and memory deficits in tau transgenic mouse models of neurodegenerative diseases. These findings are novel, timely and presented clearly. The experimental standard is very high. This reviewer has only minor comments that should be addressed before publication of the manuscript in EMBO reports.

Line 83: Ref 25 should be cited correctly as physiological localization of tau to post-synapses.

Response: Thank you very much for your positive comments. As suggested, we have rephrased the sentences with correct citations as follows: Tau proteins are largely located in the neuronal axons in physiological conditions [22]; it is also reported that the post-synaptic location of Fyn is tau-dependent [23], suggesting the dendritic distribution of tau (please see page 4, line 83-87).

Line 364: Since the data in Fig S1 is part of the supplementary, the main text should make clear that these experiments were done in transfected HEK295 cells.

Response: As suggested, we have added the following sentence: To test this, we first conducted a whole-genome mRNA chip screening in hTau-transfected HEK293 cells (please see page 18, line 364-366)

Line 560: Please change the final sentence of the discussion from "...thus induces..." to "...thus contributing to..." to provide a more balanced summary that does not exclude other disease mechanisms.

Response: We have rephrased the sentence as suggested, thank you.

Figure 4E,F: Please include the levels of control mice that have no hTau expression (but either eGFP or Cre) to compare the up-regulation of NMDAR in hTau+Cre to physiological levels and in the absence of STAT1 per se.

Response: As suggested, we have done additional experiments and added the new data in the revised paper: By quantitative analysis, we observed that knockdown STAT1 by AAV-Cre restored tau-induced reduction of GluN1 to 91%, of GluN2A to 87% and of GluN2B to 73% when compared to the AAV-eGFP control (please see Appendix Fig. S5D). Furthermore, knockdown of STAT1 by expressing AAV-Cre in STAT1$^{flox/flox}$ mice without overexpressing hTau increased the protein levels of GluN1 to 149%, of GluN2A to 201%, and of GluN2B to150% when compared with AAV-eGFP controls (please see Appendix Fig. S5C-D).

Referee #2:

Accumulation of Tau is possibly one of early steps of aggregates formation ultimately leading to neurofibrillary degeneration. A common neuropathological feature to many neurological disorders together knows as tauopathies. This early accumulation is supposed to have detrimental consequences on neuron functionality. However, early modification of gene expression or signaling pathway activation has not been determined.

In the present manuscript Li and collaborators address this question using multidisciplinary and complementary approach. The results are very interesting and suggest that accumulation of Tau is associated with a JAK2 / STAT1 activation process which is detrimental to neuronal plasticity and cognitive functions.

A major concern arises from this study. One hTau expressing system is used to demonstrate the mechanism of JAK2 / STAT1 early activation and consequent repression of NMDA receptor expression. Authors claims that this mechanism is specific to hTau. However, to demonstrate such a specificity additional experiments are needed.

If this mechanism is specific to hTau, what isoform was used in this study?

Response: We clarified that the wildtype full-length human tau (hTau, also termed tau441 or tau40 or 2N4R) was used for the study (please see page 6 line 114; page 7 line 132).

What part of Tau is necessary to induce this mechanism. Either authors should use different isoforms of Tau or generate fragments of Tau to identify the minimal fragments that induce an increase STAT1 expression.

Response: As suggested, we constructed various truncated tau plasmids covering different length of N-terminal and C-terminal tau fragments. After expressed these tau fragments in HEK293 cells, we detected the protein levels of STAT1 and p-STAT1. The results showed that the N-terminal tau (tau1-368, tau1-255, tau1-197) but not C-terminal (tau256-441) could activate STAT1 (Fig. EV1A-B, D-E); the currently identified minimal fragment able to induce STAT1 activation was tau1-197 while the shorter tau fragments including tau1-44, tau1-150, tau121-150 and tau121-197 had no stimulating effect on STAT1 (Fig. EV1A-B, D-E).

How hTau is inducing STAT1 expression is also not addressed and therefore the mechanism is not fully addressed. Is STAT1 increased expression an epigenetic mechanism?

Response: To address your question, we measured mRNA and protein levels of STAT1. The result showed that overexpressing hTau increased both protein and mRNA levels of STAT1 (please see attached Figure below). A previous study demonstrated that overexpressing hTau induced histone acetylation (Mol Neurodegener. 2017 May 4;12(1):34. doi: 10.1186/s13024-017-0178-8), therefore, the upregulated STAT1 expression may involve epigenetic mechanism. We have discussed this in the revised paper (please see page 26, line 554-558).

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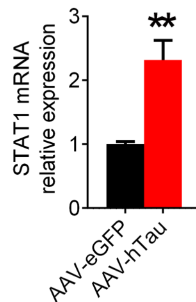

Figure legend: Overexpression of hTau upregulated mRNA levels STAT1 by qRT-PCR in mice hippocampal tissues. **, p<0.01 vs AAV-eGFP. Data were presented as mean ± SD (Mann-Whitney test).

To further demonstrate that STAT1 activation is specific to Tau, a non-related protein should be overexpressed in this system. Synuclein and TDP-43 are suggested.

Response: As suggested, we studied the effects of TDP-43 and α-synuclein on STAT1 expression and phosphorylation in HEK293 cells and primary hippocampal neurons, respectively. The results showed that overexpression of TDP-43 or α-synuclein did not significantly change the levels of STAT1 and p-STAT1 and as well as the protein levels of NMDARs (Fig. EV1), indicating that STAT1 activation may be specific to tau. We have added this in the revised paper (please see page 19, line 387-390).

In Figure 1 a significant increase of STAT1 is shown. This increase of STAT1 expression is not reflect by the western-blot of figure 3C.

Response: To address your critiques, we have replaced the blots with the more representative ones which evidently show upregulation of STAT1 by hTau (please see Fig. 6C-E).

An increase phospho-STAT1 at tyrosine 701 is clearly observed as well as the enrichment of this activated form in the nucleus. Authors used a student-t test for the comparison of means and standard deviations. A non-parametric test such Mann-Whitney is preferred because of the low number of repeated experiments. This is even more important for the statistical analysis used in animal paradigms.

Response: As suggested, we also used Mann-Whitney test for the statistical analyses of pSTAT1 and animal studies (please see the New legends), and the results stand for our previous conclusions.

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In figure 1G, the experimental control is eGFP. This is not sufficient to demonstrate that the activation of STAT1 is specific to hTau expression. Overexpression of proteins prone to form aggregates such as synuclein or TDP-43 should be used as a control of the specificity.

Response: As suggested, we have done additional experiments by overexpressing α-synuclein and TDP-43, the widely recognized misfolding-apt proteins. Unlike hTau, overexpressing TDP-43 and α-synuclein had no significant effect on STAT1 and NMDARs levels in HEK293 cells and primary neurons (please see Fig. EV1A-C).

In figure 2, on would expect to see an increase nuclear staining of STAT1 and phospho-STAT1 in the nuclei of AD brain tissue. Could author comment? Pictures selected are showing a cytoplasmic staining.

Response: To address your critiques, we have replaced the images with more representative ones (please see New Fig. 2F).

Western blot should also be performed to demonstrate that phospho-STAT1 is increase in AD brain tissue. If Western-blots are not possible a double-labelling of neurofibrillary degeneration and phospho-STAT1 should be performed to show an increase staining in degenerating neurons. A tau and amyloid staining would also suitable to show the presence of neurofibrillary tangles and amyloid plaques in the AD tissue and the absence in the ctrl brain tissue.

Response: Thank you for your suggestion. We have done additional experiments to measure pSTAT1 level in the AD brain by Western blotting, and the increased pSTAT1 in the AD patients was detected when compared with the age-matched controls (please see New Fig. 2G). The detailed information of human brain tissues used in the present study was listed in the Appendix Table S4.

In figure 3 and 4, several control experiments are lacking. What is the basal expression of STAT1 in naïve animal as well as in CRE animals.

Response: Thanks you for pointing out the insufficiency. As suggested, we have done additional experiments. No significant difference of STAT1 was found between wildtype and naïve $STAT1^{flox/flox}$ mice by expressing AAV-eGFP, and infection of AAV-CRE alone did not change the STAT1 and pY-STAT1 levels in wildtype mice (please see Appendix Fig. S5B).

In figure 3, eGFP expression was used as a control but this expression pattern of either hTau or eGFP is not shown (immunohistochemistry and western blots).

Response: We have added the expression pattern of eGFP and eGFP-hTau measured by immunohistochemistry and Western blotting (please see New Fig. 3A, B).

Importantly, in figure 4 a control of CRE expression alone should be used to demonstrate that NMDA receptor are under the control of STAT1 expression and that loss CRE expression also reduce the basal level of STAT1 expression when compared to eGFP or hTau overexpression.

Response: To address your critiques, we have done additional experiments. The results showed that expression of AAV-Cre in $STAT1^{flox/flox}$ indeed significantly decreased STAT1 level compared with

eGFP or hTau group (please see Appendix Fig. S5D), and knockdown STAT1 by AAV-Cre in STAT1$^{flox/flox}$ mice remarkably unregulated NMDARs level (please see Appendix Fig.S5C, D).

Referee #3:

In this manuscript, Li et al. provide a mechanistic explanation of tau mediated alterations in synaptic deficit and memory impairment. They show that expression of human tau activates the neuronal Jak2/Stat1 pathway and further Stat1, by directly binding to GAS elements in the promoter regions of GluN1, GluN2A and GluN2B in the neurons, suppresses the activity of NMDAR. This leads to impaired fear memory, spatial memory and synaptic plasticity. Using a dominant negative mutant Stat1 construct that restores levels of GluN1, GluN2A and GluN2B, the authors show that overexpression of human tau in mouse hippocampus does not result in previously observed tau-associated memory deficit and LTP suppression. This establishes the mechanistic link.

In previous studies, amyloid beta has been shown to result in learning and memory impairment by regulating Stat1 and laminin beta1. The authors here show that laminin beta1 is not associated in the effects observed in this study. Previous studies have also shown that tau fragments (result of proteolysis) can bind active Stat1 and activating Stat1 mediated transcription. This is very important and has broad implications in terms of Alzheimer's pathogenesis. One pertinent question therefore is whether the authors in this present study observed anything similar - whether htau expression resulted in tau proteolysis and whether these tau fragments (not full length tau per se) contributed to the Stat1 mediated effects on NMDAR function.

Response: As mentioned by the reviewer, previous studies had shown that Aβ could induce learning and memory impairments by upregulating STAT1 and β-laminin1 (Neuropsychopharmacology. 2014 Feb;39(3):746-58). Here we did not see change of β-laminin1 by overexpression of hTau (please see Appendix Fig.S6), indicating that tau affects the neural metabolisms/functions and cognitive function with the mechanisms different from Aβ. We have discussed these in the revised paper (please see page 24, line 503-505). As suggested, we did measure the effects of full-length tau and the truncated fragments on STAT1. The results showed that overexpressing both full-length and the N-terminal tau proteins could activate STAT1 (please see Fig. EV1A-B,D-E). However, we did not see significant increase of tau1-368 after hippocampal neuronal overexpression of full-length tau for one month (please see attached Figure below), suggesting that accumulation of full-length tau alone in hippocampus may be sufficient to activate STAT1. We have discussed matter in the revised paper (please see page 19, line 387-397).

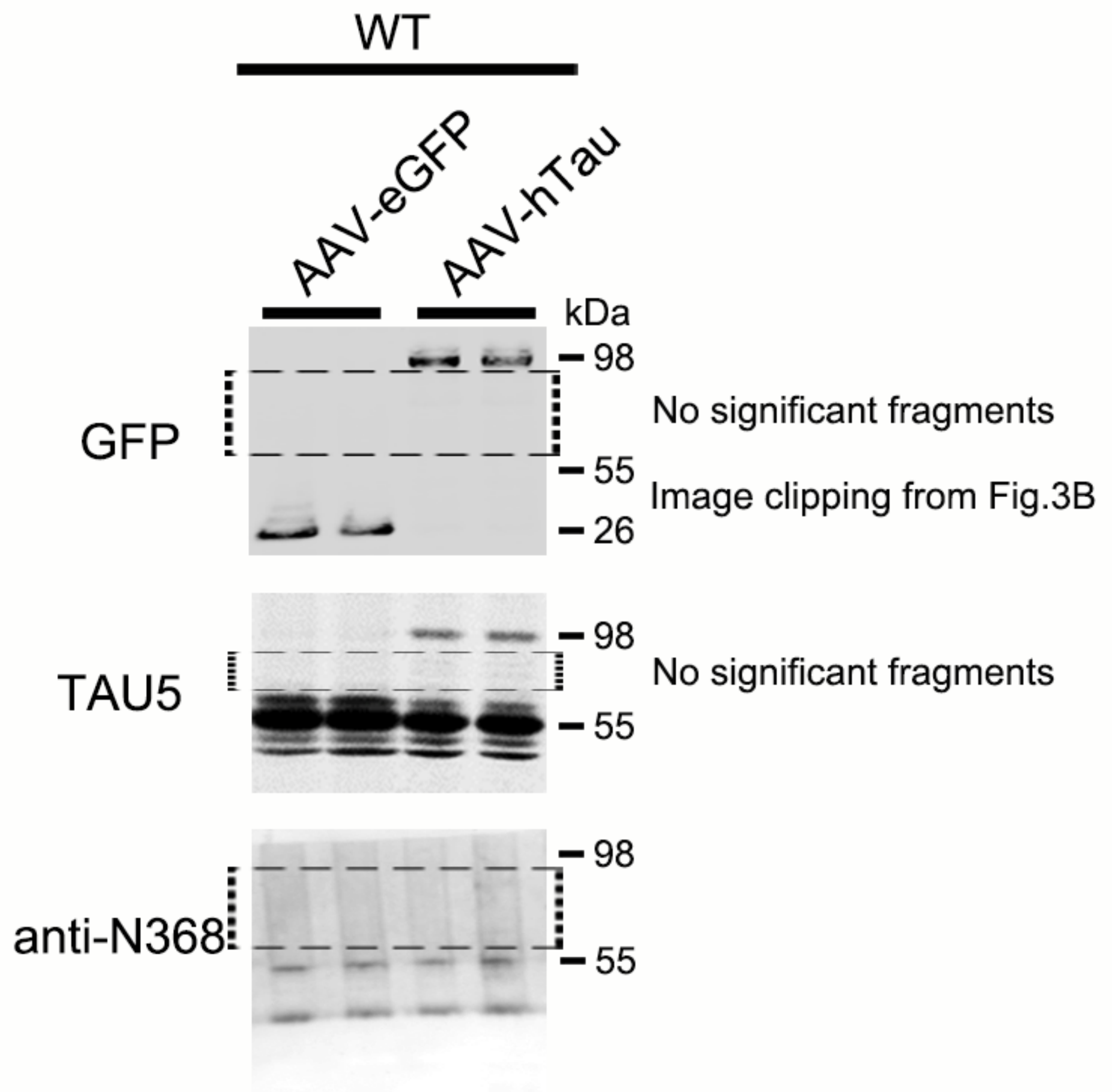

Figure legend: Adeno-associated virus (AAV)-eGFP expressing human full-length tau (AAV-eGFP-hTau) or AAV-eGFP (1.13×1013 v.g./ml) was stereotaxically injected into the hippocampal CA3 of 3 m-old C57 mice. One month later, GFP, TAU5 (reacts with total tau), tau-1-368 (anti cleaved tau 1–368 residue but not full-length tau) antibodies were used to detect potential hTau proteolysis fragments.

More importantly, the authors have stated that htau accumulation is the trigger - there is no clear data presented to show this 'accumulation' - are these sarkosyl insoluble, silver positive tangles, pre-tangles or simply accumulation of phospho tau?

Response: To address your critiques, we have done additional experiments to measure the intracellular aggregation of the misfolded tau by Thioflavin-S staining. The result showed that transfection of AAV-hTau significantly enhanced Thioflavin-S staining (please see Fig. EV2B). Simultaneously, both soluble and sarkosyl insoluble levels of the phosphorylated tau at Ser214, Ser396, Ser404 and Thr231 were also increased by overexpressing hTau (please see Appendix Fig. S8A, B).

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In the AAV-cre experiments, three crucial points stick out: 1) absence of AAV-cre as a control (not just eGFP as shown by authors),

Response: To address your critiques, we have done additional experiments. No significant difference of STAT1 was found between wildtype and naïve STAT1$^{flox/flox}$ mice in the absence of AAV-cre, and infection of AAV-CRE alone did not significantly alter the STAT1 and pY-STAT1 levels in wildtype mice (please see Appendix Fig. S5B). On the other hand, knockdown STAT1 in STAT1$^{flox/flox}$ mice significantly increased the expression of NMDARs (please see Appendix Fig. S5C, D).

2) absence of data showing that AAV-tau and AAV-cre are actually co-expressed in the same cells and

Response: To address your critiques, we have done additional experiments. Abundant co-expression of AAV-hTau and AAV-Cre (which shown no positive-STAT1 immunoreactivity) in the same cell was detected (Appendix Fig. S5A).

3) lack of data on ptau and conformationally altered tau (immunohistochemistry or western).

Response: To address your critiques, we have done additional experiments. Accumulation of misfolded hTau was shown in hippocampal CA3 subset measured by Thioflavin-S staining (please Fig. EV2B), accompanying with the increased p-hTau level in soluble and sarkosyl insoluble fractions measured by Western blotting (please see Appendix Fig. S8A, B).

In Fig. 3A, the demonstration of Stat1 knockdown by immunohistochemistry is not convincing as the panels have completely different background hues.

Response: To address your critiques, we have replaced the images with better quality of background staining (please see New Fig 3A).

On a similar note, in the shRNA experiments, the non-relevant shRNA control is missing.

Response: As suggested, we have added the control by additional experiments (please see in New Fig. 6I, J).

Another point that needs clarification is whether Stat1 activation by tau results in secretion of soluble factors from the neurons that might result in non cell autonomous changes in glia? Could the authors show whether neuronal Stat1 activation results in increased levels of canonical products associated with generalized neuroinflammation?

Response: As suggested, we measured the influence of hTau overexpressing on microglia and the role of STAT1 activation. The results showed that overexpression of AAV-hTau significantly activated microglia measured by immunohistochemical staining using anti-Iba1 and CD68, simultaneously, the levels of IL-1α and IL-6 were increased measured by ELISA (please see attached Figure); however, simultaneous knockdown of STAT1 by expressing AAV-Cre did not restore the inflammatory responses induced by hTau (please see attached Figure). These data

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suggest that overexpression of hTau may induce inflammation independent of STAT1 elevation. The detailed mechanisms may deserve a separate investigation.

Figures for referees removed.

(A, B) The mixture of AAV-hTau (1.13×1013 v.g./ml) and AAV-Cre (5×1012 v.g./ml) (1μl AAV-hTau plus 2μl AAV-Cre) was stereotaxically infused into the hippocampal CA3 of 3 m-old STAT1flox/flox mice. One month later, the levels of IL-1α and IL-6 in the hippocampal CA3 subset were detected by ELISA. *, p<0.05, **, p<0.01 vs eGFP. N=3 each group. Data were presented as mean ± SD (one-way ANOVA, Bonferroni's post hoc test).

(C, D) AAV-hTau or the empty vector (eGFP) (1.13×1013 v.g./ml) was stereotaxically injected into the hippocampal CA3 of 3 m-old C57 mice. One month later, the knockdown efficiency of STAT1 was confirmed (see Fig. 2A, B). The activated microglia was detected by immunohistochemical staining using anti-Iba1 or CD68 antibody. Scale bar, 200 μm (left panels); 100 μm (right panels).

Minor points:

1) please provide molecular weight markers on westerns.

Response: Thank you for your suggestion, and we have added molecular weight markers on all Western blots.

2) please provide patient demographic details - what were the clinical diagnosis, age, Braak staging, etc.

Response: Thank you for your suggestion, and the demographic details of the human brain tissues used in the present study were listed in the Appendix Table S4.

3) please explain why htau is in the nuclear fraction (Fig 1E) - is there a precedence?

Response: Localization of tau in the nucleus/nucleolus and its interaction with DNA and other nuclear proteins have been widely observed (Biomolecules. 2016 Jan 7;6(1):9). Both in vitro and in vivo studies show that the binding of tau to DNA plays a protective role in oxidative stress (PLoS One. 2008 Jul 2;3(7):e2600) and heat-stress conditions (J Biol Chem. 2011 Feb 11;286(6):4566-75). Furthermore, phosphorylation of tau at Thr212/Ser214 (probed by AT100) progressively increases in the nuclei of neuronal and non-neuronal cells during aging, and it decreases in the more severe AD stages (Brain Res. 2017 Dec 15;1677:129-137). We also observed that the phosphorylated tau at Thr205 and Ser214 was predominantly detected in the nuclear fraction after transient transfection of hTau plasmid in HEK293 cells (Neurosci Bull. 2018 Apr;34(2):261-269), which is consistent with

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the results observed in the current study (Fig 1E). The precedence relation between nuclear localization of tau and activation of STAT1 may deserve further investigation.

4) for fear conditioning test data, is this the result of context or tone fear memory?

Response: We are sorry for the unclear explanation. The contextual fear conditioning test was used in the current study (please see page 8, line 158; page 21, line 431).

5) is the tau tagged with GFP? What isoform?

Response: We are sorry for the unclear explanation. We used wildtype full-length human tau (hTau, also termed tau441 or tau40 or 2N4R) fused with eGFP in the N-terminal (please see page 6 line 114; page 7 line 132).

6) is there a change in mouse tau?

Response: In hTau and STAT1 inactivating groups, the change of the endogenous mouse tau was not significant (please see Appendix Fig. S8).

7) in supplementary figures 6C and 10, housekeeping standard (actin) seems missing.

Response: We have added the blots of housekeeping standard (DM1A) (please see Fig. EV2C and Appendix Fig. S4C), thank you.

8) in Fig 2F, pStat1 is mostly in the cytoplasm - please explain the absence of nuclear pStat1

Response: The pSTAT1 has been mostly located in the nuclear compartment from our observations, therefore, we replaced pSTAT1 images with more representative ones (please see New Fig. 2E, F).

9) minor typos such as 'alternation' instead of 'alteration', etc. need to be fixed

Response: We are sorry for the typos, and we have carefully revised the paper to maximally kill the typos.

2nd Editorial Decision 26th Feb 2019

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from two of the three referees that were asked to re-evaluate your study (you will find below). Referee #2 was not responsive, thus we have to proceed with two referees. As you will see, the remaining referees now support the publication of your manuscript in EMBO reports. However, referee #3 has remaining concerns or further suggestions, we ask you to address in a final revised version of your manuscript.

We also asked one of the remaining referees to assess if the points by referee #2 have been adequately addressed during the revision. The referee stated that this is the case. Nevertheless, s/he had these concerns/suggestions, we also ask you to address in the final revision:

- Please add the figure showing that overexpressing hTau increases mRNA levels of STAT1 to the main manuscript.

- In your point-by-point response to referee #2 you state that the overexpression of TDP-43 or α-synuclein did not significantly change the levels of STAT1 and p-STAT1 and as well as the protein levels of NMDARs. This contradicts the fact that alpha synuclein overexpression affects STAT1

regulated pathways (PMID: 27147665). Please comment, and discuss this in the final manuscript text.

- In your point-by-point response to referee #2 you state that overexpressing TDP-43 and α-synuclein had no significant effect on STAT1 and NMDARs levels in HEK293 cells and primary neurons. This is inconsistent with reports that synuclein overexpression affects NMDAR levels (PMID: 26392130). Please comment, and discuss this in the final manuscript text.

Further, I have these editorial requests, which I ask you to also address in the final revised version of the manuscript:

REFEREE REPORTS:

Referee #1:

This reviewer is happy with the changes made to the manuscript and suggests publication as is.

Referee #3:

This is a revised manuscript from Li et al. In this manuscript, Li et al. show that tau-induced synaptic deficits and memory impairment is dependent on Stat1 mediated suppression of NMDAR expression.
The authors have been extremely responsive to all the comments and have managed to redo all the controls that were missing in the original submission.

Some minor comments:

1) In light of the 'negative' data with tau1-368 antibody which is surprising given the robust effect on other pathological effects, please test whether there is any caspase cleaved tau (Tau C3 antibody).
2) A confusing aspect was that if their htau construct is tagged with eGFP (Page 6, line 114), why would the authors choose to do ThioS staining to determine whether there is any tau aggregation? Additionally, how did they differentiate between the green immunofluorescence of ThioS and htau-eGFP?
3) ThioS does not recognize pre-tangles efficiently. Please provide pre-tangle data in AAV-htau injected animals using antibodies or silver staining.
4) Another confusing aspect is that injection of AAV-cre is expected to downregulate Stat1, suppress cytokine expression and generally lower neuroinflammation (mimicking Stat1 KO mice). However, it seems that the levels of cytokine levels (IL6 and IL1b) as well as Iba-1 and CD68 staining is upregulated to similar levels in AAV-htau and AAV-htau+cre injected animals compared to control AAV-eGFP injected animals. Please comment.
5) In the Table S4, why is a NFT-BraakIII patient (E13-27) considered as control 'normal' sample? Please provide the Braak staging of the AD patients. Please provide the sex of all the patient cohorts.

2nd Revision - authors' response — 20th Mar 2019

Referee #1:
This reviewer is happy with the changes made to the manuscript and suggests publication as is.
Response: Thank you very much for your positive comments.

Referee #3:
This is a revised manuscript from Li et al. In this manuscript, Li et al. show that tau-induced synaptic deficits and memory impairment is dependent on Stat1 mediated suppression of NMDAR expression.
The authors have been extremely responsive to all the comments and have managed to redo all the controls that were missing in the original submission.

Response: Thank you very much for your positive comments.

Some minor comments:

1) In light of the 'negative' data with tau1-368 antibody which is surprising given the robust effect on other pathological effects, please test whether there is any caspase cleaved tau (Tau C3 antibody).

Response: We transfected vector, tau1-441 or tau1-421 (Tau C3 positive ctrl) in HEK293 cells. Western blots showed very limited of tau C3 fragments exist in tau1-441 group (Please see the attached fig below). Whether these small fractions of tau C3 fragments play a role in JAK2/STAT1/NMDARs axis may need further investigation.

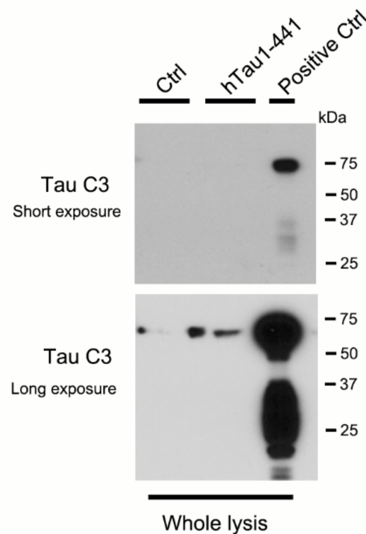

Figure legend: HEK293 cells were transfected with hTau1-441, hTau1-421 or its empty vector (Ctrl) for 48 h. Tau C3 antibodies were used to detect potential hTau proteolysis fragments.

2) A confusing aspect was that if their htau construct is tagged with eGFP (Page 6, line 114), why would the authors choose to do ThioS staining to determine whether there is any tau aggregation? Additionally, how did they differentiate between the green immunofluorescence of ThioS and htau-eGFP? ThioS does not recognize pre-tangles efficiently. Please provide pre-tangle data in AAV-htau injected animals using antibodies or silver staining.

Response: During the ThioS staining procedure, we floated brain slices in bleaching solution and blocking solution which could bleach fluorescence signal by oxidizing the molecules/complex responsible for emitting fluorescence (please see page 28 line 604 to 612), the method was also used to show amyloid plaques in previously published paper (Mol Psychiatry. 2018 Oct 31. doi: 10.1038/s41380-018-0286-z). We also applied Bielschowsky silver staining to analyze pre-tangles in AAV-hTau tissues (please see new EV. 2B)

3) Another confusing aspect is that injection of AAV-cre is expected to downregulate Stat1, suppress cytokine expression and generally lower neuroinflammation (mimicking Stat1 KO mice). However, it seems that the levels of cytokine levels (IL6 and IL1b) as well as Iba-1 and CD68 staining is upregulated to similar levels in AAV-htau and AAV-htau+cre injected animals compared to control AAV-eGFP injected animals. Please comment.

Response: It is well known that STAT1 plays a role in immune response (Nat Rev Immunol 2003; 3(11): 900-911), and inhibition of neuroinflammation ameliorates learning and memory deficits in AD animal models (Prog Neurobiol 2016; 144: 142-157; Nat Commun 2015; 6: 7967. J Neuroinflammation 2012; 9: 35). We also found that pan-neuronal overexpression of hTau activated glia cells with significantly increased levels of IL-6 and IL-1α, but simultaneous knockdown of STAT1 did not restore the inflammatory factors. These data suggest that hTau accumulation induces inflammation with STAT1-independent manner.

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5) In the Table S4, why is a NFT-BraakIII patient (E13-27) considered as control 'normal' sample? Please provide the Braak staging of the AD patients. Please provide the sex of all the patient cohorts.
Response: AD patients are diagnosed mainly dependent on NINCDS-ADRDA, Braak stage, and so on. However, a few control people also have pathophysiological change as Brakk stage (Carlson JO, et al. Antemortem Prediction of Braak Stage. J Neuropathol Exp Neurol. 2015 Nov;74(11):1061-70).
We added information of Braak stages and sex of all the patient cohorts (Please see new Appendix Table S4). As the Primary Neuropathologic Diagnosis defined patient (E13-27) as control group, and NFT-BraakIII was a Secondary Neuropathologic Diagnosis.

3rd Editorial Decision 11th Apr 2019

I have now received the report of referee #3, who was asked to assess the final revised manuscript. As you will see, the referee now supports the publication of your study. I am thus very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

REFEREE REPORTS:

Referee #3:

The authors have responded to all the queries.

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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name:Jian-Zhi Wang
Journal Submitted to: EMBO Reports
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Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes (Do not worry if you cannot see all your text once you press return)

Question	Answer
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For WB,qPCR,Luciferase assay in HEK293 cells and primary neurons:4 biological replicates and more than 3 technical replicates in all cases.For WB,qPCR,CHIP-qPCR in mice:5 biological replicates and more than 3 technical replicates in all cases.For animal behavior tests: more then 8 mice per group were used. For staining (IHC/IF) in animal :more then 3 mice per group, and more than 5 sections per animal. For electrophysiological analysis in animal:more then 4 mice per group, and more than 5 slice per animal.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For animal staining(IHC/IF):more then 3 mice per group, and more than 5 sections per animal.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Male WT C57 mice or STAT1flox/flox C57 mice were randomised to two/three groups before virus injection. All WT C57 mice or STAT1flox/flox C57 mice were 8 weeks old. And htau tg mice were 9M or 12M matched with control mice.
For animal studies, include a statement about randomization even if no randomization was used.	Male WT C57 mice or STAT1flox/flox C57 mice were randomised to two/three groups before virus injection.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No specific action was taken rather that the randomization of all animals within the 3 groups of study.
4.b. For animal studies, include a statement about blinding even if no blinding was done	We used double-blinding in animal behavior studies.
5. For every figure, are statistical tests justified as appropriate?	Yes, we use Mann-Whitney test for two-group comparison, one-way ANOVA or two-way repeated measures ANOVA followed by Bonferroni's post hoc test for multigroup analysis.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Data were expressed as mean ± SD or mean ± SEM and analyzed using SPSS 12.0 statistical software (SPSS Inc. Chicago, IL, USA). Statistical analysis was performed using Mann-Whitney test (two-group comparison), one-way ANOVA or two-way repeated measures ANOVA followed by Bonferroni's post hoc test. The level of significance was set at p<0.05.
Is there an estimate of variation within each group of data?	Yes.

Is the variance similar between the groups that are being statistically compared?	Yes, the variance was similar.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	All antibodies listed in the Appendix Table S3.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	All included in the method section.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Male WT C57 mice(8 weeks) were purchased from the animal center of Tongji Medical College, Huazhong University of Science and Technology. Male STAT1flox/flox (signal transducer and activator of transcription 1) mutant mice (B6; 129S-STAT1tm1Mam/Mmjax) and hTau transgenic mice (STOCK Mapttm1(EGFP) Klt Tg(MAPT)8cPdav/J) were purchased from Jackson lab. All mice were kept at 24 ± 2 ºC on daily 12 h light-dark cycles with ad libitum access to food and water.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal experiments were performed according to the 'Policies on the Use of Animals and Humans in Neuroscience Research' revised and approved by the Society for Neuroscience in 1995, and the Guidelines for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of the People's Republic of China, and the Institutional Animal Care and Use Committee at Tongji Medical College, Huazhong University of Science and Technology approved the study protocol.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	All animal experiments were compliant to ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	The study was approved by the Biospecimen Committee. AD was diagnosed according to the criteria of the Consortium to Establish a Registry for AD and the National Institute on Aging. Diagnoses were confirmed by the presence of amyloid plaques and neurofibrillary tangles in formalin-fixed tissue. Informed consent was obtained from the subjects.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Informed consent was obtained from the subjects.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	The human brain tissues used in the present study were provided by Dr. K Ye of the Emory University School of Medicine, USA (Appendix Table S4).
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Case Number: E07-34,E11-97,E12-06,E16-66,E05-67,E05-194,E06-18,E06-61,E08-112,A87-50,E04-34,E16-46,E06-137,E09-170,E10-142,E13-27,E14-06 (Appendix Table S4).
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
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20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
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G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA