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Connecting the Dots: Investigating the Effects of Trans-Synaptic Tau Transmission in the Hippocampus

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Connecting the Dots: Investigating the Effects of Trans-Synaptic Tau Transmission in the Hippocampus

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Anatomy and Neurobiology at Virginia Commonwealth University.

By

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Abstract

CONNECTING THE DOTS: INVESTIGATING THE EFFECTS OF TRANS-SYNAPTIC TAU TRANSMISSION IN THE HIPPOCAMPUS

Michael Adeniran Bamisile, Master of Science

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Anatomy and Neurobiology at Virginia Commonwealth University.

Virginia Commonwealth University, 2019

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Tauopathy, which results from the oligomerization of misfolded tau protein in neurons, is a feature present in a number of neurodegenerative diseases and a hallmark of Alzheimer's Disease (AD). Tau is an important phosphoprotein that regulates the assembly of microtubules, but tauopathy can occur when tau becomes hyperphosphorylated. Phosphorylation prevents tau from binding to tubulin, which results in cytosolic accumulation of tau and eventual oligomerization. This abnormal accumulation of tau leads to the spreading of hyperphosphorylated tau to downstream synaptically connected neurons through an unknown mechanism. In AD, the hippocampus is one of the first brain structures to be affected by tauopathy in humans. According to previous research, tauopathy occurs primarily between principal cells in the hippocampus. The involvement of local inhibitory interneurons in tauopathy and their potential role in AD is more controversial. Previous research suggests that tau pathogenesis primarily affects principal cells; however, given the importance, diversity, and function of interneurons in the hippocampus, it is important to gain a better understanding of the interneuron subtypes that may be impacted by the spread of trans-synaptic tau into the hippocampus. Understanding the involvement of interneurons in trans-synaptic tau transmission

is important to understanding neurodegeneration in AD and other neurodegenerative disorders. To investigate this, both male and female genetically-modified mice underwent surgery to examine the trans-synaptic spread of pathogenic tau (EGFP-Tau P301L) from the entorhinal cortex to hippocampal neurons. Histology and imaging analysis of brain sections were performed to examine the hippocampal cells impacted by trans-synaptic spread of tau. Results show that pathogenic tau can trans-synaptically spread from presynaptic neurons in the entorhinal cortex into downstream hippocampal interneurons and also that hippocampal interneurons are capable of trans-synaptically spreading tau. Future studies examining the specific subtypes of hippocampal interneurons vulnerable to trans-synaptic spread of tau will be important for a better understanding of disease progression, which could lead to uncovering new therapeutic targets for neurodegenerative diseases, like AD, which are associated with tauopathy.

Background

Alzheimer's Disease

In just half a century, the life expectancy of the world has increased significantly. According to the data collected by The World Bank, an individual's average life expectancy rose from 53 years old in 1960 to 72 in 2016 (Life expectancy, 2019). Globally, people are getting older; however, with increased longevity comes an increase in the probability of developing age-related diseases such as Alzheimer's disease (AD). In the United States alone, 5.3 million individuals have been diagnosed with AD with 5.1 million of these individuals being 65 years old or older. Thus, this is a disease predominantly affecting older individuals, and in a few years its prevalence is expected to significantly rise (Alzheimer's Association, 2015).

AD is characterized as a neurodegenerative disorder in which the progression of the disease results in memory loss and cognitive impairment (Khyade, Khyade, & Jagtap, 2016; Wilson et al., 2012). Dementia has been defined by Folstein and McHugh as "the clinical syndrome of mental life characterized by substantial global decline in cognitive function that is not attributable to alteration in consciousness" (Breitner, 2006). The degree of dementia increases as an individual goes through four stages: pre-dementia, early (mild), moderate, and advanced (severe) (Khyade, Khyade, & Jagtap, 2016). Pre-dementia and the early stage of AD are characterized by impaired learning and memory; however, individuals are still capable of living independently in these stages (Storandt & Hill, 1989). In these early stages, verbal memory is typically the first aspect of memory that is affected (Linn et al., 1995). In moderate and severe stages of AD, individuals are unable to live independently and memory/cognition are severely impaired compared to the predementia and early stages of AD (Khyade et al., 2016).

Furthermore, in these later stages, individuals have a three year life expectancy (Wimo, Winblad, Stoffler, Wirth, & Mobius, 2003).

As the sixth leading cause of death in the United States ("2015 Alzheimer's disease facts and figures," 2015), the impact of AD has motivated researchers to investigate the pathological basis of the disease. There are three popular hypothesis to explain the cause of AD: the genetic or amyloid cascade hypothesis, the cholinergic hypothesis, and the tau hypothesis (Khyade et al., 2016). The genetic or amyloid hypothesis posits that a number of genetic mutations occur in the amyloid precursor protein (APP) or in catalytic enzymes involved in processing APP (presenilin 1 and 2) (Price, Tanzi, Borchelt, & Sisodia, 1998). It is believed that in the amyloid cascade hypothesis, the amyloid precursor protein is cleaved in the endosomal-lysosomal pathway to form amyloid beta, which is then released into the extracellular space resulting in amyloid beta plaque formation (O'Brien & Wong, 2011) Accumulation of amyloid beta plaques can eventually result in neurofibrillary tangles evident in later stages of AD (Hardy & Higgins, 1992; Maccioni, Farías, Morales, & Navarrete, 2010). However, the role of amyloid beta in the disease state remains unclear because research has shown that amyloid beta plaques can exist in a normal cognitive brain (Morris, Clark, & Vissel, 2014). In contrast, no confirmed genetic mutations have been causally correlated to the far more prevalent late-onset form of AD. However, previous research suggest that expression of the APOE4 isoform of apolipoprotein E has a weak correlation to an individual acquiring late-onset sporadic AD; and, in contrast, the APOE2 isoform appears to have protective effects against AD (Strittmatter & Roses, 1996). The cholinergic hypothesis posits that cholinergic neurons, which are important for learning and memory, are some of the first neuronal subtypes to be impacted at the onset of AD (P. T Francis, Palmer, Snape, & Wilcock, 1999; Terry, 2003). This hypothesis provides a useful

pharmaceutical strategy (combatting the loss of cholinergic neurons) that has worked well for early stages of AD; however, its efficacy diminishes as AD progresses to later stages (Bartus, 2000). The most popular hypothesis amongst the three, the tau hypothesis, suggests that tau hyperphosphorylation and aggregation is the key factor for AD (Maccioni et al., 2010). Tau hyperphosphorylation, spread, and aggregation is also known as tauopathy, and many researchers are in agreement that this is likely an important underlying cause of AD (Fernández, Rojo, Kuljis, & Maccioni, 2008; Kosik, Joachim, & Selkoe, 1986; Maccioni, Rojo, Fernández, & Kuljis, 2009; Rojo et al., 2008).

Tauopathy

Tauopathy is a feature present in a number of neurodegenerative diseases associated with dementia and cognitive decline. It is a hallmark of AD (Rojo et al., 2008), but several other diseases also display tau-dependent neurofibrillary tangles such as Argyrophilic grain disease (Tolnay & Clavaguera, 2004), supranuclear palsy, corticobasal degeneration, Pick's Disease, frontotemporal dementia, (Wattez & Delacourte, 1999; Spillantini, Van Swieten, & Goedert, 2000) and Parkinson's Disease (Lei et al., 2010), all of which are associated with increased dementia and cognitive decline. The involvement of tau in many neurogenerative diseases should come as no surprise given the important function tau has in the central nervous system.

Tau is an important phosphoprotein that regulates the assembly of microtubules (Weingarten et al., 1975). In human, the coding sequence for the microtubule associated protein tau (MAPT) gene is located on chromosome 17 and is alternatively spliced to produce 6 isoforms (Goedert, Wischik, Crowther, Walker, & Klug, 1988). Studies indicate that tau, although also located in the peripheral nervous system, is predominantly located in axons of neurons of the

central nervous system (Shin et al., 1991), supporting the observation that tau is particularly important in microtubule organization of axons (Harada et al., 1994). Tau is able to regulate microtubules by being phosphorylated by a protein kinase, PKN (Taniguchi et al., 2001); however, it can also be phosphorylated and dephosphorylated by a range of proteins (Billingsley & Kincaid, 1997).

Tauopathy occurs at least in part because tau becomes hyperphosphorylated. Phosphorylation of specific residues prevents tau from binding to tubulin, which results in cytosolic accumulation of tau and eventual oligomerization (Lindwall & Cole, 1984). This abnormal accumulation of tau leads to the trans-synaptic spread of hyperphosphorylated tau by an unknown mechanism (Rojo et al., 2008). This abnormal phosphorylation is a serious concern not only because of aggregation and trans-synaptic spread, but because destabilized microtubules in neurons (axons predominantly) results in a compromised axonal transport system, which can ultimately lead to cell death (Kosik et al., 1986). This cell death helped early scientists identify which brain regions are first affected by tauopathy as cell death appears to occur in a serial fashion.

The Entorhinal Cortex, Hippocampus, and Tauopathy

In AD, the first brain structure in humans to be affected by tauopathy is the entorhinal cortex (Braak & Del Tredici, 2012; Gómez-Isla et al., 1996; Khan et al., 2014; Moreno et al., 2007; Whitwell et al., 2007), more specifically the transentorhinal cortex (Braak & Braak, 1995). The entorhinal cortex is located in the medial temporal lobe and functions as the "gate-keeper" between the neocortex and the hippocampus. The entorhinal cortex is usually separated into two regions: the medial entorhinal cortex and the lateral entorhinal cortex. Both lie next to each other

and encode different modalities. The medial entorhinal cortex is primarily involved in spatial memory (Tsao et al., 2018). This is supported by studies that have shown a correlation between spatial memory diminishing and the volume of the medial entorhinal cortex decreasing (Fox et al., 1996; Frisoni et al., 1999). The lateral entorhinal cortex is primarily involved in novel object-context recognition (Wilson et al., 2013). Layer II and layer III in both the medial and lateral entorhinal cortex each project to different region and areas in the hippocampus. Layer II of the medial entorhinal cortex projects to the middle third portion of the molecular layer in the DG region of the hippocampus and to the deep portion of the stratum lacunosum-moleculare layer in the CA3 region of the hippocampus. Layer II of the lateral entorhinal cortex projects to the outer third portion of the molecular layer in the DG region of the hippocampus and the most superficial portion of the stratum lacunosum in the CA3 region of the hippocampus. Layer III of the medial entorhinal cortex projects to the CA1 stratum lacunosum adjacent to CA3 region of the hippocampus. Layer III of the lateral entorhinal cortex projects to CA1 stratum lacunosum adjacent to the subiculum region of the hippocampus (Canto & Witter, 2012a, 2012b). *See Figure 1 and Figure 2 for connections from the entorhinal cortex.*

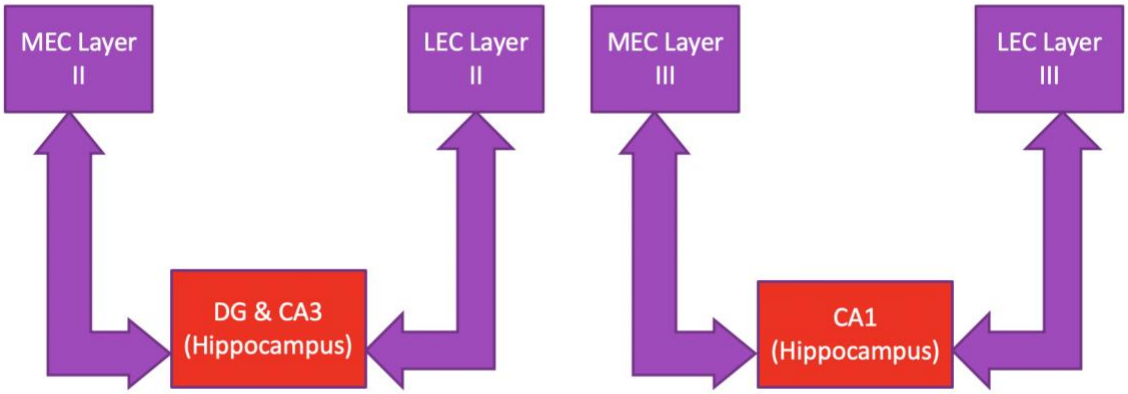

Figure 1: Entorhinal Cortex and Hippocampal connections.

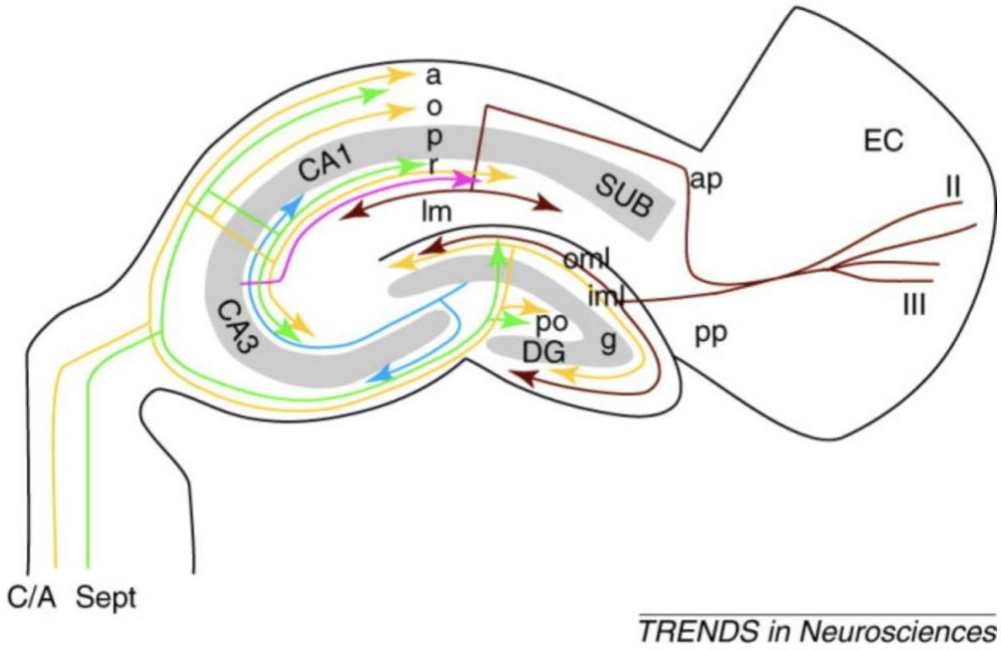

Figure 2: Skutella, T., & Nitsch, R. (2001). New molecules for hippocampal development. Trends in neurosciences, 24(2), 107-113.

The hippocampus is the next brain structure to be affected by tauopathy (Fox et al., 1996; Frisoni et al., 1999). The hippocampus is located in the medial temporal lobe and has been shown to be primarily involved in learning and memory (Erickson et al., 2011; L. R. Squire, 1986; Larry R. Squire, 1992; Winocur, Wojtowicz, Sekeres, Snyder, & Wang, 2006). Typically, the hippocampus is divided into four regions: dentate gyrus, CA3 region, CA1 region and subiculum. *See Figure 3 for an image of the hippocampus in rodents*.

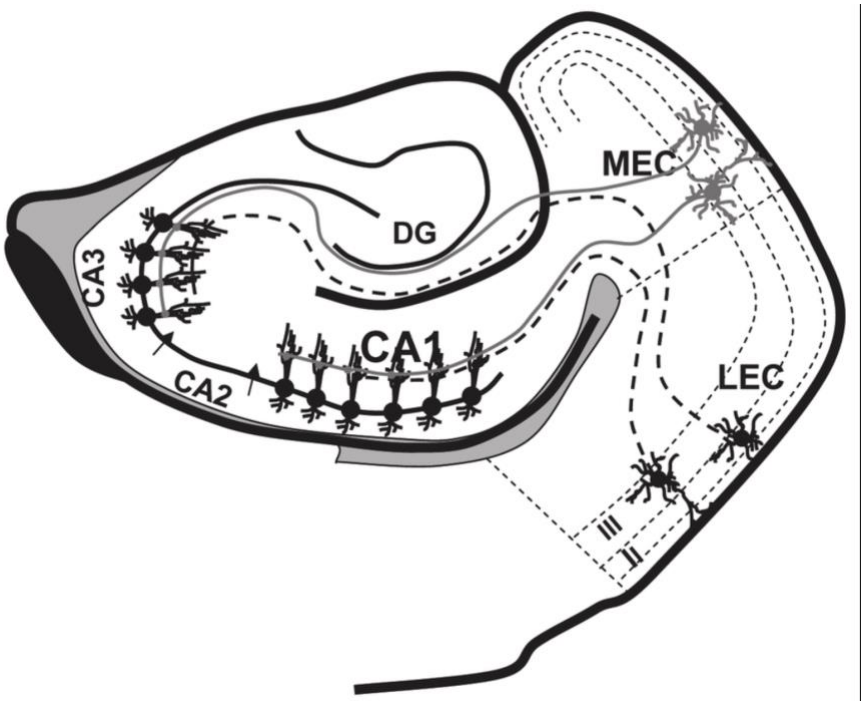

Figure 3: Torrealba, F., & Valdés, J. L. (2008). The parietal association cortex of the rat. Biological research, 41(4), 369-377.

According to previous research, the neurons affected by pathogenic tau and trans-synaptic spread in the entorhinal cortex and hippocampus are thought to be principal glutamatergic neurons (Steward & Scoville, 1976; Varga, Lee, & Soltesz, 2010). This finding is

further supported by research showing that tau trans-synaptically spreads between the principal cells of each brain structure (Paul T. Francis, 2003; Palop & Mucke, 2010). These findings suggest that principal cells are exclusively involved in tauopathy; however, concerns arise with this reasoning because principal cells are not the only cell types residing in the hippocampus and because there is evidence that interneurons may be involved in tauopathy (Koliatsos et al., 2006; Loreth et al., 2012; Levenga et al., 2013).

Interneurons and Tauopathy

In addition to principal cells, inhibitory interneurons are another group of cells found in the hippocampus. Inhibitory interneurons are crucial to the gating of inputs to the hippocampus, the integration of excitatory inputs within the dendritic trees of principal cells, and the generation of behaviorally relevant rhythms observed in principal cells of the hippocampus (Freund & Buzsáki, 1998). Dysfunction in interneurons has also been linked to disrupt cognitive function as well as play a role in the generation of seizures (Buzsáki, 2002; Chauviere et al., 2009). The scientific community continues to investigate hippocampal interneurons and the expanding list of their subpopulations, but we remain in the midst of discovering more inhibitory interneuron subpopulations that vary in morphology, function, and connectivity. Given the wide range of roles and functions of interneurons in the hippocampus, investigating how interneurons are involved in tauopathy could be essential for understanding mechanisms contributing to cognitive dysfunction in neurodegenerative diseases like AD.

The relationship between interneurons and tauopathy and their potential role in AD remains contentious. Previous research suggests that interneurons are not actively involved in trans-synaptic tau transmission (Paul T. Francis, 2003; Verret et al., 2012). Others suggest that

interneurons have no role in AD (Fu et al., 2019). However, given the diversity and function of interneuron subtypes, their importance in regulating hippocampal network function, and studies that suggest that certain interneuron subtypes may be impacted by pathogenic tau, further investigation into their role in tauopathy and AD is warranted.

Theory

This study aims to identify and classify interneurons and their subtypes impacted by the trans-synaptic spread of tau. If interneurons are affected, determining what interneuron subtypes are affected, which hippocampal subregions are being affected, and comparing the relative number of interneurons to principal neurons being affected is essential to understanding tauopathy. By providing these answers to our gap in knowledge regarding trans-synaptic spread of tau in the hippocampus may provide important information necessary for discovering new therapeutic targets for neurodegenerative disease like AD.

Materials and Methods

Animals

Both adult male and female genetically-modified mice were used to examine the trans-synaptic spread of pathogenic tau (EGFP-Tau P301L) from the entorhinal cortex to hippocampal neurons. To determine the hippocampal cell types vulnerable to the spread of EGFP-Tau P301L we used the following Cre-recombinase driver mouse lines: Vip$^{tm1(cre)Zjh}$ /J (JAX Stock No. 010908), B6;129P2-Pvalb$^{tm1(cre)Arbr}$ /J (JAX Stock No. 008069), Slc32a1 tm2(cre)Lowl/J (JAX Stock No. 016962), Cr-IRES-Cre (JAX Stock No. 010774), and C57BL/6-Tg(Pvalb-tdTomato)15Gfng/J (Jax Stock No. 027395) (*See Table 1 for purpose of each animal).* The Cre-

driver lines were crossed to a Cre-dependent tdTomato reporter line Ai14 (B6.Cg-GT(ROSA)26Sortm14(CAG-tdTomato)Hze/J) (JAX Stock No. 007914) to identify VGAT positive GABAergic interneurons (cells were classified as principal cells based off of morphology and whether it was VGAT- tdTomato negative). These animals were then crossed with animals that underwent surgery to selectively infect MEC or ECIII cells using a flex virus (an AAV1-hSYN1-DIO-GFPtauP301L-WPRE injected in to selectively express pathogenic tau in Cre-containing principal cells of layer III of the medial entorhinal cortex) that is under the control of the human synapsin promotor or tTA virus (an AAV1-TRE-GFPtauP301L-WPRE to selectively express pathogenic tau in tTA-containing principal cells of layer II of the medial entorhinal cortex) that is under the control of the tetracycline response element. The specifics of the rationale are explained later in this section. All animals were housed in an animal care facility approved by the American Association for the Accreditation of Laboratory Animal Care. Animal experimental procedures followed a protocol approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University (AD20205). This protocol adheres to the ethical guidelines described in the *Guide for the Care and Use of Laboratory Safety Animals* (8th edition). All efforts were made to minimize animal discomfort and to reduce the number of animals used.

Table 1

Mouse ID	Stock #	Strain of Origin	Purpose
Viptm1(cre)Zjh /J	JaxLab Stock # (0)10908	(C57BL/6 x 129S4/SvJae)F1	Cre targets VIP cells
B6;129P2-Pvalbtm1(cre)Arbr /J	JaxLab Stock # (00)8069	129P2/OlaHsd	Cre targets PV cells
Slc32a1 tm2(cre)Lowl/J	JaxLab Stock # (0)16962	129S6/SvEvTac	Cre targets VGAT cells
Cr-IRES-Cre	JaxLab Stock # (0)10774	C57BL/6	Cre targets Calretinin cells
C57BL/6-Tg(Pvalb-tdTomato)15Gfng/J	JaxLab Stock # (0)27395	C57BL/6NTac	tdTomato expressed in PV cells (PvTg)
B6;CBA-Tg(Cyp19a1-cre)1Dlc/J	JaxLab Stock # (0)31779	C57BL/6 x CBA	Cre recombinase expressed from aromatase promoter (MEC_tTA)
Ai14 (B6.Cg-GT(ROSA)26Sortm14(CAG-tdTomato)Hze/J)	JaxLab Stock # 007914	(129S6/SvEvTac x C57BL/6NCrl)F1	tdTomato reporter line to identify interneurons
B6.Cg-Tg(Klk8-tTA)SMmay/MullMmmh	# 031779-MU	N/A	NOP-tTA animal that allows for specific tTA expression in the layer II medial entorhinal cortex
ECIII	A gift from S. Tonegwa, MIT	N/A	a pOXR1-Cre animal that expresses Cre in MEC layer III of the entorhinal cortex

List of all animal strains and the purpose of each animal used in this experiment.

Surgery

All instruments were sterilized and an aseptic field was created before surgery. All animals were initially anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (2.5--5 mg/kg) via intraperitoneal (IP) injection. Atropine was administered to control secretions (0.04 mg/kg) via subcutaneous injection (SC). After animals were placed on the robot stereotaxic (Neurostar, Tubingen, Germany), the site of incision was wiped with Betadine (Purdue Products L.P. Stanford, CT 06901-3431) alternated with 70% ethanol three times. During the procedure, anesthesia was maintained with O_2 supplemented with 1.0% isoflurane. An incision was made along the skull, the skin was pulled back, and a very small hole was drilled over the area of interest (the entorhinal cortex or the hippocampus). An aluminosilicate glass pipette containing an adeno-associated virus (AAV) that expressed eGFP-Tau P301L under the control of the tetracylcine response element (AAV1-TRE-eGFPtau) or the human synapsin promoter (AAV1-hSYN1-DIO-GFPtauP301L) was injected into the medial entorhinal cortex (MEC) using a Hamilton syringe (Hamilton 87930, Franklin, MA) at a rate of 100 nl/min using a motorized nanoinjector (Neurostar). As a reminder, the animals injected were either a B6.Cg-Tg(Klk8-tTA)SMmay/MullMmmh (Stock # 031779-MU) strain (Mutant Mouse Resourse and Research Center), which is a NOP-tTA animal that allows for specific tTA expression in the layer II medial entorhinal cortex, or an ECIII (a gift from S. Tonegwa, MIT), which is a pOXR1-Cre animal that expresses Cre in MEC layer III of the entorhinal cortex. These mice strains were chosen based off successful previous experiments in our lab and other labs investigating tran-synaptic tau transmission in the hippocampus. After surgery was complete, animals were monitored daily for dehydration, lethargy, weight loss and signs of inflammation for minimum of 3 days.

Tissue Processing for Immunohistochemistry

At specific time points following viral injection (3 -6 months), each animal was deeply anesthetized with a combination of 200 mg/kg ketamine, 20 mg/kg xylazine IP for tissue recovery and immunofluorescence processing. These post injection time points were chosen because this is the earliest time point that we observed trans-synaptic spreading of tau. Once animals no longer responded to toe pinch, animals were transcardially perfused with ice cold carbogen-bubbled artificial cerebrospinal fluid (aCSF which contains 125 mM of NaCl, 3.0 mM of KCl, 1.2 mM of CaCl, 1.2 mM of $MgSO_4.7H_2O$, 1.25 mM of $NaH_2PO_4.H_2O$, 25 mM of $NaHCO_3$, and 25 mM of glucose) followed by 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS, Boston BioProducts, Cat # BM-155, pH 7.4 +/- 2). The aCSF was perfused at 6 mL/min and the 4% PFA was perfused at 2 mL/min using a Peri-Star Pro Pump (Sarasota, Florida), brains were stored in PFA at 4 degrees for at least 48 hours.

Microscopy was performed at the VCU- Department of Anatomy & Neurobiology Microscopy Facility, supported in part, with funding from the NIH-NCI Cancer Center Support Grant **P30 CA0116059.** Brains were washed in PBS (Quality Biological, *Cat.* # 119-069-101, Gaithersburg, Maryland). Brains were then transferred to 30% sterile-filtered sucrose for a few days until brains saturated, indicated by falling to the bottom of the scintillation vial. Brains were blocked using a mouse brain matrix and then the blocked brains were embedded in Tissue Tek O.C.T (Electron Microscopy Sciences, Cat # 62550-01) using a Tissue-Tek Cryomold (Sakura, Torrance, California). Brains were then hardened using wet ice for 15 minutes followed by dry ice until O.C.T. was opaque. Once another member of the laboratory had completed the preceding steps, I performed the following procedures. The tissue was then sectioned using

ThermoScientific cryostat (Waltham, Massachusetts) at a slice thickness of 20 microns. Cut brain sections were placed in PBS using a small bristle artist paint brush.

After brain sections were collected, wide field fluorescence microscopy was used to determine which brain sections had the brightest trans-synaptic eGFP-Tau P301L expression. Once identified, 15 brain sections encompassing the brightest expression of GFP within the hippocampus were taken in serial order. Antibodies directed toward cell type specific markers were used to identify which cell types contained trans-synaptic eGFP-Tau P301L. Primary and secondary antibodies (*See Table #2)* were then added per normal protocol using free-floating brain sections in appropriate blocking buffer. All antibody incubations other than washes were done at 4 degrees. All washes were done in PBS. Brain sections were mounted on Fisherbrand colorfrost slides (ThermoScientific, Waltham, Massachusetts) using ProLong Gold antifade (Invitrogen, Carlsbad, California).

Table 2

Name	Manufacturer	Catalog Number	Concentration (dilution)	Optimal Slice Thickness	Incubation Period
mouse- monoclonal anti GFP (1°)	Clontech	632375	(1:50)	20-30 microns	overnight
poly rabbit anti DSRED (1°)	Clontech	632496	(1:500)	20-30 microns	overnight
poly rabbit anti calretinin (1°)	Millipore	ABN2191	(1:250 - 1:1000)	20-80 microns	3 days
Goat Anti Mouse 488 (2°)	ThermoFisher	A-11001	(1:1000)	variable	overnight
Goat Anti Rabbit 568 (2°)	ThermoFisher	A11036	(1:1000)	variable	overnight
Goat Anti Rabbit 647 (2°)	ThermoFisher	A32733	(1:1000)	variable	overnight

Primary and Secondary Antibody List used for this experiment

Quantitative and Qualitative Analysis

All brain sections were stored overnight at room temperature after mounting. Each brain sections was then examined with the BX53 Upright Microscope (Olympus). During each examination, the following cell counts were collected by visualization with the OLYMPUS cellSens dimension software (Olympus): the total number of cells containing trans-synaptic

pathogenic tau in all sections that successfully underwent antibody incubation, the relative number of principal cells and inhibitory interneurons located in the dentate gyrus, CA3 and CA1 subregions of the hippocampus, in addition to the relative number of interneurons affected in each layer of each region of the hippocampus. A total number of 6 transgenic mice were used in this experiment and a total number of 1,131 cells containing trans-synaptic pathogenic tau were counted. After examination, brain sections were stored in a -20°C freezer. Brain sections that best represented the semi-quantitative data were imaged with the BX53 Upright Microscope (Olympus).

Results

For all experiments, we set out to determine the hippocampal neuronal subtypes in the hippocampus that were vulnerable to the trans-synaptic spread of tau. How this was accomplished for each group is described below. For all experiments, all interneurons described are VGAT positive and fluoresced tdTomato. All other cells were classified as principal cells once morphology was confirmed. (*See Table #3 for the Animal Information and the Counts for the Total Number of Cells Containing Trans-Synaptic Pathogenic Tau in this experiment).*

Table 3

Group	Animal	Sex	Virus Timepoint	# of slices	Antibodies	Injection Site	Total # of GFP-Tau infected Cells	Total # of Infected Principal Cells	Principal Cells DG	Interneurons DG	Principal Cells CA3	Interneuons CA3	Principal Cells CA1	Interneurons CA1	Virus
					GFP 488 + DSRED 568										
					Staining for:										
					GFP Tau				1	54	3	15	85	17	
					VGAT				SM: 0	SM: 6	SO: 0	SO: 1	SO: 77	SO: 4	
					Interneuron				SG: 0	SG: 13	SP: 1	SP: 5	SP: 7	SP: 5	
MEC	VGATtd 5	M	6 months	12	s	R MEC (Layer II MEC)	175	89	Hilus: 1	Hilus: 35	SR: 1	SR: 5	SR: 0	SR: 4	AAV1-TRE-eGFPtau
									SLM: 1		SLM: 1	SLM: 4	SLM: 1	SLM: 4	
					GFP 488 + DSRED 568 + CR 633					30		15		43	
												VGAT:		VGAT:	
					Staining for:					VGAT:		SO: 3		SO: 7	
										SM: 1		SP: 0		SP: 10	
					GFP Tau					SG: 15	0	SR: 2	119	SR: 7	
					VGAT				14	Hilus: 12		SLM: 8		SLM: 10	
					Interneuron					----------		----------		----------	
					s				SM: 0	CR:	SO: 0	CR:	SO: 107	CR:	
					Calretinin				SG: 6		SP: 0		SP: 11		
					Interneuron				Hilus: 8	SM: 0	SR: 0	SO: 0	SR: 1	SO: 1	
										SG: 0	SLM: 0	SP: 1	SLM: 0	SP: 2	
	VGATtd 5	M	6 months	12	s	R MEC (Layer II MEC)	221	133		Hilus: 2		SLM: 1		SR: 6	AAV1-TRE-eGFPtau
														SLM: 0	
					GFP 488 + DSRED 568										
					Staining for:										
					GFP Tau				3	29	1	9	95	4	
					VGAT				SM: 0	SM: 5	SO: 1	SO: 0	SO: 87	SO: 1	
					Interneuron				SG: 1	SG: 10	SP: 0	SP: 3	SP: 7	SP: 2	
					s				Hilus: 2	Hilus:14	SR: 0	SR: 2	SR: 1	SR: 1	
	VGATtd 1	F	6 months	12	s	R MEC (Layer II MEC)	141	99			SLM: 0	SLM: 4	SLM: 0	SLM: 0	AAV1-TRE-eGFPtau
					GFP 488 + DSRED 568										
					Staining for:				0	0	0	0	103	49	
					GFP Tau				SM:	SM:	SO:	SO:	SO: 24	SO: 8	
					VGAT				SG:	SG:	SP:	SP:	SP: 73	SP: 36	
ECIII	ECIIIVGATTD 14 6	F	3 months	10	s	R MEC (Layer III MEC)	152	103	Hilus:	Hilus:	SR:	SR:	SR: 5	SR: 5	AAV1-hSYN1-DIO-GFPtau/P301L-WPRE
												SLM:	SLM: 1	SLM: 0	
					GFP 488 + DSRED 568										
					Staining for:				1	12	38	26	65	61	
					GFP Tau				SM: 0	SM: 2	SO: 36	SO: 21	SO: 44	SO: 37	
					VGAT				SG: 1	SG: 7	SP: 2	SP: 4	SP: 11	SP: 9	
					Interneuron				Hilus:0	Hilus: 3	SR: 0	SR: 1	SR: 6	SR: 8	
	VGATTD 42	M	3 months	9	s	R vCA1	203	104			SLM: 0	SLM: 0	SLM: 4	SLM: 7	AAV1-hSYN1-DIO-GFPtau/P301L-WPRE
					GFP 488 + DSRED 568										
					Staining for:				6	25	1	15	43	67	
					GFP Tau				SM: 2	SM: 2	SO: 0	SO: 2	SO: 29	SO: 25	
					VGAT				SG: 1	SG: 2	SP: 0	SP: 6	SP: 8	SP: 26	
					Interneuron				Hilus: 3	Hilus: 21	SR: 1	SR: 7	SR: 4	SR: 11	
	VGATTD 43	M	3 months	8	s	R vCA1	157	50			SLM: 0	SLM: 0	SLM: 2	SLM: 5	AAV1-hSYN1-DIO-GFPtau/P301L-WPRE
					GFP 488 + DSRED 568										
					Staining for:				0	0	7	7	46	22	
					GFP Tau				SM:	SM:	SO: 6	SO: 6	SO: 30	SO: 12	
					VGAT				SG:	SG:	SP: 0	SP: 1	SP: 15	SP: 7	
					Interneuron				Hilus:	Hilus:	SR: 1	SR: 0	SR: 1	SR: 3	
VGATtd	VGATTD 54	M	6 months	8	s	R vCA1	82	53			SLM: 0	SLM: 0	SLM: 0	SLM: 0	AAV1-hSYN1-DIO-GFPtau/P301L-WPRE

Animal Information and the Counts for the Total Number of Cells Containing Trans-Synaptic Pathogenic Tau in this Experiment. Table can also be viewed in the appendix section.

MEC Injections

In this group, we found that inhibitory interneurons accumulated trans-synaptic spread of pathogenic tau (Fig. 4 and Fig. 6). In fact, accumulation of trans-synaptic spread of tau was more prevalent in inhibitory interneurons than principal cells, which is contrary to previous research concerning the involvement of interneurons in trans-synaptic spreading of tau.

We first examined trans-synaptic spread from Layer II MEC neurons into downstream hippocampal neurons. We did this by injecting an AAV that expresses GFPtau under the control of a TRE promoter in MEC-tTA driver mice. The MEC-tTA mouse line primarily expresses tTA in MEC LII projection neurons; however, a smaller cohort of neurons also express tTA in MEC LIII. All neurons that received trans-synaptic pathogenic tau by transfecting LII MEC neurons were taken into account when determining the following proportions.

The proportions that follow are out of the total number of cells that contain trans-synaptic pathogenic tau in the DG, CA3, and CA1 region of the hippocampus. Cells were classified as GABAergic (interneuron) or non-GABAergic (principal cells) based on the expression of tdTomato in GABAergic neurons (VGAT-Cre x ai14 tdTomato reporter). When examining the DG region, 26% of interneurons contained tau, as opposed to 1% of principal cells (Fig. 5A). When examining the CA3 region, 8% of interneurons contained tau, as opposed to 1% of principal cells that contained tau (Fig. 5A). When examining the CA1 region, 6% of interneurons contained tau, as opposed to 58% of principal cells that contained tau (Fig. 5A).

We next examined the proportions of all the neurons that received trans-synaptic GFPtau in the DG, CA3, and CA1 subregions of the hippocampus. Cells were classified as GABAergic (interneuron) or non-GABAergic (principal cells) based on the expression of tdTomato in GABAergic neurons (VGAT-Cre x ai14 tdTomato reporter). The proportions that follow are

from the total number of cells that contained trans-synaptic pathogenic tau in the DG, CA3, and CA1 region of the hippocampus respectively. Of all neurons in the DG region that trans-synaptically expressed GFPtau, 13% were interneurons located in the molecular layer, 27% were interneurons and 2% were principal cells located in the granule cell layer, 54% were interneurons and 4% principal cells (presumptive mossy cells) located in the dentate hilus (Fig. 5B). Surprisingly, 94% neurons that contained trans-synaptic pathogenic tau in the DG region were interneurons, which is in contrast to current hypotheses.

Of all neurons in the CA3 region that trans-synaptically expressed GFPtau, 3% were interneurons and 5% were principal cells located in the stratum oriens, 29% were interneurons and 3% were principal cells located in the stratum pyramidale, 24% were interneurons and 3% were principal cells located in the stratum radiatum, and 31% were interneurons and 3% were principal cells located in the stratum lacunosum-moleculare (Fig. 5C). Surprisingly, 87% of neurons that contained trans-synaptic pathogenic tau in the CA3 region were interneurons, which is in contrast to the current hypotheses.

Of all neurons in the CA1 region that trans-synaptically expressed GFPtau, 2% were interneurons and 82% were principal cells located in the stratum oriens, 3% were interneurons and 7% were principal cells located in the stratum pyramidale, 2% were interneurons and 11% were principal cells located in the stratum radiatum, and 2% were interneurons and 0% were principal cells located in the stratum laconosum (Fig. 5D).

Because eGFPTau-P301L was found to trans-synaptically spread into a significant number of GABAergic interneurons, we next attempted to determine whether specific subtypes of interneurons were more vulnerable to eGFPTau-P301L infection. Calretinin, a calcium-binding protein, is expressed in a small distinct subset of GABAergic interneurons that

exclusively innervate other interneurons but not principal cells. Modification of calretinin interneuron function by pathogenic tau could therefore have profound impact on the activity of the entire population of hippocampal interneurons. Thus, we used immunofluorescence to determine whether calretinin interneurons were susceptible to the trans-synaptic spread of eGFPTau-P301L. We detected no spread of eGFPTau-P301L into calretinin neurons in the molecular or granule cell layer. However, in the hilus of DG, 5% of eGFPTau-P301L expressing neurons also stained positive for calretinin (Fig. 7). In CA3, all eGFPTau-P301L positive neurons were negative for calretinin in the stratum oriens and the stratum radiatum. However, 7% of neurons in the stratum pyramidale and 7% in the stratum lacunosum-moleculare were positive for calretinin interneurons containing trans-synaptic pathogenic tau (Fig. 7). Interestingly, eGFPTau-P301L did not appear to trans-synaptically spread into CA3 principal cells in these studies. In CA1, of all the eGFPTau-P301L expressing neurons only 1% of stained positive for calretinin in the stratum oriens and stratum pyramidale and an additional 6% stained positive for calretinin in the stratum radiatum. None of the eGFPTau-P301L positive neurons in the stratum lacunosum-moleculare were also positive for calretinin (Fig. 7). Therefore, although calretinin neurons only make up a very small percentage of neurons that received the trans-synaptic spread of tau, calretinin neurons appear to be involved in trans-synaptic spreading of tau.

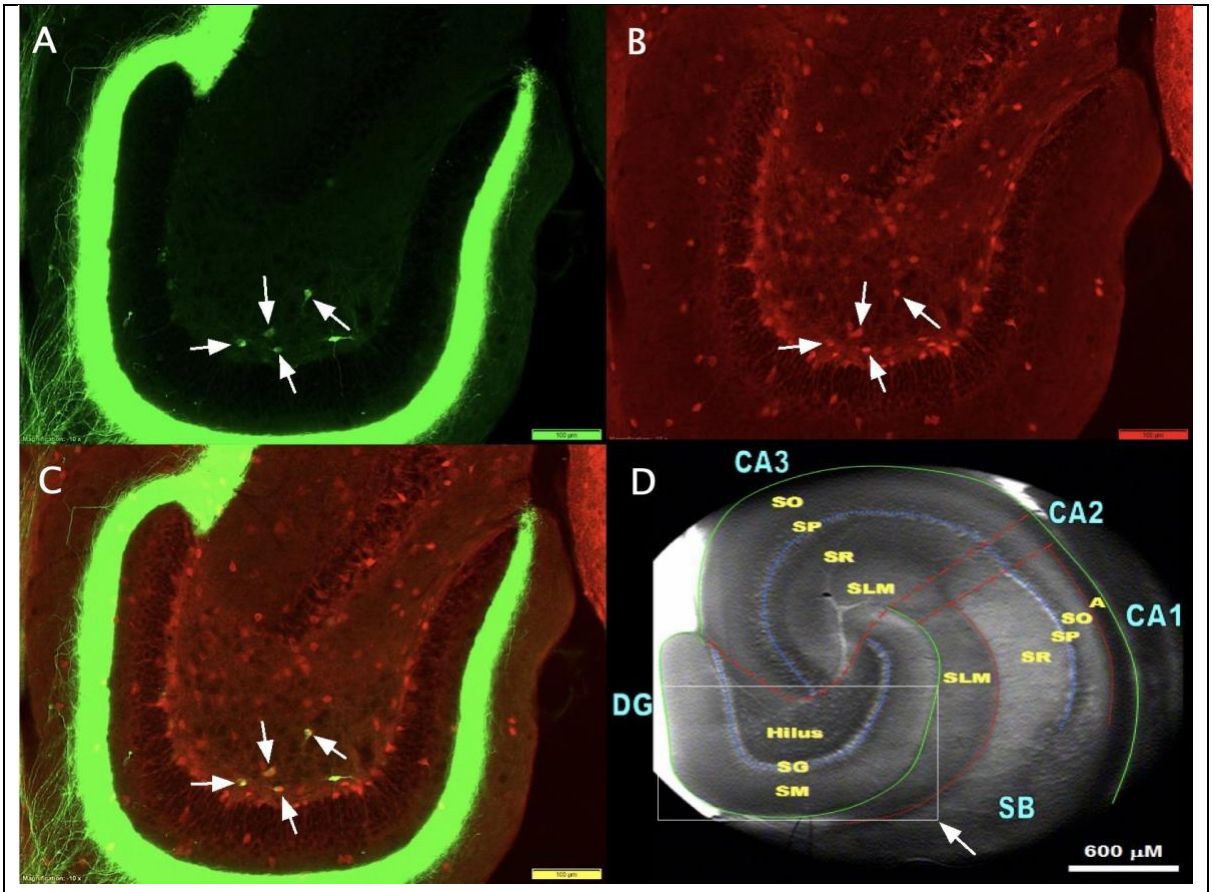

Figure 4: Medial Entorhinal Cortex Layer II Injection- Targets DG & CA3

A: Tau containing cells are labeled. Arrows point to GFP-tau containing cells that overlap with VGAT interneurons.
B: VGAT interneurons are labeled. Arrows point to GFP-tau containing cells that overlap with VGAT interneurons.
C: Arrows point to GFP-tau containing cells that overlap with VGAT interneurons.
D: Picture of the mouse hippocampus. Arrow points to hippocampal region of interest for this experiment.

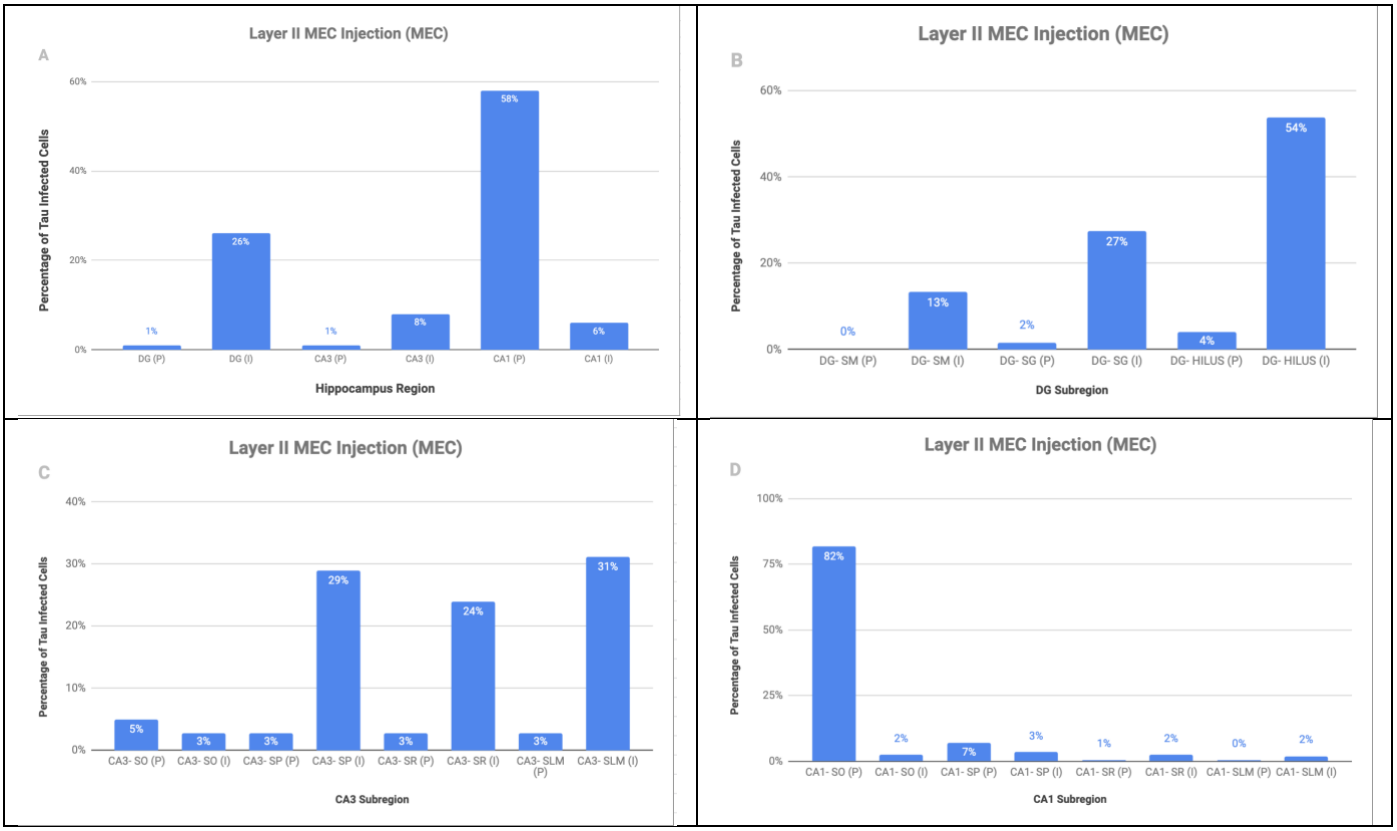

Figure 5: Percentage of Tau Containing Cells after Layer II MEC Injection

A, B, C, & D: Proportions of tau containing cells in each region and sub region of the hippocampus (P = principal cell; I = interneuron; SM = stratum molecular layer; SG = stratum granule cell layer; Hilus; SO = stratum oriens; SP = stratum pyramidale; SR = stratum radiatum; and SLM = stratum laconosum).

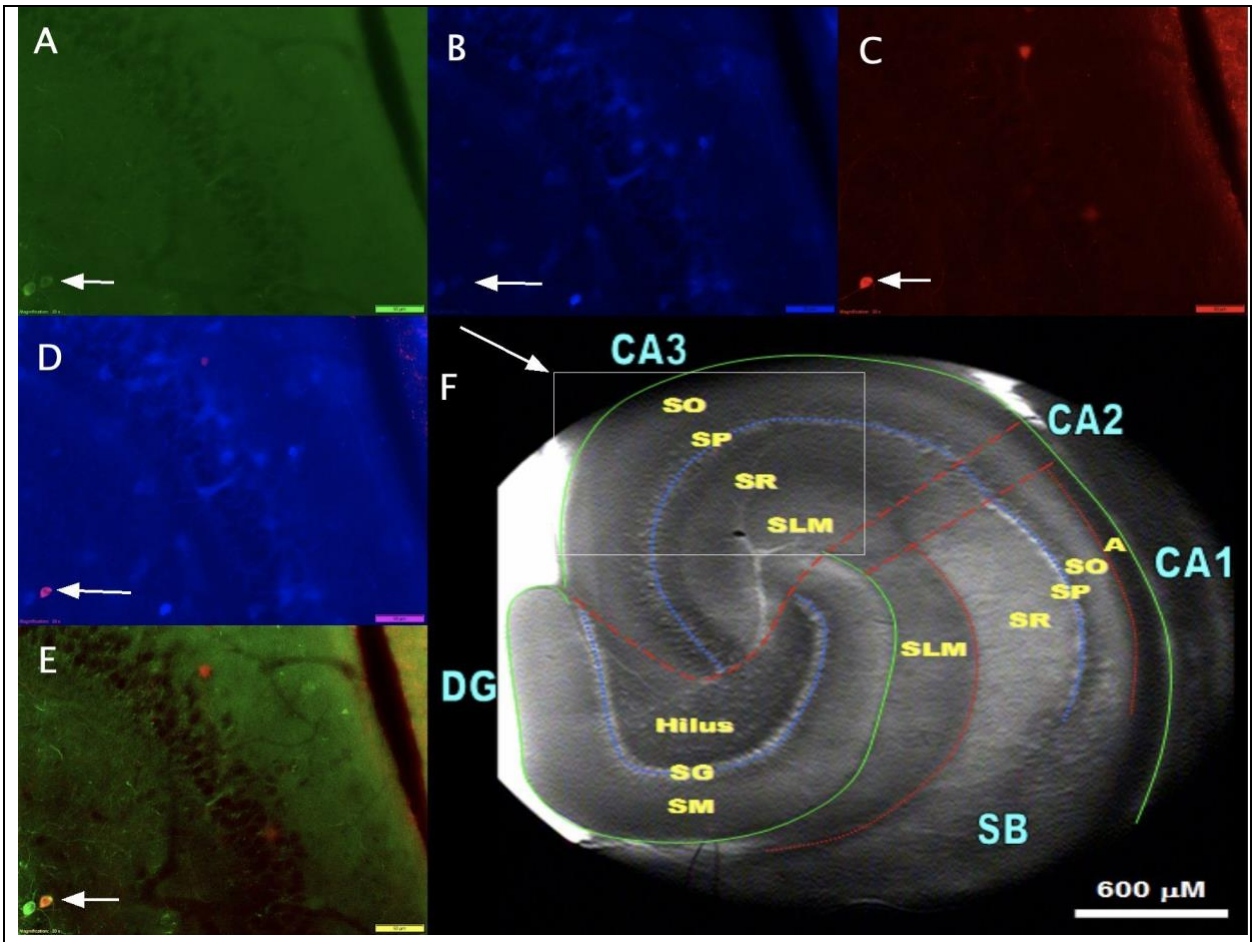

Figure 6: Medial Entorhinal Cortex Layer II Injection- Targets DG & CA3

A: Tau containing cells are labeled. Arrow points to a GFP-tau containing cell.
B: VGAT interneurons fluorescing tdTomato are labeled. Arrow points to a VGAT interneuron.
C: Calretinin interneurons are labeled. Arrow points to a calretinin interneuron.
D: Calretinin interneurons are a small subset of VGAT interneurons. Arrow points to a VGAT positive and calretinin positive interneuron.
E: Tau containing cells are labeled. Arrow points to GFP-tau containing cells that overlap with calretinin interneuron.
F: Picture of the mouse hippocampus. Arrow points to hippocampal region of interest for this experiment.

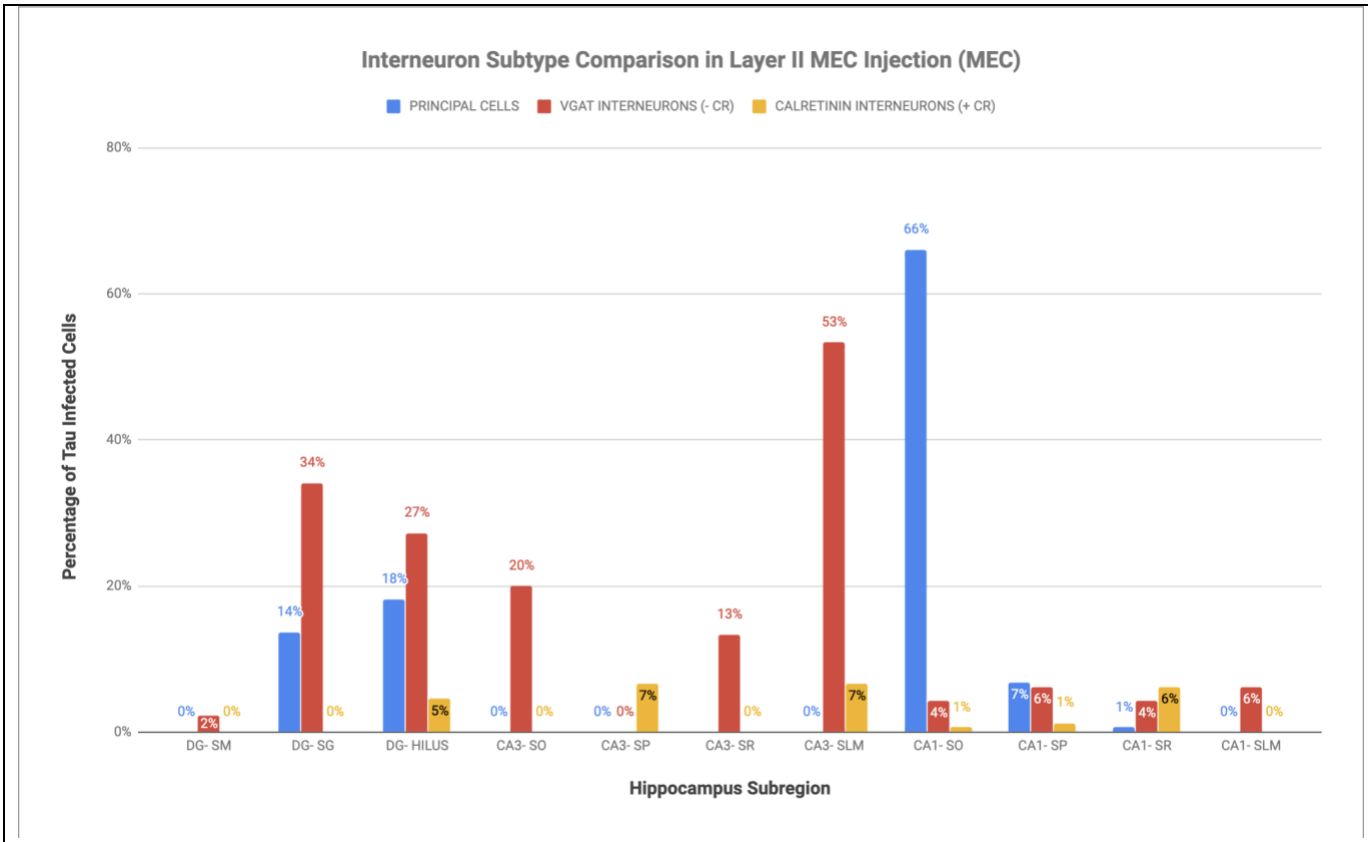

Figure 7: Percentage of Tau Containing Calretinin Positive Cells after Layer II MEC Injection

Proportions of tau containing cells in each region and sub region of the hippocampus (SM = stratum molecular layer; SG = stratum granule cell layer; Hilus; SO = stratum oriens; SP = stratum pyramidale; SR = stratum radiatum; and SLM = stratum laconosum).

ECIII Injections

In this group, we found that inhibitory interneurons accumulated trans-synaptic spread of pathogenic tau (Fig. 8). In fact, based on proportions, accumulation of trans-synaptic spread of tau was more prevalent in inhibitory interneurons than principal cells, which is contrary to previous research concerning the involvement of interneurons in trans-synaptic spreading of tau.

We next examined the trans-synaptic spread of eGFPTau-P301L into postsynaptic hippocampal neurons following the virally-mediated transfection of LIII neurons of the MEC. Expression of eGFPTau-P301L depended on Cre-mediated recombination, which was largely restricted to layer III principal cells in the MEC. Layer III MEC neurons project exclusively to the CA1 region of the hippocampus. Therefore, we expected to observe trans-synaptic spread of eGFPTau-P301L into CA1 neurons but not those of the DG or CA3.

The proportions that follow are out of the total number of cells containing trans-synaptic pathogenic tau in the CA1 region of the hippocampus. As expected, we observed no expression of eGFPTau-P301L in postsynaptic neurons in the DG or CA3 following eGFPTau-P301L transfection of LIII MEC projection neurons. When examining the CA1 region, 32% of interneurons contained trans-synaptic pathogenic tau, as opposed to 68% of principal cells that contained trans-synaptic pathogenic tau (Fig. 9A). This observation is important as interneurons as a whole make up less than 20% of the neurons in hippocampal CA1. This suggests that subsets of hippocampal CA1 inhibitory interneurons may be particular vulnerable to the trans-synaptic spread of eGFPTau-P301L.

We next examined the spread of eGFPTau-P301L into neurons of the different layers of CA1. Of all the interneurons expressing eGFPTau-P301L in CA1, 5 % were found in stratum oriens, 24% in stratum pyramidale, 3% in stratum radiatum, and none in stratum lacunosum-

moleculare. For “principal” non-GABAergic neurons, 16% were found in stratum oriens, 48% in stratum pyramidale, 3% in stratum radiatum, and 1% in stratum lacunosum-moleculare (Fig. 9B). Because inhibitory interneuron subtypes are found to be preferentially found in different layers, these findings may guide future studies toward identifying interneuron subtypes that are particularly vulnerable to the trans-synaptic spread of eGFPTau-P301L.

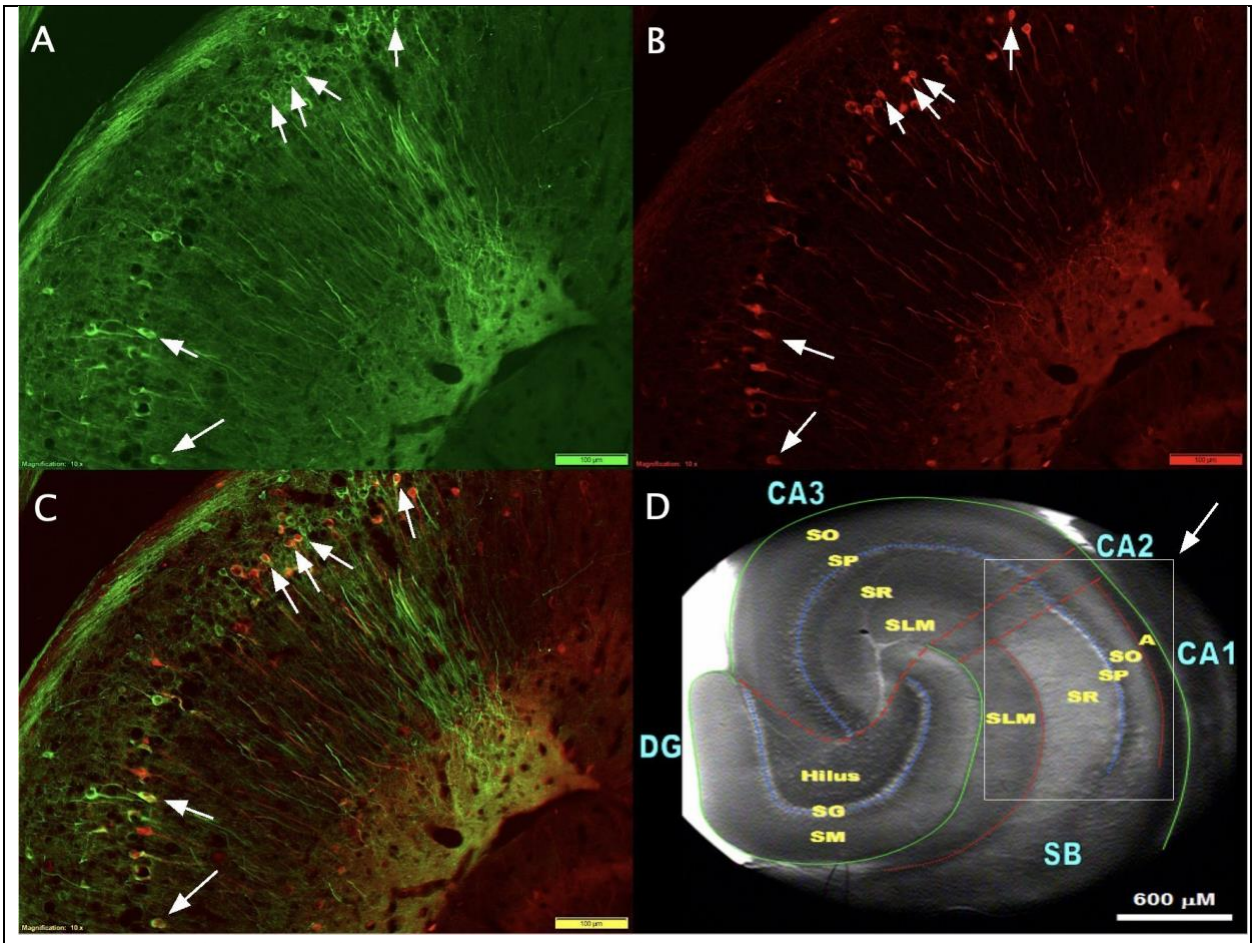

Figure 8: Medial Entorhinal Cortex Layer III Injection- Targets CA1

A: Tau containing cells are labeled. Arrows point to GFP-tau containing cells that overlap with VGAT interneurons.
B: VGAT interneurons are labeled. Arrows point to GFP-tau containing cells that overlap with VGAT interneurons.
C: Arrows point to GFP-tau containing cells that overlap with VGAT interneurons.
D: Picture of the mouse hippocampus. Arrow points to hippocampal region of interest for this experiment.

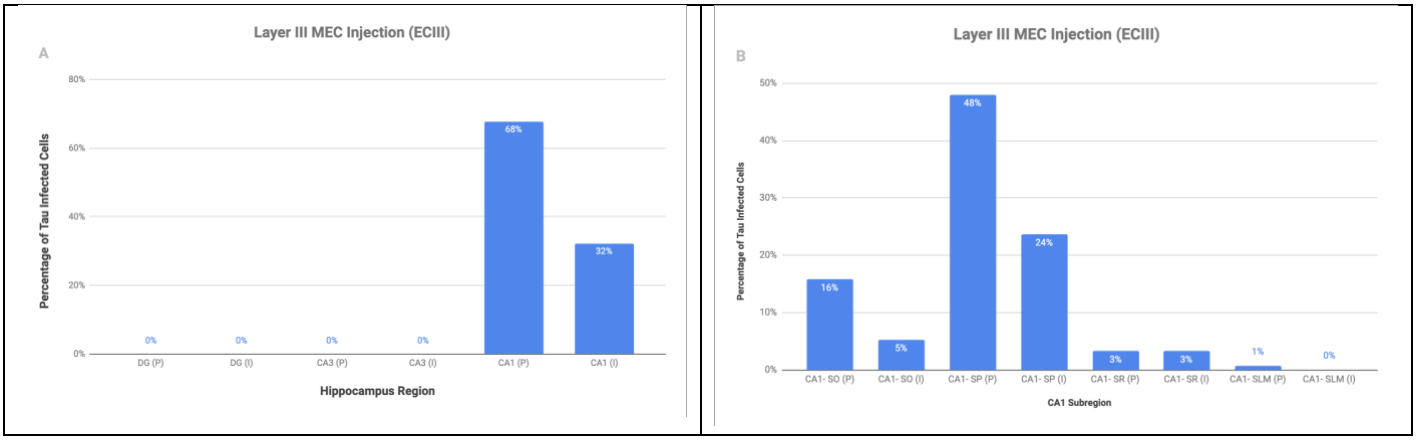

Figure 9: Percentage of Tau Containing Cells after Layer III MEC Injection

A & B: Proportions of tau containing cells in the CA1 region of the hippocampus (P = principal cell; I = interneuron; SM = stratum molecular layer; SG = stratum granule cell layer; Hilus; SO = stratum oriens; SP = stratum pyramidale; SR = stratum radiatum; and SLM = stratum laconosum).

vCA1 Injections

In this group, we found that principal cells accumulated trans-synaptic spread of pathogenic tau (Fig. 10). This suggest that inhibitory interneurons are actively involved in trans-synaptic spread of tau, which is contrary to previous research concerning the involvement of interneurons in trans-synaptic spreading of tau.

Because trans-synaptic tau spreads into a significant proportion of interneurons in hippocampal CA1, we asked whether CA1 interneurons can trans-synaptically spread tau to CA1 pyramidal cells. To do this we expressed eGFPTau-P301L in GABAergic neurons in hippocampal CA1 and assessed if eGFPTau-P301L was then passed to postsynaptic CA1 pyramidal neurons. Please refer to Figure 10 and Figure 11 for these findings.

Although we targeted hippocampal CA1 for our AAV eGFPTau-P301L injections, our injections permitted diffusion of virus into CA3 and DG resulting in expression of eGFPTau-P301L in all 3 regions of the hippocampus. When examining the hippocampus as a whole, 11% of eGFPTau-P301L was found in DG interneurons, 2% in DG principal cells (presumptive mossy cells), 11% in CA3 interneurons, 10% in CA3 principal cells, 36% in CA1 interneurons, and 30% CA1 principal cells (Fig. 11 A). Therefore, CA1 interneurons were capable of transmitting eGFPTau-P301L to postsynaptic CA1 principal cells. However, perhaps more interesting was the observation that interneurons in DG and CA3 could also trans-synaptically spread eGFPTau-P301L into principal neurons of the DG and CA3, respectively. This latter observation was interesting because local interneurons were capable of passing eGFPTau-P301L to postsynaptic principal cells in DG and CA3 whereas inputs from the entorhinal cortex appeared incapable. Therefore, it appears that vulnerability to trans-synaptic spread of eGFPTau-P301L is synapse specific and perhaps not cell type specific.

We next examined the layer specific expression of eGFPTau-P301L in each region of the hippocampus. In the DG, 11% of eGFPTau-P301L neurons were molecular layer interneurons, 3% were molecular layer principal cells, 30% were granule cell layer interneurons, 5% were granule cell layer principal neurons, 45% were hilar interneurons and 5% were hilar principal cells (Fig. 11 B). In CA3, 23% of eGFPTau-P301L neurons were in stratum oriens interneurons, 28% were stratum oriens principal cells, 22% were stratum pyramidale interneurons, 2% were stratum pyramidale principal cells, 23% were stratum radiatum interneurons, and 3% in stratum radiatum principal cells (Fig. 11 C). No interneurons or principal cells expressing eGFPTau-P301L were observed in the stratum lacunosum-moleculare of CA3. In CA1, 26% of eGFPTau-P301L neurons were stratum oriens interneurons, 31% were statum pyramidale principal cells 15% were stratum pyramidale interneurons, 8% were stratum pyramidale principal 8% were interneurons in stratum radiatum, 4% were principal cells in stratum radiatum, 5% were interneurons in stratum lacunosum-moleculare and 3% were non GABAergic neurons in stratum lacunosum-moleculare (Fig. 11 D).

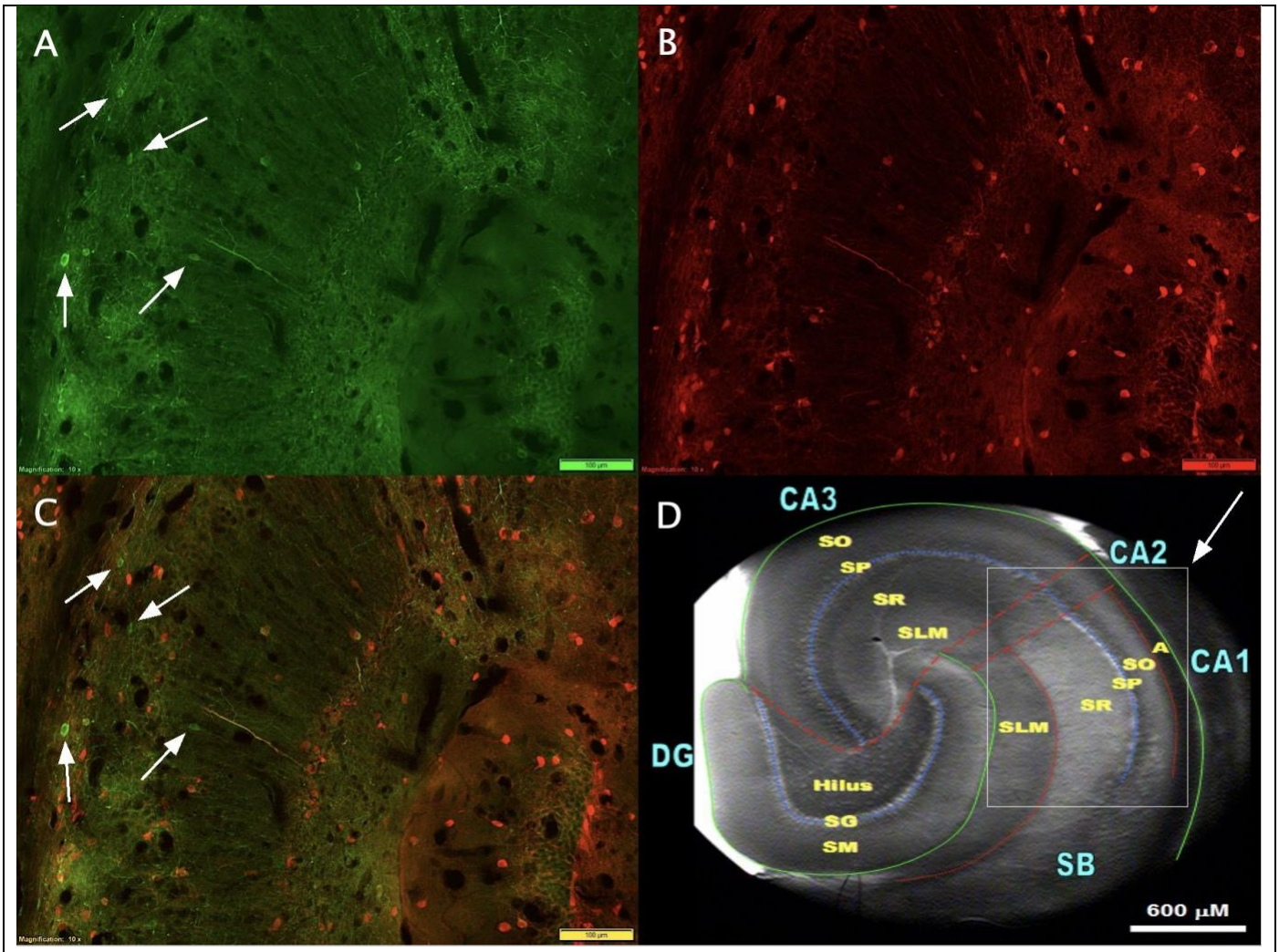

Figure 10: Ventral CA1 Injection

A: Tau containing cells are labeled. Arrows point to GFP-tau containing cells that do not overlap with VGAT interneurons.
B: VGAT interneurons are labeled.
C: Arrows point to GFP-tau containing cells that do not overlap with VGAT interneurons.
D: Picture of the mouse hippocampus. Arrow points to hippocampal region of interest for this experiment.

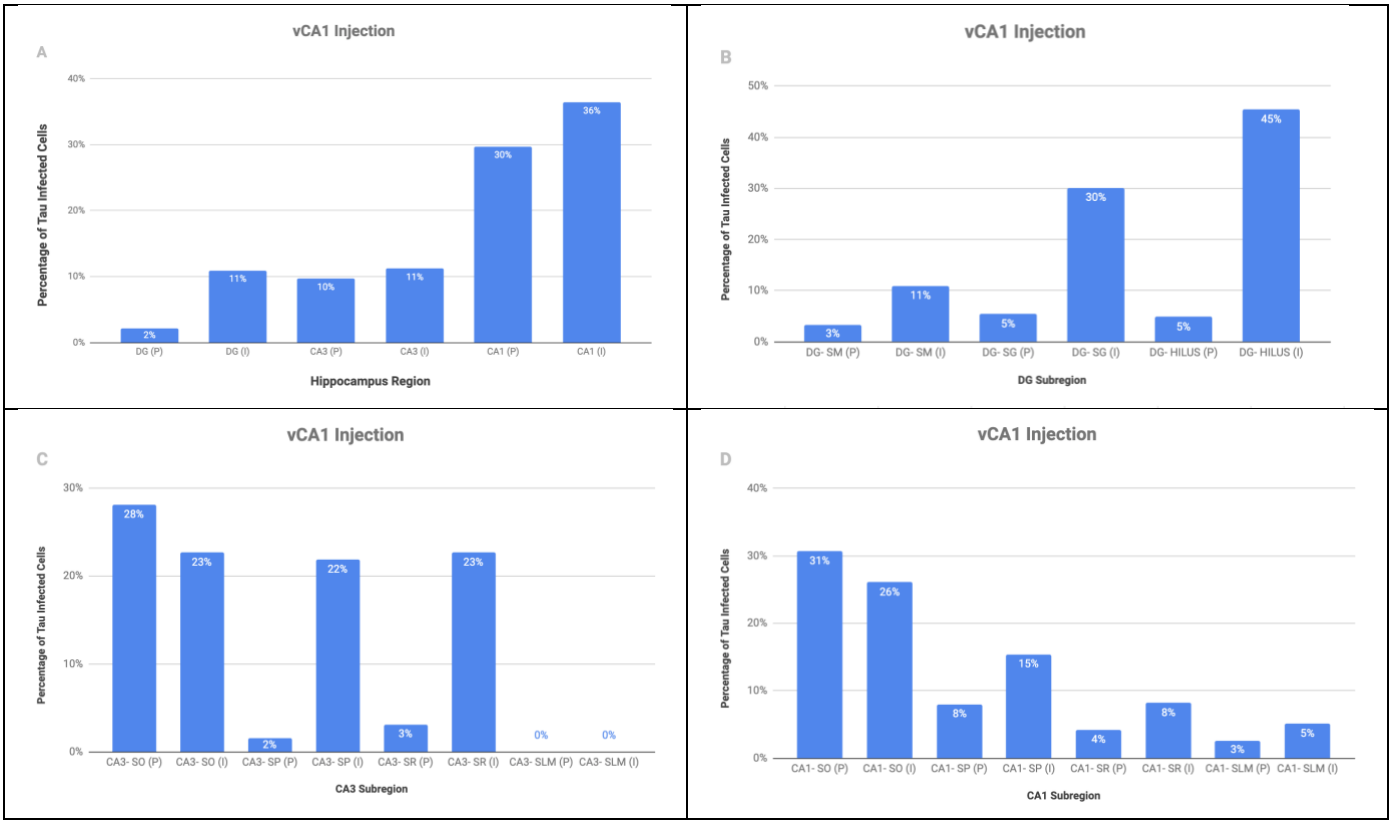

Figure 11: Percentage of Tau Containing Cells after vCA1 Injection

A, B, C, & D: Proportions of tau containing cells in each region and sub region of the hippocampus (P = principal cell; I = interneuron; SM = stratum molecular layer; SG = stratum granule cell layer; Hilus; SO = stratum oriens; SP = stratum pyramidale; SR = stratum radiatum; and SLM = stratum laconosum).

Discussion

To examine the trans-synaptic spread of eGFPTau-P301L, we used an adeno associated virus (AAV) strategy in which eGFP-Tau P301L was expressed under the control of the tetracylcine response element (AAV1-TRE-eGFPtau) or the human synapsin promoter (AAV1-hSYN1-DIO-GFPtauP301L) was injected into either layer II of the medial entorhinal cortex (MEC group), layer III of the medial entorhinal cortex (ECIII group), or ventral CA1 of the hippocampus (VGATtd group). This was done to identify and classify interneurons and their subtypes vulnerable to the trans-synaptic spread of tau. Because interneurons were trans-synaptically labelled with eGFPTau-P301L, we determined the relative susceptibility of interneurons to the trans-synaptic spread of tau of interneurons compared to principal neurons and we further explored which subpopulations of interneurons contained pathogenic tau based on their anatomical location and co-expression of calretinin. In this study, vesicular GABAergic transporter (VGAT) interneurons, which is found in all interneurons in the hippocampus, expressed tdTomato (Rudy, Fishell, Lee, & Hjerling-Leffler, 2011). So, interneurons were classified as containing pathogenic tau if cells were positive for both GFP and tdTomato.

MEC Injections

The known projection from layer II of the medial entorhinal cortex is to the DG and CA3 region of the hippocampus. We examined trans-synaptic spread from Layer II MEC neurons into downstream hippocampal neurons. We did this by injecting an AAV that expresses GFPtau under the control of a TRE promoter in MEC-tTA driver mice. The MEC-tTA mouse line expresses tTA in MEC LII projection neurons, so we expected eGFPTau-P301L to progress from layer II of the medial entorhinal cortex and only infect cells (principal cells and interneurons) in

the DG and CA3 region of the hippocampus. As expected, the DG and CA3 cells of the hippocampus were contained pathogenic tau. Based on the proportions, it would appear that interneurons in these regions are more susceptible to trans-synaptic spread of tau. This is contrary to previous research suggesting that principal cells are predominantly involved in trans-synaptic spread of tau. It was also determined that at least one subpopulation of interneurons, calretinin interneurons, are susceptible to the trans-synaptic spread of tau. GABAergic interneurons make up about 10% - 15% of cells in the hippocampus (Pelkey et al., 2017) and calretinin (CR+) interneurons make up a small proportion (~ 10%) of GABAergic interneurons (Gulyás, Hájos, & Freund, 1996). Although CR+ interneurons that contain pathogenic tau are small in numbers, the fact that this small subset of interneurons contains trans-synaptic pathogenic tau is impressive. This finding is also interesting because calretinin interneurons are interneuron selective and do not innervate principal cells. So, their effect can be significant through their influence in inhibitory interneurons through disinhibition. Future studies should determine the other interneurons subpopulations involved in trans-synaptic tau transmission because our results suggest that other subpopulations are likely to be involved.

Unexpectedly, CA1 cells contained pathogenic tau as well. CA1 cells in this group were contained tau because, in these mice, some of the cells in layer III of the medial entorhinal cortex had tTA in them (which allowed for the expression of tau in this pathway). In an ideal mouse model (one where there is no genetic variability between individuals), cells in layer III of this group would not have had tTA in them, and ultimately no cells would contain pathogenic tau in CA1. Due to limitations of the mouse line, the infection of cells in the CA1 region of the hippocampus were unavoidable.

ECIII Injections

The known projection from layer III of the medial entorhinal cortex is to the CA1 region of the hippocampus. We examined the trans-synaptic spread of eGFPTau-P301L into postsynaptic hippocampal neurons following the virally-mediated transfection of LIII neurons of the MEC. Expression of eGFPTau-P301L depended on Cre-mediated recombination, which was largely restricted to layer III principal cells in the MEC. Layer III MEC neurons project exclusively to the CA1 region of the hippocampus. Therefore, we expected to observe trans-synaptic spread of eGFPTau-P301L into CA1 neurons but not those of the DG or CA3. As expected, the CA1 cells of the hippocampus contained pathogenic tau. Upon further examination of the CA1 region, it was evident that interneurons contained pathogenic tau. Principal cells in CA1 appear to contain pathogenic tau in greater number than interneurons; however, about one third of cells that contain pathogenic tau in this region are interneurons. Keeping this in mind, since interneurons make up about 15% of neurons in CA1, this finding suggest that subsets of interneurons may be more susceptible to spread than CA1 principal cells based on probabilities. If everything was equal, we would have expected about 15% of tau containing neurons to be interneurons. Interneurons may be more susceptible to trans-synaptic spread of tau and this is contrary to previous research suggesting that principal cells are predominantly involved in tauopathy. No cells in the DG region or the CA3 region of the hippocampus contained trans-synaptic pathogenic tau because, as expected, layer III of the medial entorhinal cortex does not project to these regions.

vCA1 Injections

Ventral CA1 has many known projections outside the hippocampal system, but this experiment was done to determine if interneurons can trans-synaptically spread tau to CA1 principal cells. To do this we expressed eGFPTau-P301L in GABAergic neurons in hippocampal CA1 and assessed whether eGFPTau-P301L was trans-synaptically spread to postsynaptic CA1 pyramidal neurons. The rationale for this experiment was that when virus is injected in ventral CA1, only interneurons will express GFP- tau because only interneurons have cre-recombinase in this group of mice. If principal cells in CA1 contained tau, this would suggest that interneurons are actively participating in the trans-synaptic spreading of tau. We did not know what to expect, except that only interneurons in vCA1 would be expressing GFP-tau in them.

After a 3-month time point, interneurons and principal cells in CA1 both contained trans-synaptic pathogenic tau. Additionally, interneurons and principal cells in DG and CA3 also contained pathogenic tau. The trans-synaptic spread of tau is more than likely due to diffusion from injection site into the DG and CA3 region of the hippocampus, not due to interneurons projecting backwards into these regions. Nevertheless, these results from the CA1 region of the hippocampus suggest that interneurons play an active role in the trans-synaptic spreading of tau. This is contrary to previous research suggesting that principal cells are exclusively involved in tauopathy.

Conclusion

Because this was a discovery expedition, this study was more concerned with determining the trends of trans-synaptic tau transmission associated with hippocampal interneurons. Contrary to previous research, our results suggest that principal cells are not

exclusively involved in tauopathy and that interneurons are actively involved in trans- synaptic tau transmission within the hippocampus. This was determined by observing how tau progresses from three hippocampal pathways. This study has provided insight into the cell types that are affected by tauopathy, which is a hallmark of AD and other neurogenerative diseases. This understanding could lead to the discovery for new therapeutic targets for neurodegenerative diseases that are associated with tauopathy. More studies concerning the relationship between tauopathy and interneurons should progress for this discovery.

Limitations, however, did exist in this study. All AAV injections were done manually through stereotactic surgery and injected into either the entorhinal cortex or the hippocampus to the best of our abilities. Although mice that received a missed injection were excluded from the data, minor variability in the injected area of interest will always exist for this type of methodology. Genetic uniqueness of transgenic animals in this study should also be considered. Although the transgenic mice from this study were received from professional services and were properly tested and controlled for, genetic uniqueness for each transgenic animal could have potentially affected the way trans-synaptic tau activity occurs in each hippocampal system as well as the cells affected and involved in tauopathy. The number of animals used in this study should also be considered. About 40 mice were used for this study. Because this was an exploratory study, some mice were excluded from the data because it was too early of a timepoint for cells to be affected by tau or too late of a timepoint and tau containing cells were dead; however, the majority of mice were excluded from this study because they received a missed injection. The data from this study are from the few successful injections. In the future, collecting data with more mice will be helpful to determine significance within the study.

Future studies should also determine the relationship between the role of sex differences and the prevalence of tau containing interneurons in hippocampal trans-synaptic tau activity. This study could have potentially given light on this subject, but there were not enough successful injections to make a worthy suggestion. Future studies should also determine other interneuron subpopulations affected by trans-synaptic tau transmission. The original plan of this study was to assess whether more subpopulations, not just calretinin interneurons, contained trans-synaptic pathogenic tau; however, there was not enough successful injections to examine other subtypes. Future studies should also determine how interneurons are affected as tau progresses from other areas of the entorhinal cortex and the hippocampus. This study only examined the pathways from layer II and layer III of the medial entorhinal cortex as well as ventral CA1 of the hippocampus. Examining pathways from the lateral entorhinal cortex as well as other regions of the hippocampus could potentially give insight as to how tau progresses and the behavior of tau containing interneurons in the hippocampal system.

Appendix

Table 1: Animal Stock Information

Mouse ID	Stock #	Strain of Origin	Purpose
Viptm1(cre)Zjh /J	JaxLab Stock # (0)10908	(C57BL/6 x 129S4/SvJae)F1	Cre targets VIP cells
B6;129P2-Pvalbtm1(cre)Arbr /J	JaxLab Stock # (00)8069	129P2/OlaHsd	Cre targets PV cells
Slc32a1 tm2(cre)Lowl/J	JaxLab Stock # (0)16962	129S6/SvEvTac	Cre targets VGAT cells
Cr-IRES-Cre	JaxLab Stock # (0)10774	C57BL/6	Cre targets Calretinin cells
C57BL/6-Tg(Pvalb-tdTomato)15Gfng/J	JaxLab Stock # (0)27395	C57BL/6NTac	tdTomato expressed in PV cells (PvTg)
B6;CBA-Tg(Cyp19a1-cre)1Dlc/J	JaxLab Stock # (0)31779	C57BL/6 x CBA	Cre recombinase expressed from aromatase promoter (MEC_ITA)
Ai14 (B6.Cg-GT(ROSA)26Sortm14(CAG-tdTomato)Hze/J)	JaxLab Stock # 007914	(129S6/SvEvTac x C57BL/6NCrl)F1	tdTomato reporter line to identify interneurons
B6.Cg-Tg(Klk8-tTA)SMmay/MullMmmh	# 031779-MU	N/A	NOP-tTA animal that allows for specific tTA expression in the layer II medial entorhinal cortex
ECIII	A gift from S. Tonegwa, MIT	N/A	a pOXR1-Cre animal that expresses Cre in MEC layer III of the entorhinal cortex

Table 2: Primary and Secondary Antibody List

Name	Manufacturer	Catalog Number	Concentration (dilution)	Optimal Slice Thickness	Incubation Period
mouse- monoclonal anti GFP (1°)	Clontech	632375	(1:50)	20-30 microns	overnight
poly rabbit anti DSRED (1°)	Clontech	632496	(1:500)	20-30 microns	overnight
poly rabbit anti calretinin (1°)	Millipore	ABN2191	(1:250 - 1:1000)	20-80 microns	3 days
Goat Anti Mouse 488 (2°)	ThermoFisher	A-11001	(1:1000)	variable	overnight
Goat Anti Rabbit 568 (2°)	ThermoFisher	A11036	(1:1000)	variable	overnight
Goat Anti Rabbit 647 (2°)	ThermoFisher	A32733	(1:1000)	variable	overnight

Table 3: Animal Information for Thesis

Group	Animal	Sex	Virus Timepoint	# of slices	Antibodies	Injection Site	Total # of GFP-Tau infected Cells	Total # of Infected Principal Cells	Principal Cells DG	Interneurons DG	Principal Cells CA3	Interneurons CA3	Principal Cells CA1	Interneurons CA1	Virus
MEC	VGATtd 5	M	6 months	12 s	GFP 488 + DSRED 568 Staining for: **GFP Tau** **VGAT** **Interneuron** GFP 488 + DSRED 568 + CR 633 Staining for: **GFP Tau** **VGAT** **Interneuron** s **Calretinin** **Interneuron**	R MEC (Layer II MEC)	175	89	1 SM: 0 SG: 0 Hilus: 1 54 SM: 6 SG: 13 Hilus: 35 3 SO: 0 SP: 1 SR: 1 SLM: 1 30 VGAT: SM: 1 SG: 15 Hilus: 12 ---------- CR: SM: 0 SG: 0 Hilus: 2 15 SO: 1 SP: 5 SR: 5 SLM: 4 15 VGAT: SO: 3 SP: 0 SR: 2 SLM: 8 ---------- CR: SO: 0 SP: 1 SR: 0 SLM: 1 85 SO: 77 SP: 7 SR: 0 SLM: 1 43 VGAT: SO: 7 SP: 10 SR: 7 SLM: 10 ---------- CR: SO: 107 SP: 11 SR: 1 SLM: 0 17 SO: 4 SP: 5 SR: 4 SLM: 4	AAV1-TRE-eGFPtau
	VGATtd 5	M	6 months	12 s	GFP 488 + DSRED 568 Staining for: **GFP Tau** **VGAT** **Interneuron**	R MEC (Layer II MEC)	221	133	14 SM: 0 SG: 6 Hilus: 8	0	119				
	VGATtd 1	F	6 months	12 s	GFP 488 + DSRED 568 Staining for: **GFP Tau** **VGAT** **Interneuron**	R MEC (Layer II MEC)	141	99	3 SM: 0 SG: 1 Hilus: 2	29 SM: 5 SG: 10 Hilus: 14	1 SO: 1 SP: 0 SR: 0 SLM: 0	9 SO: 0 SP: 3 SR: 2 SLM: 4	95 SO: 87 SP: 7 SR: 1 SLM: 0	4 SO: 1 SP: 2 SR: 1 SLM: 0	AAV1-TRE-eGFPtau
ECIII	ECIIIVGATTD 14 6	F	3 months	10 s	GFP 488 + DSRED 568 Staining for: **GFP Tau** **VGAT** **Interneuron**	R MEC (Layer III MEC)	152	103	1 SM: SG: Hilus:	0 SO: SP: SR: SLM:	103 SO: 24 SP: 73 SR: 5 SLM: 1	49 SO: 8 SP: 36 SR: 5 SLM: 0	AAV1-hSYN1-DIO-GFPtauP301L-WPRE		
	VGATTD 42	M	3 months	9 s	GFP 488 + DSRED 568 Staining for: **GFP Tau** **VGAT** **Interneuron**	R vCA1	203	104	1 SM: 0 SG: 1 Hilus:0	12 SM: 2 SG: 7 Hilus: 3	38 SO: 36 SP: 2 SR: 0 SLM: 0	26 SO: 21 SP: 4 SR: 1 SLM: 0	65 SO: 44 SP: 11 SR: 6 SLM: 4	61 SO: 37 SP: 9 SR: 8 SLM: 7	AAV1-hSYN1-DIO-GFPtauP301L-WPRE
	VGATTD 43	M	3 months	8 s	GFP 488 + DSRED 568 Staining for: **GFP Tau** **VGAT** **Interneuron**	R vCA1	157	50	6 SM: 2 SG: 1 Hilus:3	25 SM: 2 SG: 2 Hilus: 21	1 SO: 0 SP: 0 SR:1 SLM: 0	15 SO: 2 SP: 6 SR: 7 SLM: 0	43 SO: 29 SP: 8 SR: 4 SLM: 2	67 SO: 25 SP:26 SR: 11 SLM: 5	AAV1-hSYN1-DIO-GFPtauP301L-WPRE
VGATtd	VGATTD 54	M	6 months	8 s	GFP 488 + DSRED 568 Staining for: **GFP Tau** **VGAT** **Interneuron**	R vCA1	82	53	0 SM: SG: Hilus:	0 SM: SG: Hilus:	7 SO: 6 SP: 0 SR: 1 SLM: 0	7 SO: 6 SP: 1 SR: 0 SLM: 0	46 SO: 30 SP: 15 SR: 1 SLM: 0	22 SO: 12 SP: 7 SR: 3 SLM: 0	AAV1-hSYN1-DIO-GFPtauP301L-WPRE

Table 4: All Animals Used for Experiments

Group	Animal	Sex	Virus Timepoint	**Missed Injection?**
MEC	VGATtd 5	M	6 months	**No**
	VGATtd 1	F	6 months	**No**
	VGATTD 14	F	6 months	**Yes. Did not process- missed injection**
	VGATTD 15	M	6 months	**Yes. Did not process- missed injection**
	VGATTD 16	F	6 moths	**Yes. Did not process- missed injection**
	VGATTD 17	F	6 months	**Yes. Did not process- missed injection**
	MEC_tTA 194	M	6 months	**Yes. Did not process- missed injection**
ECIII	PVTG 20	F	3 months	**Yes. Did not process- missed injection**
	PVTG 21	F	3 months	**No. Processed but very early timepoint**
	PVTG 22	F	3 months	**No. Processed but very early timepoint**
	PVTG 16	F	3 months	**No. Processed but very early timepoint**
	PVTG 18	F	3 months	**No. Processed but very early timepoint**
	ECIII 393	F	3 months	**No. Processed but very early timepoint**
	ECIIIVGATTD14 1	F	3 months	**Yes. Did not process- missed injection**
	ECIIIVGATTD14 3	M	4 months	**No. Processed but very early timepoint**
	ECIIIVGATTD14 4	M	4 months	**No. Processed but very early timepoint**
	ECIIIVGATTD14 5	M	4 months	**Yes. Did not process- missed injection**
	ECIIIVGATTD 14 6	F	3 months	**No**
	ECIIIVGATTD 14 9	F	3 months	**Yes. Processed, but missed injection**
	ECIII 353	M	9 months	**Yes. Processed, but missed injection**
	ECIII 350	F	9 months	**Yes. Did not process- missed injection**
rTG-Tau	rTgtauEC_ChF 289	M	19.2 months	**No injection required for this mice. Tau activation through breeding. Timepoint to late and all tau infected cells were dead.**
	rTgtauEC_ChF 291	M	19.2 months	**No injection required for this mice. Tau activation through breeding. Timepoint to late and all tau infected cells were dead.**
	rTgtauEC_ChF 292	F	19.2 months	**No injection required for this mice. Tau activation through breeding. Timepoint to late and all tau infected cells were dead.**
	rTgtauEC_ChF 295	F	18.5 months	**No injection required for this mice. Tau activation through breeding. Timepoint to late and all tau infected cells were dead.**
VGATtd	VGATTD 42	M	3 months	**No**
	VGATTD 43	M	3 months	**No**
	VGATTD 49	M	6 months	**Yes. Processed, but missed injection**
	VGATTD 51	M	6 months	**Yes. Processed, but missed injection**
	VGATTD 52	M	6 months	**Yes. Processed, but missed injection**
	VGATTD 50	M	6 months	**Yes. Processed, but missed injection**
	VGATTD 54	M	6 months	**No**
	VGATTD 37	M	6 months	**Yes. Processed, but missed injection**
	VGATTD 38	M	6 months	**Yes. Processed, but missed injection**

Table 5: Materials

Material	Company	Catalog Number (if available)	Purpose
Robot Stereotaxic	Neurostar	SD467	Surgery
BX53 Upright Microscope	Olympus	N/A	Quantitative/Qualitative Analysis
OLYMPUS cellSens dimension software 1.13	Olympus	Build: 13479	Quantitative Analysis
ThermoScientific Cryostat	Fischer	N/A	Tissue Recovery
Beladine	Purdue Products L.P.	06901-3431	Pre Surgery
Hamilton Syringe	Hamilton Company	87930	Surgery
Peri-Star Pro Pump	World Precision Instrument	N/A	Perfusion
Tissue Tek O.C.T embedding compound	Electron Microscopy Sciences	62550-01	Tissue Recovery
Tissue Tek Cryomold	Sakura	4566	Tissue Preparation
ProLong Gold Antifade Reagent	Invitrogen	P36930	Tissue Preparation
ColorFrost Slide	ThermoFisher Scientific	9951T-006	Tissue Preparation
Mice	The Jackson Laboratory	Varied	Animals

References

2015 Alzheimer's disease facts and figures. (2015). Alzheimer's & Dementia, 11(3), 332–384. https://doi.org/10.1016/j.jalz.2015.02.003

Bartus, R. T. (2000). On Neurodegenerative Diseases, Models, and Treatment Strategies: Lessons Learned and Lessons Forgotten a Generation Following the Cholinergic Hypothesis. Experimental Neurology, 163(2), 495–529. https://doi.org/10.1006/exnr.2000.7397

Billingsley, M. L., & Kincaid, R. L. (1997). Regulated phosphorylation and dephosphorylation of tau protein: effects on microtubule interaction, intracellular trafficking and neurodegeneration. Biochemical Journal, 323(Pt 3), 577–591.

Booker, S. A., & Vida, I. (2018). Morphological diversity and connectivity of hippocampal interneurons. Cell and Tissue Research, 373(3), 619–641. https://doi.org/10.1007/s00441-018-2882-2

Braak, H., & Braak, E. (1995). Staging of alzheimer's disease-related neurofibrillary changes. Neurobiology of Aging, 16(3), 271–278. https://doi.org/10.1016/0197-4580(95)00021-6

Braak, H., & Del Tredici, K. (2012). Alzheimer's disease: Pathogenesis and prevention. Alzheimer's & Dementia, 8(3), 227–233. https://doi.org/10.1016/j.jalz.2012.01.011

Breitner, J. C. S. (2006). Dementia—Epidemiological Considerations, Nomenclature, and a Tacit Consensus Definition. Journal of Geriatric Psychiatry and Neurology, 19(3), 129–136. https://doi.org/10.1177/0891988706291081

Buzsáki, G. (2002). Theta Oscillations in the Hippocampus. Neuron, 33(3), 325–340. https://doi.org/10.1016/S0896-6273(02)00586-X

Canto, C. B., & Witter, M. P. (2012a). Cellular properties of principal neurons in the rat entorhinal cortex. I. The lateral entorhinal cortex. Hippocampus, 22(6), 1256–1276. https://doi.org/10.1002/hipo.20997

Canto, C. B., & Witter, M. P. (2012b). Cellular properties of principal neurons in the rat entorhinal cortex. II. The medial entorhinal cortex. Hippocampus, 22(6), 1277–1299. https://doi.org/10.1002/hipo.20993

Chauviere, L., Rafrafi, N., Thinus-Blanc, C., Bartolomei, F., Esclapez, M., & Bernard, C. (2009). Early Deficits in Spatial Memory and Theta Rhythm in Experimental Temporal Lobe Epilepsy. Journal of Neuroscience, 29(17), 5402–5410. https://doi.org/10.1523/JNEUROSCI.4699-08.2009

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., … Kramer, A. F. (2011). Exercise training increases size of hippocampus and improves memory, 6.

Fernández, J. A., Rojo, L., Kuljis, R. O., & Maccioni, R. B. (2008). The Damage Signals Hypothesis of Alzheimer's Disease Pathogenesis. Journal of Alzheimer's Disease, 14(3), 329–333. https://doi.org/10.3233/JAD-2008-14307

Fox, N. C., Warrington, E. K., Freeborough, P. A., Hartikainen, P., Kennedy, A. M., Stevens, J. M., & Rossor, M. N. (1996). Presymptomatic hippocampal atrophy in Alzheimer's disease: A longitudinal MRI study. Brain, 119(6), 2001–2007. https://doi.org/10.1093/brain/119.6.2001

Francis, P. T, Palmer, A. M., Snape, M., & Wilcock, G. K. (1999). The cholinergic hypothesis of Alzheimer's disease: a review of progress. Journal of Neurology, Neurosurgery & Psychiatry, 66(2), 137–147. https://doi.org/10.1136/jnnp.66.2.137

Francis, Paul T. (2003). Glutamatergic systems in Alzheimer's disease. International Journal of Geriatric Psychiatry, 18(S1), S15–S21. https://doi.org/10.1002/gps.934

Freund, T. F., & Buzsáki, G. (1998). Interneurons of the hippocampus. Hippocampus, 6(4), 347–470. https://doi.org/10.1002/(SICI)1098-1063(1996)6:4<347::AID-HIPO1>3.0.CO;2-I

Frisoni, G. B., Laakso, M. P., Beltramello, A., Geroldi, C., Bianchetti, A., Soininen, H., & Trabucchi, M. (1999). Hippocampal and entorhinal cortex atrophy in frontotemporal dementia and Alzheimer's disease. Neurology, 52(1), 91–91. https://doi.org/10.1212/WNL.52.1.91

Fu, H., Possenti, A., Freer, R., Nakano, Y., Villegas, N. C. H., Tang, M., … Duff, K. E. (2019). A tau homeostasis signature is linked with the cellular and regional vulnerability of excitatory neurons to tau pathology. Nature Neuroscience, 22(1), 47. https://doi.org/10.1038/s41593-018-0298-7

Goedert, M., Wischik, C. M., Crowther, R. A., Walker, J. E., & Klug, A. (1988). Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. Proceedings of the National Academy of Sciences, 85(11), 4051–4055. https://doi.org/10.1073/pnas.85.11.4051

Gómez-Isla, T., Price, J. L., McKeel Jr., D. W., Morris, J. C., Growdon, J. H., & Hyman, B. T. (1996). Profound Loss of Layer II Entorhinal Cortex Neurons Occurs in Very Mild Alzheimer's Disease. The Journal of Neuroscience, 16(14), 4491–4500. https://doi.org/10.1523/JNEUROSCI.16-14-04491.1996

Gulyás, A. I., Hájos, N., & Freund, T. F. (1996). Interneurons Containing Calretinin Are Specialized to Control Other Interneurons in the Rat Hippocampus. Journal of

Neuroscience, 16(10), 3397–3411. https://doi.org/10.1523/JNEUROSCI.16-10-03397.1996

Harada, A., Oguchi, K., Okabe, S., Kuno, J., Terada, S., Ohshima, T., ... & Hirokawa, N. (1994). Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature*, *369*(6480), 488.

Hardy, J. A., & Higgins, G. A. (1992). Alzheimer's Disease: The Amyloid Cascade Hypothesis. Science; Washington, 256(5054), 184.

Iqbal, K., Liu, F., Gong, C.-X., & Grundke-Iqbal, I. (2010). Tau in Alzheimer Disease and Related Tauopathies. Current Alzheimer Research, 7(8), 656–664.

Khan, U. A., Liu, L., Provenzano, F. A., Berman, D. E., Profaci, C. P., Sloan, R., … Small, S. A. (2014). Molecular drivers and cortical spread of lateral entorhinal cortex dysfunction in preclinical Alzheimer's disease. Nature Neuroscience, 17(2), 304–311. https://doi.org/10.1038/nn.3606

Khyade, V. B., Khyade, S. V., & Jagtap, S. G. (2016). Alzheimer's Disease: Overview, 3(12), 16.

Knierim, J.J. (2015). The hippocampus. *Current Biology, 25*, R1116-R1121.

Koliatsos, V. E., et al., 2006. Early involvement of small inhibitory cortical interneurons in Alzheimer's disease. Acta Neuropathol. 112, 147-62.

Kosik, K. S., Joachim, C. L., & Selkoe, D. J. (1986). Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. Proceedings of the National Academy of Sciences, 83(11), 4044–4048. https://doi.org/10.1073/pnas.83.11.4044

Lei, P., Ayton, S., Finkelstein, D. I., Adlard, P. A., Masters, C. L., & Bush, A. I. (2010). Tau protein: Relevance to Parkinson's disease. The International Journal of Biochemistry & Cell Biology, 42(11), 1775–1778. https://doi.org/10.1016/j.biocel.2010.07.016

Levenga, J., et al., 2013. Tau pathology induces loss of GABAergic interneurons leading to altered synaptic plasticity and behavioral impairments. Acta Neuropathol Commun. 1, 34.

Loreth, D., et al., 2012. Selective degeneration of septal and hippocampal GABAergic neurons in a mouse model of amyloidosis and tauopathy. Neurobiol Dis. 47, 1-12.

Life expectancy at birth, total (years). (2019). Retrieved November 10, 2018, from https://data.worldbank.org/indicator/SP.DYN.LE00.IN

Lindwall, G., & Cole, R. D. (1984). Phosphorylation Affects the Abilityof Tau Protein to Promote Microtubule Assembly, 6.

Linn, R. T., Wolf, P. A., Bachman, D. L., Knoefel, J. E., Cobb, J. L., Belanger, A. J., … D'Agostino, R. B. (1995). The "Preclinical Phase" of Probable Alzheimer's Disease: A 13-Year Prospective Study of the Framingham Cohort. Archives of Neurology, 52(5), 485–490. https://doi.org/10.1001/archneur.1995.00540290075020

Maccioni, R. B., Farías, G., Morales, I., & Navarrete, L. (2010). The Revitalized Tau Hypothesis on Alzheimer's Disease. Archives of Medical Research, 41(3), 226–231. https://doi.org/10.1016/j.arcmed.2010.03.007

Maccioni, R. B., Rojo, L. E., Fernández, J. A., & Kuljis, R. O. (2009). The Role of Neuroimmunomodulation in Alzheimer's Disease. Annals of the New York Academy of Sciences, 1153(1), 240–246. https://doi.org/10.1111/j.1749-6632.2008.03972.x

McBain, C. J., & Fisahn, A. (2001). Interneurons unbound. Nature Reviews Neuroscience, 2(1), 11–23. https://doi.org/10.1038/35049047

McHugh P, Folstein MF. Dementia syndrome in depression. In: Katzman R, Terry RD, Bick K, eds. Aging. Vol 7, New York, NY: Raven Press; 1977:94 -96.

Moreno, H., Wu, W. E., Lee, T., Brickman, A., Mayeux, R., Brown, T. R., & Small, S. A. (2007). Imaging the Aβ-Related Neurotoxicity of Alzheimer Disease. Archives of Neurology, 64(10), 1467–1477. https://doi.org/10.1001/archneur.64.10.1467

Morris, G. P., Clark, I. A., & Vissel, B. (2014). Inconsistencies and Controversies Surrounding the Amyloid Hypothesis of Alzheimer's Disease, 21.

O'Brien, R. J., & Wong, P. C. (2011). Amyloid Precursor Protein Processing and Alzheimer's Disease. Annual Review of Neuroscience, 34(1), 185–204. https://doi.org/10.1146/annurev-neuro-061010-113613

Palop, J. J., & Mucke, L. (2010). Amyloid-β Induced Neuronal Dysfunction in Alzheimer's Disease: From Synapses toward Neural Networks. Nature Neuroscience, 13(7), 812–818. https://doi.org/10.1038/nn.2583

Pelkey, K. A., Chittajallu, R., Craig, M. T., Tricoire, L., Wester, J. C., & McBain, C. J. (2017). Hippocampal GABAergic Inhibitory Interneurons. Physiological Reviews, 97(4), 1619–1747. https://doi.org/10.1152/physrev.00007.2017

Price, D. L., Tanzi, R. E., Borchelt, D. R., & Sisodia, S. S. (1998). ALZHEIMER'S DISEASE: Genetic Studies and Transgenic Models. Annual Review of Genetics, 32(1), 461–493. https://doi.org/10.1146/annurev.genet.32.1.461

Rojo, L. E., Fernández, J. A., Maccioni, A. A., Jimenez, J. M., & Maccioni, R. B. (2008). Neuroinflammation: Implications for the Pathogenesis and Molecular Diagnosis of Alzheimer's Disease. Archives of Medical Research, 39(1), 1–16. https://doi.org/10.1016/j.arcmed.2007.10.001

Rudy, B., Fishell, G., Lee, S., & Hjerling-Leffler, J. (2011). Three Groups of Interneurons Account for Nearly 100% of Neocortical GABAergic Neurons. Developmental Neurobiology, 71(1), 45–61. https://doi.org/10.1002/dneu.20853

Shin, R. W., Iwaki, T., Kitamoto, T., & Tateishi, J. (1991). Hydrated autoclave pretreatment enhances tau immunoreactivity in formalin-fixed normal and Alzheimer's disease brain tissues. *Laboratory investigation; a journal of technical methods and pathology*, *64*(5), 693-702.

Skutella, T., & Nitsch, R. (2001). New molecules for hippocampal development. *Trends in neurosciences*, *24*(2), 107-113.

Spillantini, M. G., Van Swieten, J. C., & Goedert, M. (2000). Tau gene mutations in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Neurogenetics, 2(4), 0193–0205. https://doi.org/10.1007/s100489900084

Squire, L. R. (1986). Mechanisms of memory. Science, 232(4758), 1612–1619. https://doi.org/10.1126/science.3086978

Squire, Larry R. (1992). Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. Psychological Review, 99(2), 195. https://doi.org/10.1037/0033-295X.99.2.195

Steward, O., & Scoville, S. A. (1976). Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. The Journal of Comparative Neurology, 169(3), 347–370. https://doi.org/10.1002/cne.901690306

Storandt, M., & Hill, R. D. (1989). Very Mild Senile Dementia of the Alzheimer Type: II. Psychometric Test Performance. Archives of Neurology, 46(4), 383–386. https://doi.org/10.1001/archneur.1989.00520400037017

Strittmatter, W. J., & Roses, A. D. (1996). Apolipoprotein E and Alzheimer's Disease, 25.

Taniguchi, T., Kawamata, T., Mukai, H., Hasegawa, H., Isagawa, T., Yasuda, M., … Tanaka, C. (2001). Phosphorylation of Tau Is Regulated by PKN. Journal of Biological Chemistry, 276(13), 10025–10031. https://doi.org/10.1074/jbc.M007427200

Terry, A. V. (2003). The Cholinergic Hypothesis of Age and Alzheimer's Disease-Related Cognitive Deficits: Recent Challenges and Their Implications for Novel Drug Development. Journal of Pharmacology and Experimental Therapeutics, 306(3), 821–827. https://doi.org/10.1124/jpet.102.041616

Tolnay, M., & Clavaguera, F. (2004). Argyrophilic grain disease: A late-onset dementia with distinctive features among tauopathies. Neuropathology, 24(4), 269–283. https://doi.org/10.1111/j.1440-1789.2004.00591.x

Torrealba, F., & Valdés, J. L. (2008). The parietal association cortex of the rat. *Biological research*, *41*(4), 369-377.

Tsao, A., Sugar, J., Lu, L., Wang, C., Knierim, J. J., Moser, M.-B., & Moser, E. I. (2018). Integrating time from experience in the lateral entorhinal cortex. Nature, 561(7721), 57. https://doi.org/10.1038/s41586-018-0459-6

Varga, C., Lee, S. Y., & Soltesz, I. (2010). Target-selective GABAergic control of entorhinal cortex output. Nature Neuroscience, 13(7), 822–824. https://doi.org/10.1038/nn.2570

Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., … Citron, M. (1999). ᴺʟ-Secretase Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE, 286, 8.

Verret, L., Mann, E. O., Hang, G. B., Barth, A. M. I., Cobos, I., Ho, K., … Palop, J. J. (2012). Inhibitory Interneuron Deficit Links Altered Network Activity and Cognitive

Dysfunction in Alzheimer Model. Cell, 149(3), 708–721. https://doi.org/10.1016/j.cell.2012.02.046

Weingarten, M. D., Lockwood, A. H., Hwo, S. Y., & Kirschner, M. W. (1975). A protein factor essential for microtubule assembly. Proceedings of the National Academy of Sciences, 72(5), 1858–1862. https://doi.org/10.1073/pnas.72.5.1858

Whitwell, J. L., Przybelski, S. A., Weigand, S. D., Knopman, D. S., Boeve, B. F., Petersen, R. C., & Jack, C. R. (2007). 3D maps from multiple MRI illustrate changing atrophy patterns as subjects progress from mild cognitive impairment to Alzheimer's disease. Brain, 130(7), 1777–1786. https://doi.org/10.1093/brain/awm112

Wilson, D. I. G., Langston, R. F., Schlesiger, M. I., Wagner, M., Watanabe, S., & Ainge, J. A. (2013). Lateral entorhinal cortex is critical for novel object-context recognition. Hippocampus, 23(5), 352–366. https://doi.org/10.1002/hipo.22095

Wilson, R. S., Segawa, E., Boyle, P. A., Anagnos, S. E., Hizel, L. P., & Bennett, D. A. (20120903). The natural history of cognitive decline in Alzheimer's disease. Psychology and Aging, 27(4), 1008. https://doi.org/10.1037/a0029857

Wimo, A., Winblad, B., Stoffler, A., Wirth, Y., & Mobius, H. (2003). Resource Utilisation and Cost Analysis of Memantine in Patients with Moderate to Severe Alzheimer??s Disease: PharmacoEconomics, 21(5), 327–340. https://doi.org/10.2165/00019053-200321050-00004

Winocur, G., Wojtowicz, J. M., Sekeres, M., Snyder, J. S., & Wang, S. (2006). Inhibition of neurogenesis interferes with hippocampus-dependent memory function. Hippocampus, 16(3), 296–304. https://doi.org/10.1002/hipo.20163

Wirt, R., & Hyman, J. (2017). Integrating spatial working memory and remote memory: interactions between the medial prefrontal cortex and hippocampus. *Brain sciences*, *7*(4), 43.

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