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TITLE: Mechanisms of Cortical Excitability Changes in Frontotemporal Degeneration Onset

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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

None listed.

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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) **not due**

(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) **not started** 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 due**

What was accomplished under these goals?

IRB/IACUC approval was received as stated in SOW, prior to the beginning of work with mice.

Mice expressing Cre under the parvalbumin promoter were crossed to mice expressing ChR2-EYFP (a green-shifted fluorophore).

The plasmid expressing human wild-type microtubule associated protein Tau (2N4R) was obtained from addgene (https://www.addgene.org/140424/) and used to make the constructs described in the experimental design including the control by Gibson assembly cloning and site directed mutagenesis (Gibson et al., 2008) . Additionally the EGFP (green) fluorophore was substituted with mRuby (red) to fit the experimental design of animals crossed to green reporter. To increase the amount of the plasmid for the adeno-associated viruses production we used QIAgen maxi prep kits with overnight cultured (12-16 hours) transfection of dH5alpha competent cells. In addition to wild-type (WT) constructs, four others were made for the study: (1) mutant P301L (affecting microtubule binding domain 2 – R2), (2) mutant S320F (R3), (3) double mutant P301L-S320F, and (4) fluorophore only without Tau (control to WT). All Tau constructs were made to be isoform 4, a more active isoform, as described in the literature, and have 0 (zero) 29 amino-acid N-terminal repeats and include all 4 microtubule binding domains, 0N4R (reviewed in Boyarko and Hook, 2021). The absence of other mutations not specified in the experimental design were confirmed with DNA sequencing of the constructs using multiple primers. The adeno-associated (AAVs) viruses serotype 2/5 (capsid) were produced from those plasmids in HEK293 cells with purity confirmed by SDS-PAGE and densitometry by the university Magee viral core facility as described in the budget documents. The result is the 5 AAVs that can be used in our experimental design:

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

The expression of AAVs was validated in mice. Mastery of stereotaxic surgery involving injections of AAVs in specific coordinates of mouse cortex was accomplished as stated in SOW. The results of injections are shown in figures (Figure 1, the validation work flow; Figure 2, validation of all the AAV constructs with single PHF1 anti-Tau antibody; Figure 3, validation of AAV2/5.CBA.mRuby.2A.hP301L.Tau with 8 different anti-Tau antibodies) with all the AAV constructs tested and validated with Tau antibodies.

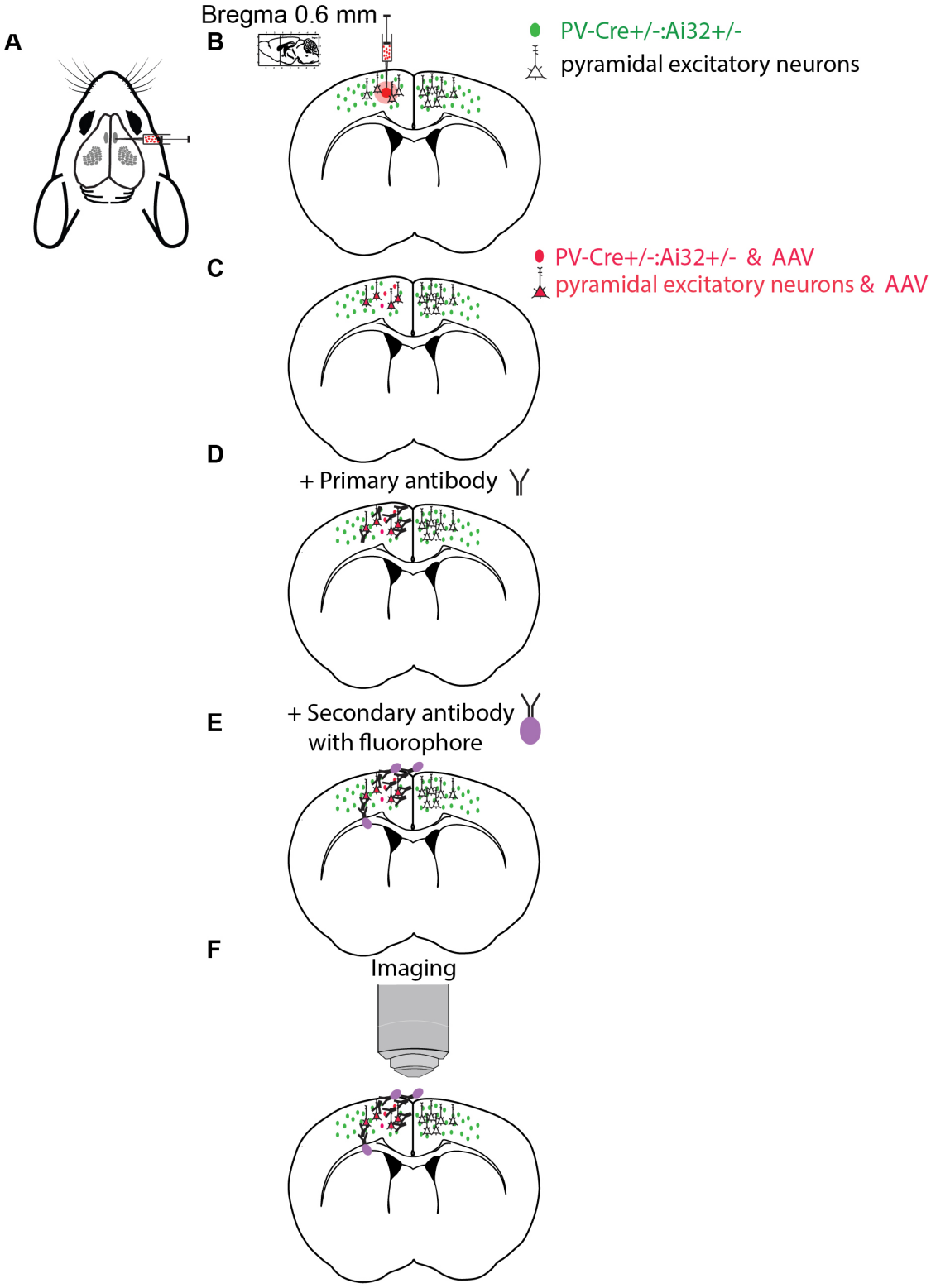

Figure 1. AAVs validation in mouse cortical slices of vibrissal motor cortex (vM1) workflow.
A. Injection of AAVs into mouse vibrissal motor cortex +0.6 mm Bregma, +1.5 mm lateral, 0.5-0.8mm depth. **B.** Illustration of injection in coronal brain slice. **C.** Starting a week after the injection the slices are processed for immunohistochemistry. **D.** After blocking with goat serum and permeabilization with triton-X the slices are incubated with primary antobody against Tau for overnight. **E.** The next day the slices are incubated with the secondary antibody conjugated with fluorophore with 647 nm emission, then the slices are mounted on slides and coverglassed. **F.** The immunostained AAV injected slices are imaged.

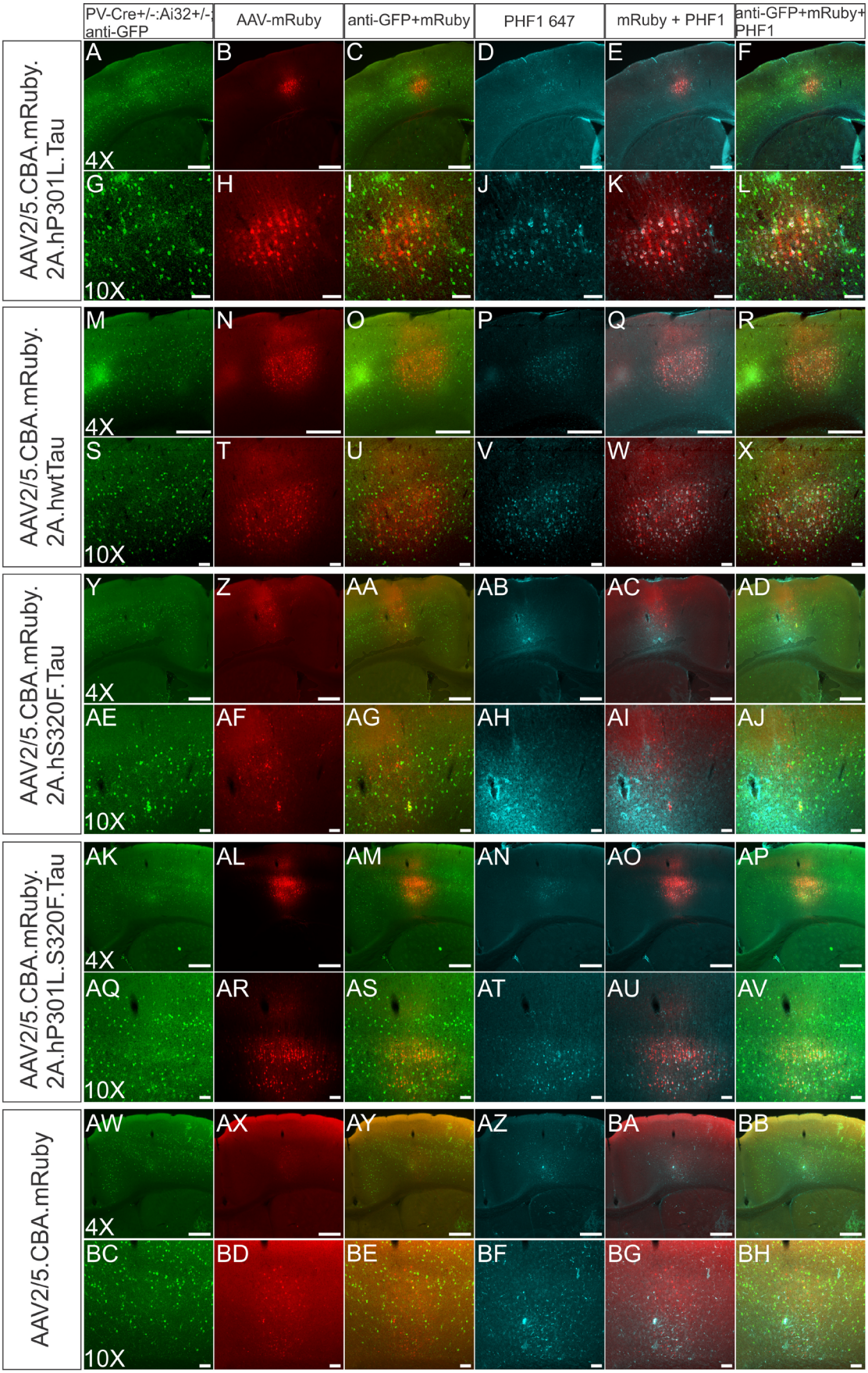

Figure 2. AAVs expression validation in mouse cortical slices of vibrissal motor cortex (vM1). **A.-L.**Expression of AAV carrying hTau-P301L mutation. **M.-X.** Expression of AAV carrying wild-type hTau. **Y.-AJ.** Expression of AAV carrying hTau-S320F mutation. **AK.-AV.** Expression of AAV carrying hTau-P301L-S320F double mutant. **AW.-BH.** Expression of AAV carrying mRuby fluorophore only. Left most column is the anti-GFP(488 nm) staining, the 2nd column is the mRuby (568 nm) in the AAV; the 3rd column is two first columns merged; 4th column is PHF1 anti-Tau antibody staining (647 nm); 5th column is the merge of the 2nd and 4th; 6th column is the merge of 1st, 2nd and 3rd. Scale bars 500 μm for 4X and 100 μm for 10X magnification images.

AAV2/5.CBA.mRuby.2A.hP301L.Tau

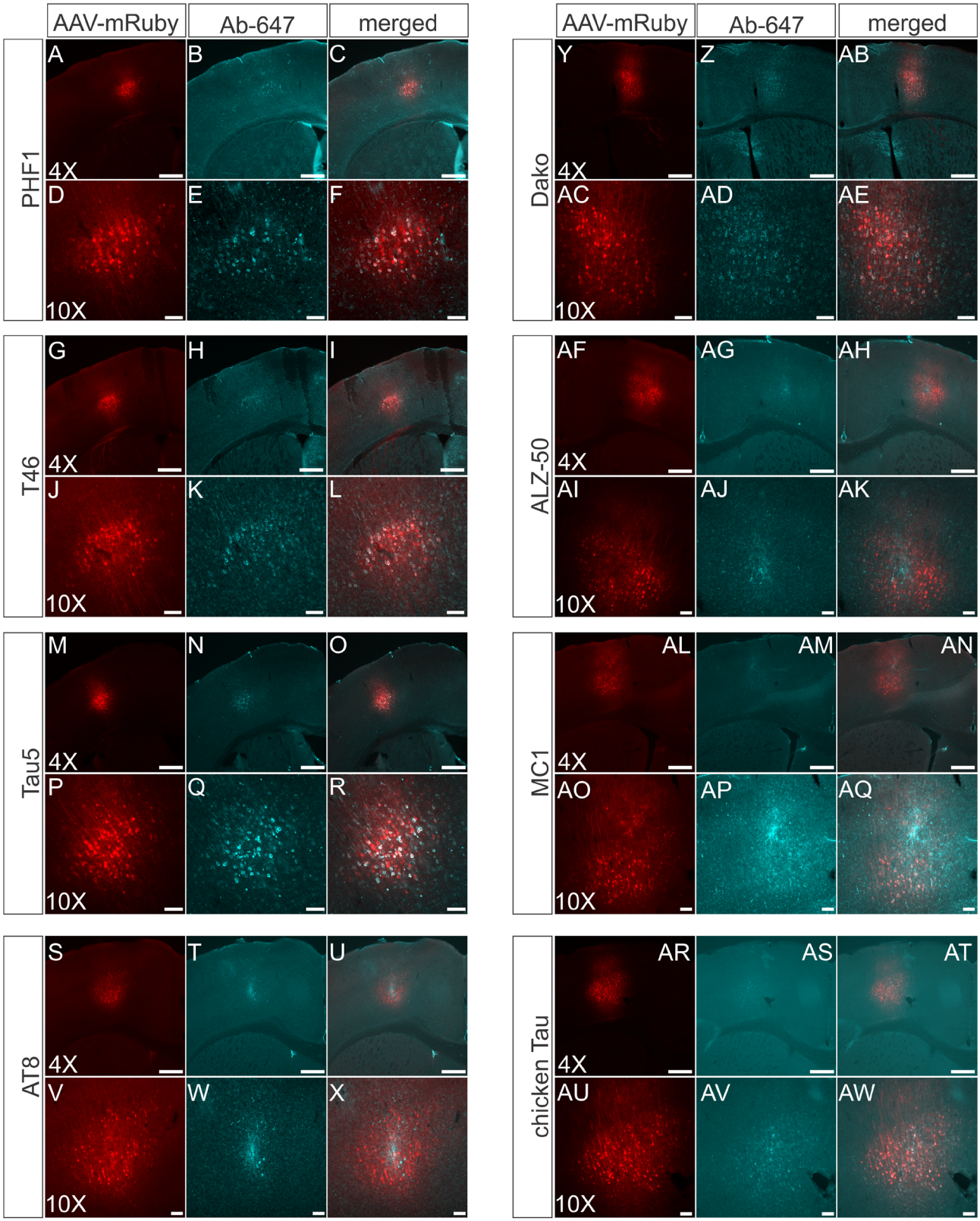

Figure 3. AAV (AAV2/5.CBA.mRuby.2A.hP301L.Tau) expression validation in mouse cortical slices of vibrissal motor cortex (vM1). **A.-F.** PHF1 anti-Tau Ab. **G.-L.** T46 anti-Tau Ab. **M.-R.** Tau5 anti-Tau Ab. **S.-X.** AT8 anti-Tau Ab. **Y.-AE.** Dako anti-Tau Ab. **AF.-AK.** ALZ-50 anti-Tau Ab. AL.-AQ. MC1 anti-Tau Ab. **AR.-AW.** chicken Tau anti-Tau Ab. Left most column is the AAV.mRuby (568 nm), middle column is the anti-Tau antibody staining (647 nm); right most column is left and middle columns merged. Scale bars are 500 μm for the 4X and 100 μm for the 10X images.

We have not yet started the injections to begin the electrophysiological recordings. We are planning to begin this month (April). The experiments are well thought through, and the equipment (the rig) is functioning, that means that the progress will be fast, and the results will show up before the end of the project.

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?

The plan is to start stereotaxic injections into PV-Cre$^{+/-}$;Ai32$^{+/-}$ (ChR2-EYFP) mice and start electrophysiological recording starting a week after the injections of AAVs. We will collect the data, do the analysis to compare changes of excitability in pyramidal excitatory cells and parvalbumin positive inhibitory interneurons between the mutant and control conditions. For the second aim of testing the changes in feedforward inhibition, we will collect the data including the optogenetic stimulation of the PV+ cells that express channelrhodopsin and analyze the results to compare changes in mutant and control conditions. Once appropriate sample size is obtained (As described in project narrative, ~20 cells per condition, 4 conditions, 2 cell types) we will move on to SOM-Cre$^{+/-}$:Ai32$^{+/-}$ animals which are also bred in our mouse colony and start collecting the data in SOM-Cre animals.

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

Nothing to Report

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

COVID-19 related delay in full functioning in the university located in the hospital building complex. We plan to expedite the electrophysiological recording, if need be we are planning to get help from the people in the lab for these recording and analysis.

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

10. References

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

Gibson DG, Benders GA, Andrews-Pfannkoch C, Denisova EA, Baden-Tillson H, Zaveri J, Stockwell TB, Brownley A, Thomas DW, Algire MA, Merryman C, Young L, Noskov VN, Glass JI, Venter JC, Hutchison CA, Smith HO (2008) Complete chemical synthesis, assembly, and cloning of a mycoplasma genitalium genome. Science 319:1215-1220.