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TITLE: Mechanisms of Cortical Excitability Changes in Frontotemporal Degeneration Onset

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CONTRACTING ORGANIZATION: The University of Pittsburgh, Pittsburgh, PA

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Head injuries, including combat-related trauma like TBI, increase lifetime risk of acquired dementias, including Frontotemporal Degeneration (FTD). A major pathological mechanism of dementias like FTD is misfolding and aggregation of Tau isoforms. But there is not yet an effective treatment targeting Tau aggregation that addresses clinical symptoms or slows disease progression. Early pathological changes include hyperexcitability and increased seizure incidence, which precede substantial Tau aggregation and FTD diagnosis. There is a significant knowledge gap about how changes in circuits lead to hyperexcitability. Understanding the pathogenesis in the cortical circuit might help identify potential therapeutic targets to treat or slow the progression of FTD. We hypothesize that pre-tangle mutant Tau induces circuit changes, resulting in weakened inhibition or increased neuronal excitability in specific circuit components. Our specific aims test this hypothesis.
To model FTD, we will express a mutant Tau isoform (P301L) in a mouse model to dissect cell-type specific circuitry. This approach allows use of transgenic lines to fluorescently label and manipulate targeted neuron populations, allowing the study of mutant Tau effects in specific connections. Use of viral vectors also allows the study of direct effects on neuronal circuits in defined cortical areas, eliminating the need to control for compensatory mechanisms as in transgenic mutant Tau mice and enabling control of the onset time of mutant Tau expression. Stereotaxic injections of viral vectors expressing mutant Tau will be made in the primary motor cortex (M1), a brain region whose general circuit connectivity is understood. We will measure changes in inhibitory connection strength as well as intrinsic excitability by targeted whole-cell recordings in mouse brain slice.

15. SUBJECT TERMS
15.4.2022-14.4.2023

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1. Introduction

Brain injuries such as mild TBI increase the lifetime risk for dementias, including Frontotemporal Degeneration (FTD). These disorders lack effective medical treatments. FTD research concentrates on molecular mechanisms of toxic protein aggregation and spread of Tau protein, which forms neurofibrillary tangles in late stages of FTD. However, increased excitability, including susceptibility to epileptic seizures, occurs at much earlier stages. Uncovering early changes in neural circuitry causing hyperexcitability by using methods for cell-type specific circuit mapping may provide better diagnostic markers and identify specific cellular targets for treatment. Hyperexcitability in neuronal circuits may result from reduced inhibition due to weakening of inhibitory synapses or degeneration of inhibitory neurons. Stronger excitatory connections or changes in intrinsic excitability of specific cells might also cause hyperexcitability. Our proposal tests the hypothesis that Tau-induced changes will cause hyperexcitability by changing connectivity of specific inhibitory cell types. By targeting recordings to specific cortical neurons using transgenic mouse models labeling specific inhibitory and excitatory cells, this proposal will establish the specific circuit location of early changes causing hyperexcitability. Expression of mutant Tau by viral injection (AAV) will be used to induce a model of FTD-like changes in mouse frontal cortex. State-of-the-art optogenetic tools (light sensitive channelrhodopsin molecules expressed in live cells) will be used to excite specific connections for circuit mapping. Recordings will be targeted to genetically defined subsets of inhibitory or excitatory neurons, allowing reliable identification of the same cell types (circuit nodes) across experiments. Changes in excitability and connectivity will be quantified and compared for each cell type in mutant Tau-expressing and neighboring neurons. The circuit location where changes in connectivity and excitability occur are then targets for therapeutic intervention to slow FTD progression.

2. Keywords

Frontotemporal degeneration, FTD, Tau, dementia, early onset, frontal cortex, hyperexcitability, inhibition, inhibitory interneurons, parvalbumin, somatostatin, optogenetic, adeno-associated viruses.

3. Accomplishments

What were the major goals of the project?

Two major goals:

(1) Identify alteration in intrinsic excitability in defined excitatory and inhibitory neurons

a. Master stereotaxic surgery for viral transfection (1-3 months) **complete** b. Gather and validate AAV for mutant Tau model (1-6 months) **complete** c. Local IRB/IACUC approval (1 months) **complete** d. Data collection in FTD model (AAV injected mice) (3-8 and 15-20 months) **in progress** e. Data analysis (17-20 months) **in progress.**

(2) Identify changes in feedforward inhibition from defined cell types a. Import animals and establish breeding colony of PV-Cre or SOM-Cre mice x ChR2 reporter (1-4 months) **complete** b. Data collection in control mice (1-2 months) **in progress** c. Data collection in FTD model (AAV injected) (3-8 months) **in progress** d. Data Analysis (7-10 months) **in progress** e. Data for lab meeting and local (Dept. or University-level) presentation (10 months) **delayed due to COVID-19** f. Drafting of manuscript (22-24 months) **not started.**

What was accomplished under these goals?

The excitability changes in response to mutant and wild type Tau were estimated in PV-Cre:Ai32 animals expressing channelrhodopsin-2 in PV inhibitory interneurons, no changes from the controls were observed.
The excitability changes in pyramidal neurons was estimated in the same animals, no changes were observed here as well.
The excitability changes were estimated in putative somatostatin interneurons in PV-Cre:Ai32 animals, suggesting possible changes in input-output of the Tau affected cells.

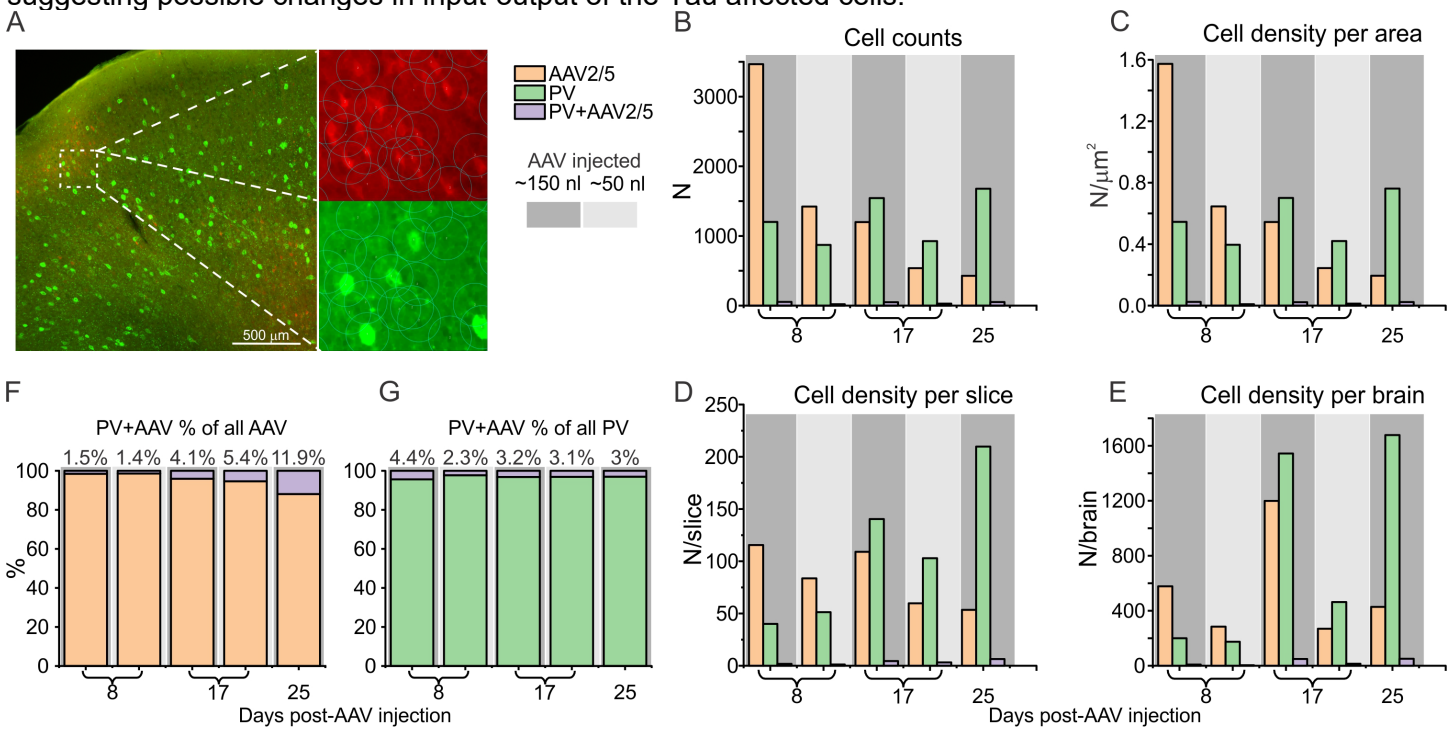


Figure 1. Cell types percent with AAV2/5 in PV-Cre:Ai32 animals. **A.** immunohistochemistry against Ai32 epitope showing PV inhibitory interneurons in green, together with AAV2/5 infected cells in red. **B.** Total cell counts from those images. **C.** Cell density per µm^2. **D.** Cell density per slice. **E.** Cell density per brain. **F.** Percent of PV (green-Ai32+AAV2/5) out of all AAV2/5 positive (red) cells. **G.** Percent of PV (green-Ai32+AAV2/5) out of all PV (green-Ai32) positive cells.

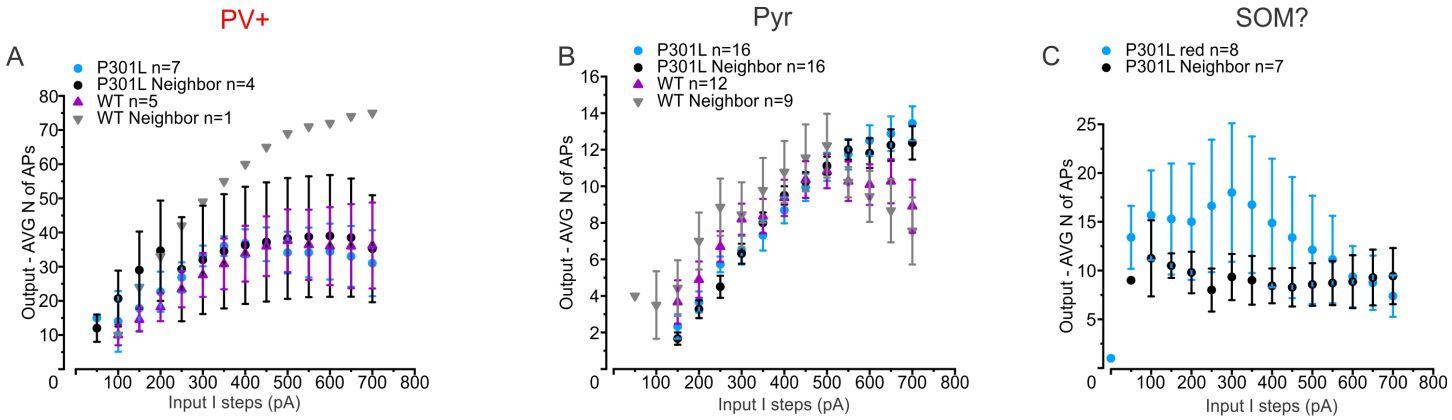


Figure 2. Input-output properties of PV+ pyramidal and putative somatostatin cells. A. PV+ input-output according to the condition, P301L – AAV2/5.hTau.P301L.mRuby, Neighbor is a control, WT-AAV2/5.hTau.WT.mRuby. **B.** pyramidal cells **C.** putative somatostatin interneurons.

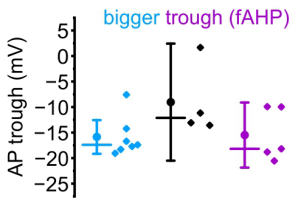
Tau-WT
Tau-P301L
Mean ± 95% CI

not statistically significant, but there is a trend

PV+ | Pyr | SOM?

less excitability | ??? | more excitability

A



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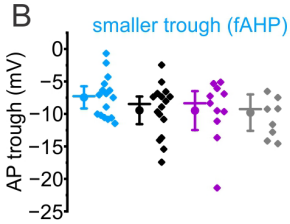

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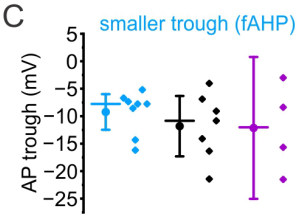

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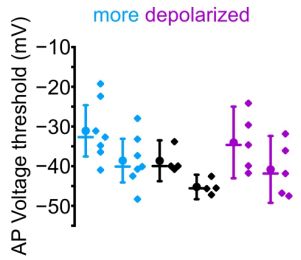

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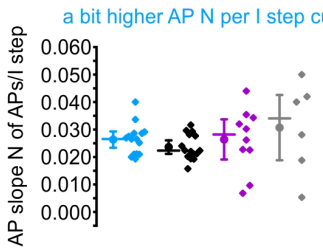

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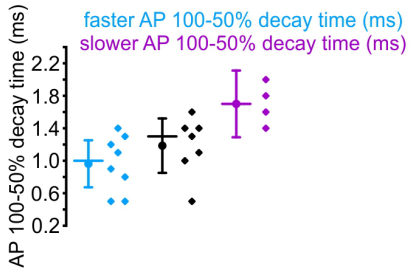

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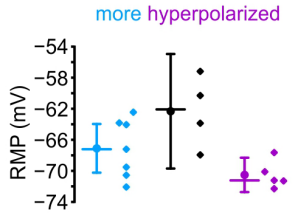

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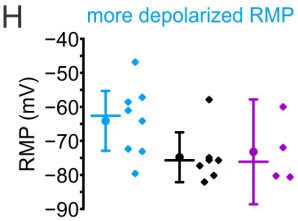

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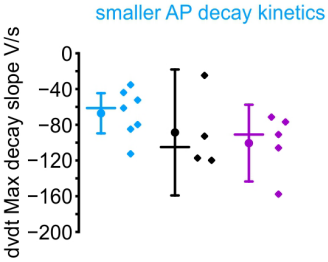

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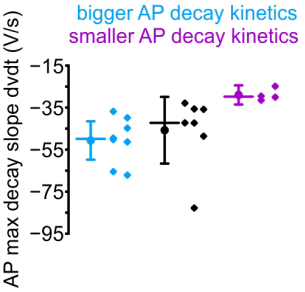

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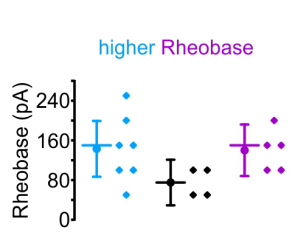

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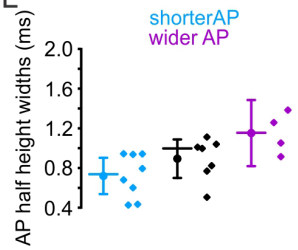

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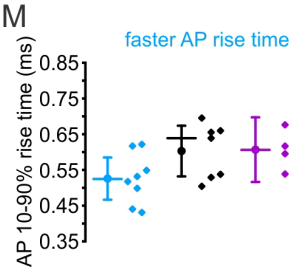

Figure 3. Excitability properties measured in PV+ pyramidal and putative somatostatin interneurons did not show statistically significant differences. A,D,G,I,K – PV cells; **B,E,** pyramidal cells; **C,F,H,J,L,M** – putative somatostatin cells. **A., B., C.** Action potential trough. **D.** Action Potential voltage threshold. **E.** AP slope, number of Action Potentials per injected current. **F.** Action Potential Decay Time. **G., H.** Resting Membrane Potential. **I., J.** Phase-space plot of Action Potential, dV/dt max decay slope. **K.** Rheobase, minimal current required to make cell fire Action Potential. **L.** Action Potential half-height width. **M.** Action Potential rise time.

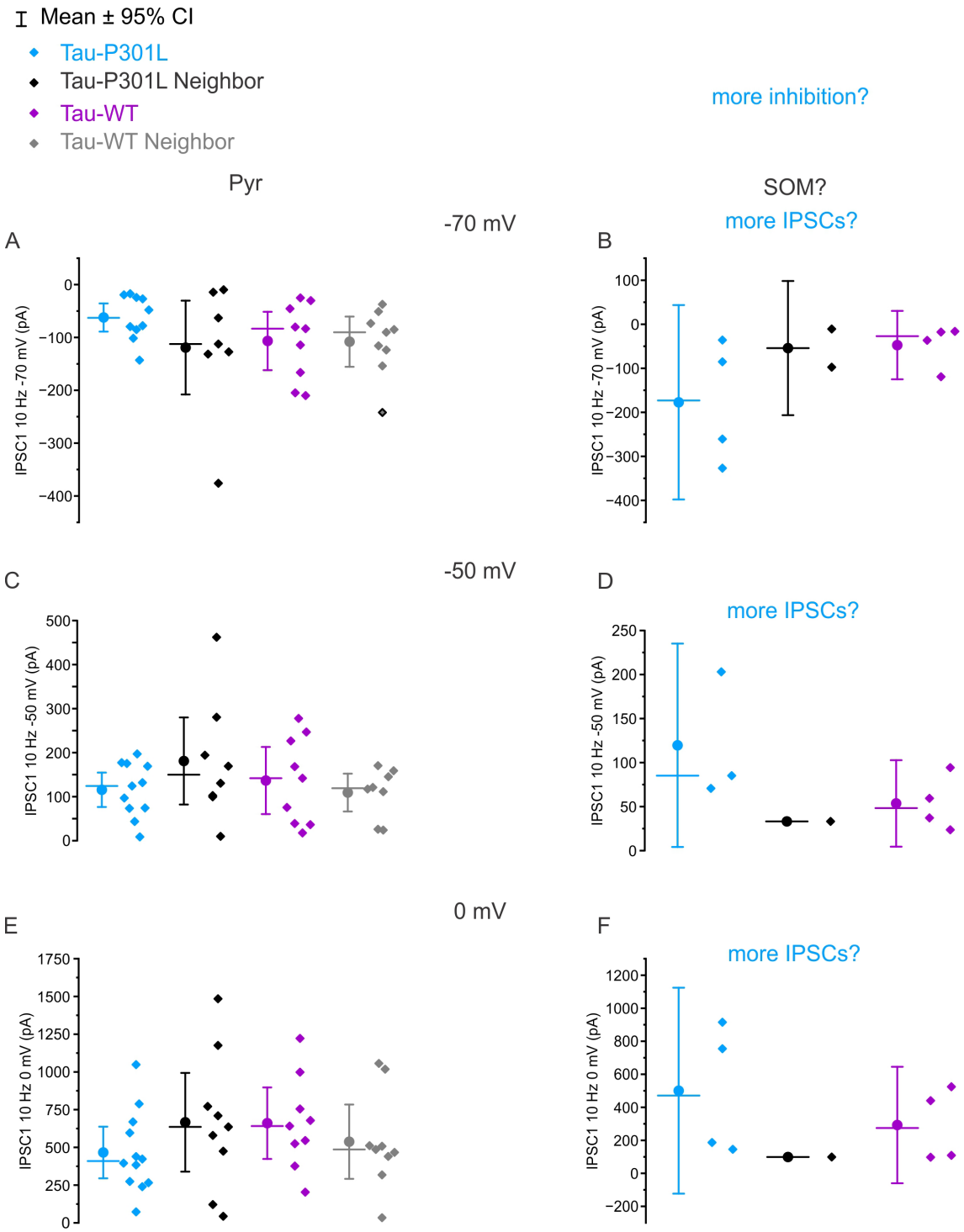

Figure 4. IPSCs from PV inhibitory interneurons recorded in pyramidal and putative somatostatin interneurons show no significant difference between control and experimental (Tau P301L and WT conditions). A., B. IPSC stimulated at 10 Hz recording in pyramidal and putative somatostatin cells at -70 mV holding potential**. C.,D.** at -50 mV holding potential, and **E. F**. at 0 mV holding potential**.**

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

4. Impact

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. Changes/Problems

Changes in approach and reasons for change

AAV mediated human mutant or wild-type Tau seems not to affect excitability in tested cells withing 4 weeks of expression.

Actual or anticipated problems or delays and actions or plans to resolve them

End of tenure. No current plan.

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. Products

- **Publications, conference papers, and presentations**

Journal publications. *Nothing to Report*

Books or other non-periodical, one-time publications. *Nothing to Report*

Other publications, conference papers, and presentations. *Nothing to Report*

Website(s) or other Internet site(s)
Nothing to Report

Technologies or techniques
Nothing to Report

Inventions, patent applications, and/or licenses
Nothing to Report

- **Other Products**

research material - AAVs, plasmids

- AAV2/5.CBA.mRuby.2A.hwtTau
- AAV2/5.CBA.mRuby.2A.hP301L.Tau
- AAV2/5.CBA.mRuby.2A.hS320F.Tau
- AAV2/5.CBA.mRuby.2A.hP301L.S320F.Tau
- AAV2/5.CBA.mRuby

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	*Roman Goz*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	2-7629-6544
Nearest person month worked:	*9*
Contribution to Project:	*No change*
Funding Support:	

Name:	*Bryan (Mac) Hooks*
Project Role:	*2nd PI*
Researcher Identifier (e.g. ORCID ID):	3-0135-4284
Nearest person month worked:	*2*
Contribution to Project:	*No change*
Funding Support:	NIH/NINDS

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. Special Reporting Requirements

Nothing to Report

9. Appendices

No appendices.

10. References

Boyarko B, Hook V (2021) Human tau isoforms and proteolysis for production of toxic tau fragments in neurodegeneration. Front Neurosci 15:702788.

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