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THE ROLE OF MICROTUBULE-ASSOCIATED PROTEIN TAU IN NEURONAL EXCITABILITY AND EPILEPTOGENESIS

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Dr. Bret N. Smith, Major Professor

Dr. Kenneth S. Campbell, Director of Graduate Studies

THE ROLE OF MICROTUBULE-ASSOCIATED PROTEIN TAU IN NEURONAL EXCITABILITY AND EPILEPTOGENESIS

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Medicine
at the University of Kentucky

By

Ryan Adam Cloyd

Lexington, Kentucky

Co- Directors: Dr. Bret N. Smith Professor of Neuroscience

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Lexington, Kentucky

2021

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ABSTRACT OF DISSERTATION

THE ROLE OF MICROTUBULE-ASSOCIATED PROTEIN TAU IN NEURONAL EXCITABILITY AND EPILEPTOGENESIS

Tauopathies, including Alzheimer's disease (AD), are devastating diseases with an immense burden on society which is predicted to increase in coming decades. In addition to progressive loss of memory and cognitive function, patients with tauopathies have a 6-10 fold increase in lifetime risk for seizures, and many are diagnosed with epilepsy. The presence of epileptiform activity on electroencephalogram (EEG) recordings from patients with AD predicts faster cognitive decline compared to patients without abnormal EEG readings. Electrophysiological measurements in murine models of AD have identified neuronal hyperexcitability. Furthermore, reducing tau phosphorylation or expression confers seizure resistance in animal epilepsy models. Although evidence suggests the presence of common mechanisms contributing to both tauopathy and epilepsy, more work is needed to understand how this interaction works and whether tau can be effectively targeted to improve patients' lives. This study investigated the relationship between tauopathy using transgenic mice that expressed no tau protein ($tau^{-/-}$) or expressed non-mutant, human tau protein without expressing murine tau (htau). The htau mice develop progressive tauopathy with age. Non-transgenic C57BL/6J mice were used as controls. Whole-cell patch-clamp electrophysiology was used to define tau's role in neuronal excitability in vivo in dentate gyrus granule cells. Both transgenic mouse strains exhibited a lower frequency of evoked action potentials and reduced likelihood of neurotransmitter release from perforant pathway inputs as measured by the paired pulse ratio compared to control at 1.5 months of age, but these differences were lost with age. The similarities between the $tau^{-/-}$ and htau mice suggest that hyperexcitability is related to the amount of normally functioning tau rather than the presence of pathological tau, and that the presence of normal murine tau may influence the results of other studies involving models of tauopathy. Furthermore, tau's role in epileptogenesis was studied using intrahippocampal injection of kainate (i.ie., IHK) to induce status epilepticus, a model that induces temporal lobe epileptogenesis, in $tau^{-/-}$, htau, and C57BL/6J mice. The process of epileptogenesis appeared to be modified compared to control in both transgenic strains, but did not appear to be prevented. Compared to either $tau^{-/-}$ or C57BL/6J mice, htau mice experienced significantly greater mortality after IHK. Modifications in tau expression, wither deletion or humanization, partially abrogated synaptic excitability that developed following IHK.

In conclusion, this study showed that neuronal excitability is affected similarly by either deletion or humanization of tau, with the notable exception of survival after IHK. This

study provides clearer understanding of tau's role in acquired epilepsy and suggests novel therapeutics targeting tau may be effective for the treatment of epilepsy.

KEYWORDS: Microtubule associated protein tau, htau, whole-cell patch-clamp electrophysiology, temporal lobe epilepsy, intrahippocampal kainate, dentate granule cell

Ryan Adam Cloyd

(Name of Student)

05/28/2021

Date

THE ROLE OF MICROTUBULE-ASSOCIATED PROTEIN TAU IN NEURONAL EXCITABILITY AND EPILEPTOGENESIS

By
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05/28/2021
Date

This dissertation is dedicated to the memory
of my father, Robert Donald Cloyd

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CHAPTER 1. INTRODUCTION

1.1 Microtubule associated protein tau

The microtubule-associated protein tau (MAPT, tau) is a key microtubule- binding protein predominantly found in neurons. Although tau is typically thought of primarily as a microtubule stabilizer, it serves additional key functions. Tau is extremely versatile due to a combination of multiple isoforms arising from alternative splicing (section 1.1.1) and extensive post-translational modification (section 1.1.3). This versatility allows tau to serve many functions in addition to stabilizing microtubules, but also allows for significant pathologic potential. Tauopathies are a growing list of diseases which are caused by or involve tau dysfunction. Although tauopathies are diverse and overall not fully understood, disruptions in the normal splicing and post-translational modification have been heavily implicated in their pathogenesis (section 1.2.1)

1.1.1 Expression and alternative splicing

Tau is encoded by the gene *MAPT* on chromosome 17q21 (Neve et al., 1986). Human *MAPT* contains 16 exons which are alternatively spliced to produce 6 isoforms of tau protein (Andreadis et al., 1992). Eight of these exons (1, 4, 5, 7, 9, 11, 12, and 13) are constitutively translated in the brain, while 3 exons (2, 3, and 10, discussed below) are subject to alternative splicing. Three exons (4a, 6, and 8) are translated only in ganglia of the peripheral nervous system (Couchie et al., 1992; Goedert et al., 1992). The last two exons (0 and 14) are located in the 5' and 3' untranslated regions (Figure 1.1A).

The isoforms of tau arise from the presence or absence of 2 inserts near the amino-terminus coded by exons 2 and 3 (called the amino-terminal inserts) and from the inclusion

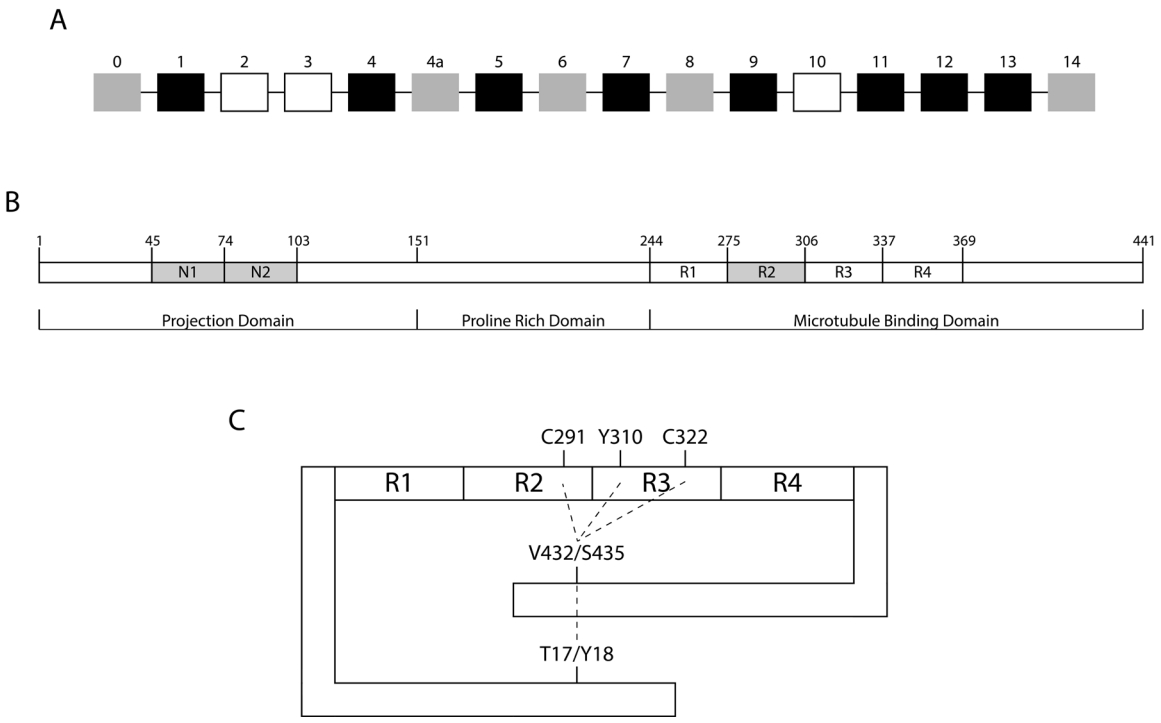

Figure 1.1 Structure of *MAPT* and microtubule associated protein tau
(A) *MAPT* contains 16 exons. In neurons, 8 exons (black boxes) are constitutively translated and 5 exons (gray boxes) are not translated. Three exons (white boxes) are subject to alternative splicing. (B) Structure of 2N4R tau including demarcations between major domains. N1, N2, and R2 (marked in gray) are be absent in shorter isoforms of tau. (C) Approximate representation (not to scale) of the "paperclip" conformation adopted by soluble tau in solution. Dotted lines denote estimated interactions between residues responsible for conformation.

of either 3 or 4 repeat domains in the microtubule binding domain near the carboxy terminus. The amino-terminus may exclude both exons 2 and 3 (giving rise to 0N tau), include exon 2 but not exon 3 (giving rise to 1N tau), or include exons 2 and 3 (giving rise to 2N tau) (Goedert and Jakes, 1990). Exon 3 is not observed in the absence of exon 2 in the human brain (Goedert et al., 1989a). The additional isoforms arise from alternative splicing of exon 10, which results in either 3 (3R) or 4 (4R) repeat domains near the carboxy terminus (Goedert et al., 1989b; Goedert et al., 1988). The resulting isoforms range from 352-441 residues. Unless otherwise noted, all residue numbers listed in this work will refer to 2N4R tau.

The relative expression of each isoform of tau depends on developmental stage and brain region. In the fetal brain, 0N3R tau is the primary isoform (Goedert and Jakes, 1990; Goedert et al., 1989a; Goedert et al., 1989b), although 4R tau expression begins late in fetal development (Kosik et al., 1989). Because 4R tau has a higher affinity for microtubules (discussed more in section 1.1.4), the development of 4R tau may promote axon growth by stabilizing the abundant microtubules in the axonal growth cone (Bamburg et al., 1986). In the adult brain, 3R and 4R tau are normally present in roughly equal amounts (Hong et al., 1998). This ratio can become perturbed in some disease, resulting in an excess of one isoform or the other depending on the disease (Chambers et al., 1999; Connell et al., 2005; Ishizawa et al., 2000). Pathologic changes in the ratio of 3R to 4R tau will be discussed in more depth in section 1.2.1.

Tau expression within the neuron is normally concentrated in the axons (Binder et al., 1985). Several mechanisms have been implicated in maintaining tau's localization. Unlike mRNA for MAP2 or tubulin, which localize to the neuron body or dendrites, tau

mRNA extends into the proximal axon (Litman et al., 1993), suggesting some degree of regional specificity in translation. Furthermore, biotinylated tau injected into the cell body of cultured neurons migrates preferentially to the axon (Hirokawa et al., 1996). Tau's localization to the axons is at least partially dependent on its microtubule binding domain. Chimeric proteins containing the N-terminal region of MAP2 (normally found in the neuron body and dendrites) and microtubule binding domain of tau localize to the axon, whereas chimeric proteins containing the N-terminal region of tau and microtubule binding domain of MAP2 localize to the neuron body and dendrites (Kanai and Hirokawa, 1995).

1.1.2 Tau structure and structural domains

Physiological tau is a "natively unfolded" protein, meaning it has little stable secondary or tertiary structure (Schweers et al., 1994). As a notable exception, dynamic interactions between tau's amino-terminus, carboxy terminus, and repeat domains results in adoption of a "paperclip" superstructure in solution (Jeganathan et al., 2006). Tau's naturally disordered nature was first recognized due to the protein's resistance to denaturing methods that render most proteins nonfunctional (Cleveland et al., 1977). This general lack of higher order structure renders x-ray crystallography impractical and detailed structural information must be gleaned through other methods. Nuclear magnetic resonance (NMR) spectroscopy studies have produced single-residue resolution of tau, revealing a highly dynamic structure (Mukrasch et al., 2009). Overall tau is basic and highly hydrophilic, although these properties vary by region and post-translational modification. Tau consists of 3 major structural regions: the projection domain, the proline rich domain, and the microtubule binding domain (Figure 1.1B).

Tau's projection domain comprises residues 1-151 in 2N4R tau and contains the amino-terminal inserts coded by exons 2 and 3 (if present). The projection domain is acidic and extends into the cytoplasm when tau is bound to microtubules (Hirokawa et al., 1988). The functions of the projection domain are not fully understood, but include interacting with the dynein-activator complex dynactin (Magnani et al., 2007), spacing and crosslinking microtubules (Chen et al., 1992; Hirokawa et al., 1988), and forming microtubule bundles (Gustke et al., 1994; Kanai et al., 1992). Tau's projection domain also mediates interaction with cellular components other than microtubules. The projection domain binds to and mediates interaction with the plasma membrane (Brandt et al., 1995). Interactions between microtubules and the plasma membrane are especially important at the axonal growth cone, where microtubules concentrate during axon elongation (Bamburg et al., 1986). GSK3β, a key disease-related tau kinase, binds in the projection domain (Sun et al., 2002). As the least studied domain of tau, the projection domain may play additional as yet undiscovered roles.

The proline-rich domain of tau, so called for its 7 PxxP motifs, comprises residues 151-244 in 2N4R tau. This domain is relatively rigid, likely due to the reduced flexibility of proline compared to other amino acids (Mukrasch et al., 2009). The proline rich domain is involved in interactions with cytoskeletal fibers. Although not the primary binding microtubule site, this region of tau contributes significantly to microtubule binding (Brandt and Lee, 1993; Goode et al., 1997; Gustke et al., 1994). In particular, two short sequences in this domain, ^{215}KKVAVVR^{221} and ^{225}KVAVVRT^{231}, have a critical role in microtubule assembly, increasing binding affinity through conformational changes and interactions with microtubules (Goode et al., 1997; Mukrasch et al., 2007). The proline rich domain is

also capable of binding actin (He et al., 2009) and the projection domain of other tau molecules (Rosenberg et al., 2008). In addition to containing many well-described phosphorylation sites (discussed more in section 1.1.3.1), the proline rich domain serves as a binding site for some key regulators of tau phosphorylation such as the src-family kinases including Fyn (Lee et al., 1998; Reynolds et al., 2008).

Of tau's 3 major structural domains, the microtubule binding domain has been the most extensively studied. It comprises residues 245-441 in 2N4R tau and contains the repeat segments that are the primary microtubule binding sites (Butner and Kirschner, 1991; Gustke et al., 1994; Himmler et al., 1989; Lee et al., 1989). Tau contains up to four imperfectly repeated segments, encoded by exons 9-12 (Lee et al., 1989). The number of repeat segments depends on the alternative splicing of exon 10, which codes for the second of four possible repeat sequences (Andreadis et al., 1992; Goedert et al., 1989b). The microtubule binding domain forms transient beta-structure, leading to regions with high rigidity (Mukrasch et al., 2009). The major features of the microtubule binding domain are the repeat domains, each consisting of 31 or 32 residues (Goedert et al., 1989b). Each repeat domain contains a conserved PGGG and KxGS motif. The PGGG motifs form hairpin turns that ensure proper conformation for efficient binding (Kadavath et al., 2015b). The KxGS motifs, particularly the one in the first repeat, form important interactions with microtubules (Biernat et al., 1993; Drewes et al., 1995). In addition to microtubules, this region of tau contains binding sites for many other targets including protein phosphatase 2A (Sontag et al., 1999; Xu et al., 2008), chaperone proteins including Hsc70 and Hsp90 (Sarkar et al., 2008; Tortosa et al., 2009), α-synuclein (Jensen et al., 1999), presenillin-1 (Takashima et al., 1998), and apolipoprotein E3 (Strittmatter et al., 1994).

1.1.3 Post-translational modifications

In addition to six different isoforms, tau is subject to extensive post-translational modification that further increases its diversity. These modifications include phosphorylation, acetylation, ubiquitination, methylation, and glycosylation. Although phosphorylation has long been the center of focus, the importance of other modifications has been increasingly recognized recently. Tau post-translational modification impacts its function in normal physiology and disease pathology by changing its microtubule binding affinity, aggregation potential, and degradation. In some cases, competition between modification mechanisms may occur at a residue. For example, some lysine residues can be subject to acetylation, ubiquitination, and methylation, and occurrence of one modification precludes the others (Morris et al., 2015; Thomas et al., 2012). Several key post-translational modifications will be reviewed here.

1.1.3.1 Phosphorylation

The longest tau isoform contains 85 potential phosphorylation sites: 44 serine residues, 36 threonine residues, and 5 tyrosine residues (Martin et al., 2013b). Out of these 85 potential sites, 31 are associated with normal physiological function, 28 are disease-specific, and 16 are found in both healthy and diseased brains (Hanger et al., 1998; Hanger et al., 2007; Martin et al., 2013b). Tau's ability to bind microtubules is heavily influenced by phosphorylation at key residues. In general, increasing phosphorylation reduces tau's binding affinity (Lindwall and Cole, 1984). Several specific phosphorylation sites have been studied for their associations with pathogenesis, particularly in the development of Alzheimer's disease (AD). Several of these sites serve as the binding epitopes for common tauopathy-related antibodies (summarized in Table 1.1).

Table 1.1 Common tau antibodies and associated epitopes

Antibody Name	Epitope	Reference
AT8	pS202 and pT205	(Goedert et al., 1995b)
AT100	pT212 and pS214	(Hoffmann et al., 1997)
AT180	pT231	(Amniai et al., 2011)
12E8	pS262	(Seubert et al., 1995)
PHF1	pS396 and pS404	(Otvos et al., 1994)

The specific functional outcome has not been identified for every phosphorylation site on tau, but the effects of phosphorylation at some key sites has been well-characterized. Phosphorylation at several sites, including S214, T231, and S396, promotes detachment of tau from microtubules and may be involved in normal physiology and disease (Illenberger et al., 1998; Kadavath et al., 2018; Sengupta et al., 1998). In contrast, phosphorylation at some sites, particularly S262, is a key step in disease processes (Biernat et al., 1993; Drewes et al., 1995; Martin et al., 2013b; Sengupta et al., 1998; Trinczek et al., 1995). A number of kinases and phosphatases are involved in maintaining physiological phosphorylation of tau and involved in pathogenesis. Some critical regulators of tau phosphorylation associated with key disease-related phosphosites will be briefly reviewed here. Important disease related phosphorylation sites for each kinase or phosphatase are summarized in Table 1.2.

Glycogen synthase kinase 3 (GSK3) is a key tau kinase originally discovered for its role in glycogen metabolism (Embi et al., 1980). Two isoforms of GSK3 (α and β) have been described which share 85% homology (Woodgett, 1990). The β isoform is strongly associated with hyperphosphorylation of tau. Overexpression of GSK3β induces tau hyperphosphorylation (Lucas et al., 2001), but this effect is prevented by administration of the GSK3β inhibitor lithium chloride (Engel et al., 2006). GSK3β activity is upregulated by administration of amyloid-beta (Aβ), demonstrating GSK3β can contribute to AD related tau hyperphosphorylation (Takashima et al., 1996; Terwel et al., 2008). GSK3β is a key AD-related tau kinase and phosphorylates tau at numerous disease-related phosphosites. Pre-phosphorylation of tau by PKA increases the GSK3β's ability to phosphorylate tau (Liu et al., 2006; Liu et al., 2004b).

Table 1.2 Key Tau Kinases/Phosphatases and Associated Phosphorylation Sites

Kinase/Phosphatase	Key Confirmed Phosphorylation Sites	References
GSK3β	S199, S202, T205, T231, S262, S404	(Hanger et al., 2007; Liu et al., 2006; Reynolds et al., 2000; Wang et al., 1998)
CK1δ	S202, T205, S262, S396, S404	(Hanger et al., 2007; Li et al., 2004; Singh et al., 1995)
PKA	S202, T205, S214, S262	(Andorfer and Davies, 2000; Benitez et al., 2021; Ko et al., 2019; Liu et al., 2004b)
PP2A	pS199, pS202, pT205, pS262, pS396, pS404	(Gong et al., 1994; Liu et al., 2005)

Casein kinase (CK) 1 and 2, which each have multiple isoforms, are involved in regulation of apoptosis and cell survival through regulation of β-catenin in the wnt-signaling pathway (Liu et al., 2002a; Marin et al., 2003; Sakanaka, 2002). The delta isoform of CK1 is especially important in tau phosphorylation. Phosphorylation by CK1δ impairs tau binding to microtubules (Li et al., 2004). CK1/2 are involved in AD beyond their role in tau phosphorylation. Different isoforms of CK1/2 are both activated by and contribute to production of Aβ (Chauhan et al., 1993; Flajolet et al., 2007; Pigino et al., 2009), phosphorylate ApoE (Raftery et al., 2005), and can activate or inhibit PP2A (Heriche et al., 1997; Pérez and Avila, 1999).

Protein kinase A (PKA), also called cAMP-dependent protein kinase, contributes to numerous signaling cascade pathways involving a broad array of processes. PKA is known to phosphorylate many other kinases, contributing to many physiologic and pathologic processes (Embogama and Pflum, 2017; Jung et al., 2017). Tau is one of PKA's many substrates (Steiner et al., 1990). Intrahippocampal injection of PKA activators results in tau hyperphosphorylation and memory deficits (Sun et al., 2005; Tian et al., 2009). Pre-phosphorylation of tau by PKA primes tau for phosphorylation by GSK3β and cdk5 (Liu et al., 2006; Liu et al., 2004b)

Although tau can be dephosphorylated by a number of phosphatases, one tau phosphatase, PP2A, stands out as the primary tau phosphatase. PP2A is the most prominent tau phosphatase, constituting approximately 70% of the total phosphatase activity (Goedert et al., 1995a; Liu et al., 2005). Treatment with PP2A dephosphorylates tau sequestered in insoluble deposits associated with AD (Drewes et al., 1993; Wang et al., 1995). PP2A activity is decreased in brains of people with AD, contributing to the accumulation of

hyperphosphorylated tau (Gong et al., 1995; Gong et al., 1993). In addition to directly dephosphorylating tau, PP2A also regulates the activity of major tau kinases (Chung and Brautigan, 1999; Kins et al., 2003; Liauw and Steinberg, 1996; Louis et al., 2011). Dysregulation of PP2A therefore contributes to tau hyperphosphorylation by multiple mechanisms.

1.1.3.2 Acetylation

Acetylation of lysine residues is an important reversible post-translational modification that regulates key cellular functions (Choudhary et al., 2009). Tau contains up to 44 lysine residues (Goedert et al., 1989a) and at least 23 of these lysine residues are subject to acetylation (Min et al., 2010). Tau is acetylated primarily by the closely related acyltransferases p300 and Creb-binding protein (CBP) (Kamah et al., 2014; Min et al., 2010) and deacetylated primarily by HDAC6 and SIRT1 (Choi et al., 2020; Cook et al., 2014). Tau also possess intrinsic acetyltransferase activity, using C291 and C322 as intermediates to transfer an acetyl group from acetyl CoA to lysine residues within tau or other proteins (Cohen et al., 2016; Cohen et al., 2013b).

The importance of tau acetylation in disease is becoming increasingly recognized. Tau acetylation increases relatively early in tauopathies, and acetylated tau resists proteasome-mediated degradation (Min et al., 2010). Several specific acetylation sites have been identified in association with AD. In particular, acetylation at K174 slows tau turnover and worsens tau-mediated deficits in animal models (Min et al., 2015). Acetylation at K274 and K281 is associated with cytoskeletal destabilization leading to memory impairment, reduced synaptic plasticity, and tau mislocalization (Sohn et al., 2016; Tracy et al., 2016). Acetylation at K280 impairs interaction with microtubules and increases the rate of tau

aggregation (Cohen et al., 2016; Cohen et al., 2011; Haj-Yahya and Lashuel, 2018). While acetylation at several sites is associated with promoting disease, acetylation of the KxGS motifs, especially K259, is associated with reduced aggregation of tau, likely through reduction in phosphorylation at S262 (Cook et al., 2014). Inhibition of HDAC6, which deacetylates the KxGS motifs, promotes tau clearance and may represent a novel therapeutic strategy (Carlomagno et al., 2017; Choi et al., 2020; Cook et al., 2014).

1.1.3.3 Ubiquitination

Ubiquitin is a 76 residue protein that is expressed by nearly all eukaryotic cells (Hershko and Ciechanover, 1998). Lysine residues on ubiquitin bind covalently to lysine residues on the target protein or other ubiquitin proteins, allowing chains of ubiquitin to develop. Ubiquitin binding is mediated through a multi-step process which culminates in an E3 ligase catalyzing the binding of ubiquitin to the target protein (Zheng and Shabek, 2017). Ubiquitin contains 7 lysine residues, and the fate of the target protein depends on which ubiquitin lysine binds (Ikeda and Dikic, 2008). Binding via K48 or K63 marks the target protein for degradation via the 26S proteasome or lysosome-autophagy systems, respectively (Ikeda and Dikic, 2008). Three E3 ligases are capable of ubiquitinating tau. The C-terminus of the Hsc70-interacting protein (CHIP) ubiquitinates tau via K48 or K63, marking it for degradation by the proteasome or lysosome system (Petrucelli et al., 2004). The TNF receptor-associated factor 6 (TRAF6) ubiquitinates tau via K63, marking it for lysosomal degradation (Babu et al., 2005). Axotrophin, also called MARCH7, ubiquitinates tau's microtubule binding domain and impairs microtubule binding, but as of yet this process is not well understood (Flach et al., 2014). The cysteine protease Otub1 is

the only deubiquitinase known to target tau, removing K48-linked ubiquitin units (Wang et al., 2017).

Tau ubiquitination primarily occurs in the microtubule binding domain. Mono- or poly-ubiquitination occurs at K254, K257, K311, K317, and K353 in brains from patients with AD (Cripps et al., 2006; Morishima-Kawashima et al., 1993). Insoluble tau is generally ubiquitinated via K48 linage, indicating a preference for proteasome mediated degradation (Cripps et al., 2006). Ubiquitinated tau can be found in mature neurofibrillary tangles but not in pre-tangles, suggesting ubiquitination is a compensatory mechanism against accumulation of aggregated tau (Bancher et al., 1991; Garcia-Sierra et al., 2012; Iwatsubo et al., 1992; Perry et al., 1987).

1.1.3.4 Methylation

Protein methylation involves covalently attaching a methyl group to a residue, typically lysine or arginine, changing its charge and altering its interactions with other residues. Methylation in tau has been observed primarily on lysine residues, although some arginine methylation occurs (Morris et al., 2015). Lysine methylation in tau is a recently recognized factor contributing to pathogenesis. Tau can be methylated *in vitro* at up to 23 different lysine residues (Funk et al., 2014). Tau from paired-helical filaments exhibits methylation at up to 7 lysine residues, while soluble tau can be methylated at 11 different lysine residues (Funk et al., 2014; Thomas et al., 2012). Lysine methylation occurs most often in the proline rich domain and micro tubule binding domains, particularly at K180 and K267 (Thomas et al., 2012). The mechanisms of tau methylation, including specific the methyltransferases and demethylases involved, have not been identified.

Lysine methylation in tau modulates its microtubule binding and aggregation potential. Within the microtubule binding domain, the lysine residues in the KxGS motifs are subject to methylation, which will affect other modifications within these motifs (Funk et al., 2014). However, the specific effect of methylation on tau binding is unclear. Tau methylation is enriched in neurofibrillary tangles and methylation at K267 is associated with increased phosphorylation at S262, which drastically reduces tau's affinity for microtubules (Thomas et al., 2012). However, *in vitro* methylation of tau has also been found to reduce the rate of aggregation (Funk et al., 2014). A likely explanation for the apparently discrepant results is the non-specific nature of the *in vitro* reductive methylation, indicating that the specific pattern of methylation is important in determining the effect. The specific role of tau methylation will likely become clearer as more research is conducted into this relatively newly recognized modification.

1.1.3.5 Glycosylation

Glycosylation, the addition of a carbohydrate to the side chains of residues, is the most common type of post-translational modification. Two broad types of glycosylation reactions have been identified: N-glycosylation, which attaches a carbohydrate chain to the side chain of an asparagine residue separated by one residue from a serine or threonine (NxS or NxT motifs), and O-glycosylation, which attaches a carbohydrate chain to the side chain of a serine or threonine residue (Haukedal and Freude, 2020). Tau can undergo N-glycosylation at N167, N359, and N410 (Sato et al., 2001). N-glycosylated tau is only found in AD brains, suggesting it is a pathology-specific modification (Liu et al., 2002c; Wang et al., 1996). N-glycosylation stabilizes tau aggregates and promotes hyperphosphorylation via modulation of PKA and PP2A activity (Liu et al., 2002b; Liu et

al., 2002c; Wang et al., 1996). Tau can undergo O-glycosylation at T123, S208, S400, and either S409, S412, or S413 (Yuzwa et al., 2012; Yuzwa et al., 2011). Unlike N-glycosylation, O-glycosylation of tau occurs in healthy brains, and O-glycosylated tau exhibits a reduced propensity for hyperphosphorylation and aggregation (Liu et al., 2004a; Yuzwa et al., 2012). O-glycosylation and phosphorylation apparently compete for residues on tau, and occurrence of one modification is frequently associated with a reduction of the other (Lefebvre et al., 2003).

1.1.4 Physiologic functions of tau

Physiological function of tau depends on coordination between the major structural domains and is significantly influenced by various post-translational modifications, especially phosphorylation. Tau's primary function is stabilization of microtubules. Tau binds longitudinally to the microtubule, at the interface between tubulin heterodimers (Al-Bassam et al., 2002; Kadavath et al., 2015a). Tau's binding to microtubules is significantly reduced by the microtubule-inhibitor vinblastine, suggesting tau and vinblastine bind the same site on microtubules (Kadavath et al., 2015a). The primary sites of direct interaction with microtubules are the repeat domains within the microtubule binding domain (Gustke et al., 1994; Himmler et al., 1989; Lee et al., 1989). NMR analysis of tau bound to microtubules has shown tau binds α-tubulin through a series of short sequence motifs which correspond largely, but not exclusively, with the repeat domains (Kadavath et al., 2018; Kadavath et al., 2015a). Interestingly, the specific order of the various binding sequences has little overall effect on binding affinity as long as all sequences are present (Trinczek et al., 1995). The number of repeat domains contributes to binding affinity, with 4R tau binding more strongly to tubulin than 3R tau (Butner and Kirschner, 1991; Goedert

and Jakes, 1990; Gustke et al., 1994). The increased binding affinity of 4R tau is apparently not simply due to the additional microtubule binding sequence, however. Rather, the sequence ^{216}KVQIINK^{223}, which falls between the first and second repeat sequences and is present only in 4R tau, exhibits a higher binding affinity than any of the repeat sequences and contributes significantly to tubulin binding in full-length tau (Goode and Feinstein, 1994; Panda et al., 1995).

While the repeat domains are the primary microtubule binding site, the regions flanking the repeats improve binding, described as a "jaws" model of binding (Gustke et al., 1994; Mandelkow et al., 1996; Mukrasch et al., 2007; Preuss et al., 1997). In this jaws model, the binding of the repeat domains is strengthened significantly by the flanking regions, resulting in much stronger binding than observed from the repeat domains alone. Specifically, the sequences ^{225}KVAVVRT^{231} and ^{243}LQTA^{246} in the proline rich domain and ^{370}KIETHKTFREN^{380} in the microtubule binding domain contribute to the jaws model of binding (Mukrasch et al., 2007). Synthetic tau peptides composed of 4 repeat domains but lacking the flanking regions bind tubulin weakly, indicating these additional interactions are crucial to normal tau binding (Gustke et al., 1994). Additional sequences contribute to binding by promoting optimal conformation of tau to increase binding affinity. To promote binding, conformational changes mediated by the sequence ^{215}KKVAVVR^{221} in the proline rich domain and by the PGGG motifs at the end of repeats 1 and 2 ensure the binding sequences align optimally (Goode et al., 1997; Kadavath et al., 2015b).

Although tau's primary physiological function is stabilizing and promoting assembly of microtubules, it is involved in many additional processes. Tau's projection domain,

which has a major direct role in microtubule binding, plays a key role in microtubule bundling, forming cross bridges with the projection domains of tau bound to nearby microtubules (Hirokawa et al., 1988; Kanai et al., 1992). Tau with a longer projection domain, as determined by the number of amino-terminal inserts, is more efficient at promoting formation of microtubule bundles (Kanai et al., 1992). Tau's projection domain has not been studied as thoroughly as the microtubule binding domain, so it may have additional as yet unidentified functions.

Tau may contribute axonal transport through modulation of the motor protein kinesin. Microtubule-bound tau promotes the detachment of kinesin proteins *in vitro* (Dixit et al., 2008; Vershinin et al., 2007). Taken in the context of tau's concentration gradient the effect of tau on kinesin acts to promote transport toward the synapse. Kinesin more easily binds microtubules in the soma, where tau concentration is low, and is more likely to disassociate as it approaches the synapse as tau concentration rises along the axon (Dixit et al., 2008). However, tau's role in axonal transport is not straightforward as axon transport has been found to be unaffected in retinal nerve axons of mice which lack or overexpress tau (Yuan et al., 2008). Although increased expression alone does not affect axonal transport, pathological tau has been shown to impair axonal transport (Kanaan et al., 2011). Tau's role in axonal transport *in vivo* therefore seems to depend on several factors beyond normal physiology.

Although tau is primarily located in the axon, it has some key somatic and dendritic functions as well. Small amounts of tau are normally found in the nucleus, where it protects DNA and RNA against oxidative and hyperthermic damage and may participate in nucleolar organization (Loomis et al., 1990; Sjoberg et al., 2006; Sultan et al., 2011; Violet

et al., 2014). Abnormal accumulations of nuclear tau have been identified in brains of patients with Huntington's disease (HD), and mouse models of HD show improvement of motor abnormalities when tau is deleted, suggesting a role for nuclear tau in HD (Fernandez-Nogales et al., 2014). Dendritic tau has an important role in synaptic function and contributes a role in dysfunction associated with Alzheimer's disease (Frandemiche et al., 2014; Ittner et al., 2010; Mondragon-Rodriguez et al., 2012; Tai et al., 2014). Tau's functions beyond its role as a microtubule stabilizer have been increasingly recognized over the past 10 years, and it seems likely that future studies will continue to implicate tau in additional cellular processes.

1.2 Tauopathies

Since neurofibrillary tangles were first identified as a defining feature of Alzheimer's disease, tau has implicated in a growing list of neurodegenerative diseases collectively called tauopathies (Alzheimer et al., 1995). Although development and presentation differ across tauopathies, these conditions generally share deposition of pathologic tau and development of dementia. Tauopathies are generally divided into two classes depending on the nature of tau's role in disease pathogenesis. In primary tauopathies, which include Pick's disease (PiD), progressive supranacular palsy (PSP), corticobasal degeneration (CBD), argyrophillic grain disease (AGT), primary age-related tauopathy (PART), and globular glial tauopathy (GGT), pathologic tau is the primary factor driving pathogenesis (Kovacs, 2015). In secondary tauopathies, including Alzheimer's disease and Down's syndrome, tau pathology develops and contributes to pathogenesis but is not thought to be the primary disease-causing factor (Josephs, 2017). Furthermore, tau pathology has been identified in other disease processes that have not traditionally been considered tauopathies

(Fernandez-Nogales et al., 2014; Loomis et al., 1990; Puvenna et al., 2016; Tai et al., 2016), but the extent to which tau pathology may contribute to these processes or merely be a byproduct is not clear.

Research involving secondary tauopathies, especially Alzheimer's disease, has been instrumental in revealing many mechanisms of tau pathology. A discussion of tauopathy would be incomplete without considering tau's interactions with disease related proteins, particularly amyloid-β. However, the following section will focus on pathological tau itself, and additional pathological processes will only be considered as they pertain to development of tau pathology.

1.2.1 Tau pathophysiology

The transition from physiological to pathological tau is diverse but can involve a combination of mutations, post-translational modifications, and conformational changes which culminates in pathology. In the course of its pathological changes, tau's solubility changes, leading to the deposition of soluble tau into insoluble aggregations. Although the specific roles of soluble and insoluble tau in disease are not fully understood, current evidence suggests each form of tau has a distinct set of roles.

1.2.1.1 *MAPT* mutations

Numerous mutations in *MAPT* on chromosome 17 have been associated with development of familial frontotemporal dementia (Goedert, 2005). Diseases arising from mutations in *MAPT* were initial collectively termed "frontotemporal lobar degeneration with tau-immunopositive inclusions (FTLD-tau)" or "frontotemporal degeneration with parkinsonism linked to chromosome 17 (FTDP-17)." However, it is now recognized that

many of these mutation-related cases are pathologically indistinguishable from sporadic cases of primary tauopathies which lack a clear genetic cause, leading some to suggest FTLD-tau should be categorized as early-onset, familial varieties of PiD, PSP, CBD, or GGT rather than as a separate disease process (Forrest et al., 2019; Forrest et al., 2018; Josephs, 2018). To avoid confusion with previous studies, this work will use the term FTDP-17 to refer to cases of dementia associated with *MAPT* mutations, but it should be noted that this nomenclature may not accurately capture the relationship between these familial and sporadic cases of dementia. The term "frontotemporal dementia" will also be used when referring collectively to FTDP-17 and the sporadic tauopathies (PiD, PSP, CBD, etc.).

Although tauopathies caused by an identified *MAPT* mutation are less common than sporadic cases, studying the specific mutations has elucidated important mechanistic aspects of tau pathophysiology. *MAPT* mutations associated with FTDP-17 are typically found throughout the microtubule-binding domain and affect splicing or alter microtubule binding (Goedert, 2005). Mutations which promote aggregation of tau will be discussed in section 1.2.1.3. Approximately half of identified *MAPT* mutations exhibit their effect at the RNA level (Goedert, 2005).

In normal adult human brain, 3R and 4R isoforms of tau are expressed in roughly equal amounts (Hong et al., 1998). However, this ratio is frequently perturbed in cases of frontotemporal dementia (Chambers et al., 1999; Connell et al., 2005; Hong et al., 1998). The predominant isoform of tau in most tauopathies is 4R, although PiD is a notable exception characterized by 3R tau (Gotz et al., 2019). Several specific *MAPT* mutations affect tau splicing and may be at least partially responsible for the change in the ratio

between 3R and 4R tau. N296 plays an important role in splicing of exon 10, and mutations at this site can promote alternative splicing of exon 10 and increase the ratio of 4R to 3R tau (Grover et al., 2002; Iseki et al., 2001; Spillantini et al., 2000; Yoshida et al., 2002). Several other mutations (including G272V, N279K, L284L, S305N, R406W, and mutations in the 5' splice site of exon 10 and the following intron) similarly increase splicing of exon 10, presumably by influencing the folding of mRNA and altering interactions with regulatory elements during post-transcriptional processing (D'Souza et al., 1999; Hasegawa et al., 1999; Hutton et al., 1998; Miyamoto et al., 2001; Spillantini et al., 1998). Although *MAPT* mutations affecting tau splicing typically promote alternate splicing of exon 10, the ΔK280 abolishes expression of 4R tau, most likely by disrupting the binding site of a splicing enhancer (D'Souza et al., 1999).

In addition to altering splicing of *MAPT* mRNA, many mutations associated with FTDP-17 reduce tau's binding affinity for microtubules. Unsurprisingly, mutations located in the microtubule binding domain or flanking regions greatly inhibit microtubule binding (Hasegawa et al., 1998). The greatest reduction in binding has been observed with mutations affecting N280 and P301, both within exon 10 (Barghorn et al., 2000; D'Souza et al., 1999; Grover et al., 2002; Rizzu et al., 1999). The relative impact of these mutations on tau's microtubule binding affinity depends in part on which isoform of tau is examined. Several mutations (G272V, V337M, and R406W) more significantly inhibit microtubule binding in 3R tau than 4R tau (Hasegawa et al., 1998). Interestingly, at least two mutation associated with FTDP-17 (S305N and Q336R) are associated with an increased microtubule binding affinity, demonstrating that excessive tau binding can also be detrimental (Hasegawa et al., 1999).

1.2.1.2 Hyperphosphorylation

Changes in the normal post-translational modification of tau, especially development of hyperphosphorylation, are a key steps in the development of tau pathogenesis. Since tau bound to microtubules may not be readily accessible for modification, some have suggested mutations which reduce tau's affinity for microtubules promote aberrant modification of tau by increasing the pool of soluble tau (Rizzu et al., 1999). Phosphorylation at several key residues has also been shown to strongly inhibit tau's binding to microtubules. In particular, phosphorylation at S214 (Illenberger et al., 1998), T231 (Sengupta et al., 1998), S262 (Biernat et al., 1993; Drewes et al., 1995; Sengupta et al., 1998), and S396 (Kadavath et al., 2018) reduces tau's ability to bind microtubules. Hyperphosphorylation is also associated with abnormal redistribution of tau to the soma and dendrites (Hoover et al., 2010). Phosphorylation is strongly implicated in aggregation of tau into insoluble paired helical filaments (PHFs), which is discussed in more detail in the next section (1.2.1.2).

1.2.1.3 Aggregation and filamentous tau

Aggregation of tau into filaments and tangles is a key step in development of tauopathic diseases. Tau aggregation depends on the hexapeptide sequences ^{275}VQIINK^{280} (located in the second repeat domain) and ^{306}VQIVYK^{311} (located in the third repeat domain) which form β-sheets with adjacent tau molecules (von Bergen et al., 2000). The propensity to form β-sheets directly correlates with the rate of aggregation (Eckermann et al., 2007; Mocanu et al., 2008). Adjacent tau molecules form anti-parallel dimers which then assemble with other dimers to form larger sheets (Wille et al., 1992). Formation of β-

sheets *in vitro* can be promoted by addition of polyanions to offset charge repulsion between the positively charged microtubule binding domains, suggesting these compounds may contribute to tau aggregation *in vivo* (Giustiniani et al., 2014; Goedert et al., 1996; Hernandez et al., 2004; Kampers et al., 1996).

Phosphorylation at specific sites can promote aggregation, although the exact role and mechanism is debated. Abnormal phosphorylation of tau precedes aggregation into tangles (Braak et al., 1994), and dephosphorylation of tau by PP2A promotes release of tau from PHFs (Drewes et al., 1993; Wang et al., 1995). Aggregation requires tau to be disassociated from microtubules, so phosphorylation at certain epitopes commonly found in PHFs, including T231, S262, and S396, which reduce tau's affinity for microtubules may contribute to aggregation (Biernat et al., 1993; Drewes et al., 1995). However, the relationship between phosphorylation and aggregation is more complicated than these studies might suggest. Phosphorylation at some sites associated with decreased microtubule affinity have been shown to inhibit aggregation (Schneider et al., 1999), and PHF-like phosphorylation occurs naturally and reversibly during hibernation in some species (Arendt et al., 2003). These studies suggest the effect of tau phosphorylation on aggregation may be contextual. Furthermore, phosphorylation may influence aggregation through by inducing conformational changes. Phosphorylation at S202/T205 or S396/S404 (but not at both sites) loosens tau's paperclip conformation, which may promote tau aggregation by exposing the repeat domains (Jeganathan et al., 2008; von Bergen et al., 2000). Phosphorylation at S202/T205 does not impact microtubule binding affinity, indicating the relationship between phosphorylation and aggregation is not simply dependent on microtubule binding (Amniai et al., 2009). Further work is needed to better

understand how phosphorylation influences aggregation of tau, but the answer is likely to be highly context dependent.

Several *MAPT* mutations (including G272V, ΔK280, ΔN296, P301L, V337M, and R406W) which are associated with impaired microtubule affinity also promote tau aggregation (Barghorn et al., 2000; Grover et al., 2002; Iseki et al., 2001; Nacharaju et al., 1999). Decreased microtubule binding increases the amount of tau in solution (Nagiec et al., 2001), therefore increasing the opportunities for tau-tau binding to occur. However, mutations that promote aggregation likely contribute to pathology beyond simply increasing the amount of tau available for aggregation. Pro-aggregation mutations increase the rate and extent of tau aggregation, suggesting they change tau in a way that directly promotes aggregation beyond simply releasing it from microtubules (Gamblin et al., 2000; Nacharaju et al., 1999). Further supporting this idea, the P301L, G272V, and V337M mutations also increase heparin-filament formation (Goedert et al., 1999). Furthermore, the ΔK280 and P301L mutations both affect the hexapeptide sequences which are central to aggregation, increasing their propensity to form β-sheets (von Bergen et al., 2001). Finally, at least one *MAPT* mutation promotes aggregation without inhibiting microtubule binding. The mutation Q336R increases tau's binding affinity for microtubules as well as promoting aggregation (Pickering-Brown et al., 2004). This mutation would ostensibly cause more tau to be bound to microtubules at any given time, but also leads to increased tau-tau interactions between unbound tau molecules.

Although PHFs are a characteristic feature of tauopathies, some evidence suggests these filamentous aggregations are not lethal to neurons. Although neuron death and neurofibrillary tangle (NFT) formation often occur together, NFTs correlate poorly with

neuron death in some models of tauopathy (Andorfer et al., 2005; Spires-Jones et al., 2008). Neuron death exceeds NFT formation in Alzheimer's disease, and neurons can live for years with stable NFTs (Gomez-Isla et al., 1997; Morsch et al., 1999). Furthermore, functional deficits do not correlate well with presence of NFTs and repression of tau expression often ameliorates deficits despite the persistence of tangles (Crimins et al., 2012; Rocher et al., 2010; Santacruz et al., 2005; Sydow et al., 2011). Rather than NFTs, many now believe tau oligomers are the primary toxic species responsible for much of the observed pathology. Tau oligomers can form from hyperphosphorylated tau and exhibit toxicity at low concentrations (Tepper et al., 2014; Tian et al., 2013). Tau oligomers are also an early stage in the aggregation process which culminates in NFT formation, so that the processes shown to contribute to NFT formation also likely promote oligomerization (Lasagna-Reeves et al., 2012; Maeda et al., 2007).

1.2.1.4 Trans-synaptic spread of tau

Each distinct tauopathy typically exhibits a consistent, disease-specific pattern of development, usually primarily affecting the same, often interconnected brain regions. In cases of Alzheimer's disease, tau pathology first develops in the entorhinal cortex before spreading to the hippocampus and other limbic structures, and finally to the temporal lobe and cortex broadly (Braak and Braak, 1991). The sequential development of tau pathology in anatomically connected brain areas has led some to suggest tau spreads directly from neuron to neuron by a variety of mechanisms. Injection of pathologic tau into the hippocampus of mice induces tau pathology that can spread to distant brain areas (Clavaguera et al., 2009; Peeraer et al., 2015). Entorhinal cortex-specific expression of 4R P301L tau results in development of tau pathology along the circuitry of the hippocampus,

suggesting synaptic transmission of pathologic tau (de Calignon et al., 2012; Liu et al., 2012). Microfluidic experiments have further supported this idea, demonstrating that pathologic tau travels along axons and spreads to post-synaptic neurons (Dujardin et al., 2014). Tau release into the synapse is associated with neuronal activity, and tau may be released with pre-synaptic vesicles (Pooler et al., 2013; Yamada et al., 2014). The ability of tau to spread across synapses is consistent with the apparent spread of pathology observed in clinical cases and suggests tau pathology may initially develop in specific, focal brain areas.

1.2.2 Mouse models of tauopathy

Many different mouse models of tauopathy have been developed to study specific aspects of tau function and pathology. Broadly, these can be divided into mice that are deficient in or entirely lacking tau, and those that express various forms of pathological tau. Additionally, to better understand tau's role in secondary tauopathies, many mouse models have been developed which express pathological tau alongside other types of disease pathology. In the following sections, several important mouse models of tauopathy will be discussed in detail.

1.2.2.1 Mouse models expressing pathological tau

Mouse models of tauopathy have been an indispensable tool in the study of tauopathy. These model are usually characterized by expression/overexpression of human tau protein, often with disease-associated mutations. The first transgenic tau mouse model expressed non-mutant 2N4R human and developed somatodendritic mislocalization, hyperphosphorylation, and additional pathology consistent with early tauopathy (Gotz and Nitsch, 2001; Gotz et al., 1995; Probst et al., 2000). Following the discovery of *MAPT*

mutations, additional transgenic models were developed which expressed human tau with the G272V (Gotz et al., 2001c), P301L (Gotz et al., 2001a; Lewis et al., 2000), P301S (Allen et al., 2002), V337M (Tanemura et al., 2001), K369I (Ittner et al., 2008), and R406W mutations (Tatebayashi et al., 2002; Zhang et al., 2004). Mice expressing the P301L mutation in particular have since been used in many studies of AD and FTDP-17 (David et al., 2005; Eckert et al., 2008; Gotz et al., 2001b; Kohler et al., 2010; Pennanen et al., 2004; Pennanen et al., 2006). Many transgenic tau models, including all of those discussed here, express only a single isoform of (usually 4R) tau. One notable exception to this trend is the 8c mouse, which overexpresses all six isoforms of human tau without any mutations (Duff et al., 2000).

Another major breakthrough in the development of transgenic tau models came with the development of the first regulatable transgenic model. The rTG4510 mouse model expresses 0N4R P301L tau under the control of a tetracycline-off promoter (Ramsden et al., 2005; Santacruz et al., 2005). Treatment with doxycycline suppresses tau expression, resulting in a rapid decrease in transgene mRNA and a halt in progression of tau pathology (Santacruz et al., 2005). However, any pretangle or tangle pathology that formed prior to doxycycline treatment usually remains stable. In addition to allowing tau expression to be controlled, this model allows the effects of tangle pathology to be studied independently of soluble tau. Although subsequent research has found that placement of the transgene disrupts the Fgf14 gene and may introduce pathology that is not tau-related (Gamache et al., 2019), the model has still been useful in demonstrating tau expression can be regulated in transgenic models. Additional regulatable transgenic models of tauopathy have since been developed (Eckermann et al., 2007; Mocanu et al., 2008; Sydow et al., 2011).

1.2.2.2 Tau deficient mouse models

The first strain of tau$^{-/-}$ mice was developed by deleting exon 1 through the use of a targeting vector (Harada et al., 1994). The same approach was later used to generate 2 additional tau$^{-/-}$ strains via deletion of exon 1 (Dawson et al., 2001; Fujio et al., 2007). A fourth strain of tau$^{-/-}$ mice has also been developed by inserting cDNA encoding EGFP into the *MAPT* gene to disrupt exon 1 (Tucker et al., 2001). While the effect on tau expression is the same in this model as in others, it has an advantage of also labeling neurons throughout the developing nervous system. Subsequent studies have primarily used the models developed by Tucker et al. and Dawson et al. to study the effects of tau deletion.

Initial studies of tau$^{-/-}$ mice surprisingly found no overt phenotypic changes, although further testing has identified some deficits. Other microtubule associated proteins, particularly MAP1A, were found to be elevated in young tau$^{-/-}$ mice and thought to compensate for the loss of tau (Dawson et al., 2001; Fujio et al., 2007; Harada et al., 1994). Fear conditioning is impaired in tau$^{-/-}$ mice (Ikegami, 2000), though cognitive function is broadly unaffected as late as 1 year of age (Dawson et al., 2010; Ikegami, 2000; Roberson et al., 2007). Short term memory was impaired in a tau$^{-/-}$ model on a different background strain, indicating memory and learning may be affected differently across strains/species (Biundo et al., 2018). Tests of motor function have produced varied results, with deficits identified in some studies (Ikegami, 2000; Lei et al., 2012; Ma et al., 2014) but not others (Dawson et al., 2010; Li et al., 2014). These discrepancies in motor testing could indicate that loss of tau has subtle effects on motor function, and minor differences in testing conditions may contribute to the differences observed.

Although gross effects of tau deletion are relatively minor, the developmental and cellular changes are more significant. Axonal development and organization are impaired (Dawson et al., 2010; Dawson et al., 2001; Harada et al., 1994), and these effects are exacerbated if MAP1B is also absent (Takei et al., 2000). Tau deficiency also increases expression of BAF-57, which normally represses expression of neuron-specific genes in non-neuronal cells and may further contribute to developmental deficits (de Barreda et al., 2010). $Tau^{-/-}$ mice exhibit EEG changes including impaired sleep and hippocampal theta slowing, indicating tau plays a role in synchronization of signals between brain regions (Cantero et al., 2010; Cantero et al., 2011). Synaptic function and neuronal excitability is significantly affected by loss of tau expression, and this topic will be covered in detail in section 1.4.6.

1.2.2.3 The htau mouse model

Most transgenic tau models express human tau protein in addition to endogenous murine tau. These models are good for studying toxic functions gained by pathological tau, but could potentially miss effects from the loss of tau function that can also develop in advanced tauopathy. The htau mouse model addresses this issue. The htau mouse was developed by crossing the human tau expressing 8c mouse (Duff et al., 2000) with a $tau^{-/-}$ mouse (Tucker et al., 2001) to produce a mouse that expresses six isoforms of human tau *without* endogenous murine tau. The htau mice are hemizygous for the transgene encoding human tau, and homozygous transgene expression is assumed to be embryonic lethal (Andorfer et al., 2003). To account for this, only one mouse in each breeding pair expresses the transgene, although both lack murine tau expression. As a result, approximately half of the offspring produced lack both human and murine tau, and are essentially the same as the

tau$^{-/-}$ model developed by Tucker et al. While this breeding scheme reduces the number of htau mice per litter, it has the advantage of also producing litter-mate tau$^{-/-}$ mice and is especially useful for comparative studies of tau function.

These mice develop progressive tau pathology including hyperphosphorylation by 1.5 months of age, somatodendritic distribution by 3 months of age, and tangle pathology by 9 months of age (Andorfer et al., 2003; Dickstein et al., 2010). They undergo a dramatic increase in neuronal death after 12 months of age, and exhibit dramatic atrophy and enlargement of the ventricles by 18 months of age (Andorfer et al., 2005). Adult neurogenesis is also impaired compared to non-transgenic mice, especially when comparing young mice (Komuro et al., 2015). No deficits in neuronal function have been described to date in young mice, but synaptic dysfunction develops in CA1 by 1 year of age (Alldred et al., 2012; Polydoro et al., 2009). In the prefrontal cortex, basal dendrite length is shorter and dendrite spine morphology is altered in htau mice compared to non-transgenic mice (Dickstein et al., 2010). Cognitive, sensory, and behavioral testing is largely normal, although deficits in visuospatial processing and food burrowing have been identified (Geiszler et al., 2016; Phillips et al., 2011).

The htau mouse model has also been used in conjunction with several other models of tauopathy and AD. Increasing expression of 4R isoforms of tau, which occurs in several tauopathies, exacerbates tau phosphorylation/aggregation and impairs nesting behavior (Schoch et al., 2016). This group also showed that 4R tau expression could be reduced by administration of an antisense oligonucleotide (ASO) to induce exon skipping, but did not demonstrate whether this reduction abrogates the changes observed. Models have also been developed which introduce AD-related pathology to the htau mouse. Crossing htau mice

with mice expressing AD-related mutations in the amyloid precursor protein (APP) and presenilin 1 (PS1) produces a triple-transgenic mouse which develops age-dependent tau and Aβ pathology (Guo et al., 2013). These APP/PS1/htau mice exhibit synergistic interaction between Aβ and tau pathology, and may better recapitulate aspects of AD than models that express only 1 or 2 of these genetic modifications. Interestingly, another group crossed the htau mouse with a different strain of APP mice, and found no evidence of interaction between the tau and Aβ pathology (Yetman et al., 2016). While the difference in results could be attributable to the different APP mutations expressed in each model, it is also possible that the inclusion of the PS1 mutation in the APP/PS1/htau mouse is necessary for interaction to occur. Given the lack of murine tau which could confound efforts at studying human disease, the htau mouse is a powerful tool for generating models of disease.

1.3 The Dentate Gyrus of the Hippocampus

The hippocampus has long been recognized for its crucial role in regulation of memory. The hippocampus receives its major inputs from the entorhinal cortex via the perforant pathway and sends its major outputs via the fornix to the rest of the limbic system (Andersen, 1975). The hippocampus is composed of the dentate gyrus and Ammon's Horn, which itself is usually subdivided into three major regions (CA1, CA2, and CA3). In overly simple terms, information passes through the hippocampus along the "trisynaptic circuit", entering from the entorhinal cortex via the perforant pathway to the synapse at the dentate gyrus, then to passing to CA3 via the mossy fibers, and finally passing to CA1 via the Schaffer collateral fibers (Andersen, 1975). In reality, the passage of information through the hippocampus is much more complicated, with feedback loops and additional signaling

pathways between different regions of the hippocampus and extrahippocampal structures. However, the simplified trisynaptic circuit captures the overall unidirectional flow of information through the hippocampus. Due to the key roles of the dentate gyrus in both tauopathy and epilepsy (discussed further in sections 1.2 and 1.4), the current section will focus primarily on the dentate gyrus granule cells and the major pathways associated with their function.

1.3.1 Dentate gyrus organization and major cell types

The dentate gyrus is a V-shaped formation folded around the end of CA3 (Amaral et al., 2007). Grossly, the dentate gyrus is composed of two blades connected by a region called the crest. The portion of the dentate gyrus that projects between CA1and CA3 is the suprapyramidal blade, and the other portion is called the infrapyramidal blade. It is separated into three principal layers: the molecular layer, the granule cell layer, and the polymorphic layer. The molecular layer, which is furthest away from CA3, is mostly acellular, composed primarily of the perforant pathway fibers and dendrites of the granule cells. The granule cell layer is mostly composed of tightly packed granule cells, although some other cell types can be found at the boundary with the polymorphic layer, a region called the subgranular zone. The polymorphic layer/hilus (sometimes called CA4), which is the closest layer to CA3, is composed of the axons from the granule cells in addition to a variety of additional cell types including the mossy cell. Detailed drawings originally produced by Ramón y Cajal can be found in published works and are valuable resources for understanding hippocampal anatomy (Andersen, 1975; Ramón y Cajal, 1909).

Three principal dentate gyrus neuron types will be discussed here. Many other classes of neurons/interneurons have been identified, primarily in the hilus/subgranular

zone. These neurons will be not discussed in detail here, but they can influence hippocampal function in important ways and are an area of ongoing study.

1.3.1.1 Dentate granule cells

The dentate granule cell (DGC) is the principal neuron type in the dentate gyrus. Each DGC has 1-4 primary dendrites that project from the soma toward the molecular layer, branching to form extensive dendrite trees that extend throughout the molecular layer to form synapses with the perforant pathway fibers (Claiborne et al., 1990). DGCs in the suprapyramidal blade have longer dendrite trees than those in the infrapyramidal blade (Claiborne et al., 1990). DGCs are the smallest and most tightly packed neuron type in the hippocampus, with 8-15 rows of neurons in the granule cell layer (West et al., 1991). DGCs exhibit a low basal rate of action potential firing, typically less than 0.5Hz (Jung and McNaughton, 1993). DGCs are also hyperpolarized compared to most neurons, with a resting membrane potential that is typically below -70mV (Cronin et al., 1992; Staley et al., 1992). The low firing rate and hyperpolarized membrane potential of DGCs is likely due to high degree of inhibitory signaling and diversity of $GABA_A$ subunits expressed by DGCs (Brooks-Kayal et al., 1998). DGCs express a specific subtype of extrasynaptic α4βδ $GABA_A$ receptors which have a high affinity for GABA and do not desensitize to any significant degree (Mtchedlishvili and Kapur, 2006; Stell and Mody, 2002; Wei et al., 2003). These receptors respond to spillover GABA from the synapse and are thought to contribute to the tonic GABA inhibition tight control over firing patterns.

DGCs are primarily glutamatergic, though they form numerous synapses with inhibitory neurons so that DGC activity frequently drives inhibitory signaling (Acsády et al., 1998; Crawford and Connor, 1973). Furthermore, DGCs can synthesize and release

other compounds including γ-aminobutyric acid (GABA) under specific conditions, leading some to challenge the glutamatergic characterization (Gutierrez, 2003; Walker et al., 2002). The dentate gyrus is one of the few brain regions with persistent neurogenesis throughout adulthood, producing new DGCs and other cell types (Altman and Das, 1965; Cameron and McKay, 2001; Kaplan and Hinds, 1977).

1.3.1.2 Dentate basket cells

The hilus and subgranular zone contain a large number of primarily inhibitory interneurons that modify neuronal function (Houser, 2007). Inhibitory neurons are particularly abundant and are crucial to maintaining tight inhibitory regulation over dentate gyrus function (discussed further in sections 1.3.4 and 1.4.4). One important class of inhibitory interneurons are the dentate basket cells. Dentate basket cells are key GABAergic inhibitory neurons found primarily at the border of the granule cell layer and polymorphic layer (Amaral, 1978). Basket cells display varied morphology, with 5 different subtypes identified (Ribak and Seress, 1983; Seress and Pokorny, 1981). Although these subtypes vary in gross morphology, they share key characteristics including aspinous dendrites which extend through all layers of the dentate gyrus, axons which extend into the granule cell and molecular layers, and GABAergic signaling (Ribak and Seress, 1983; Ribak et al., 1986). Basket cells are far less abundant than DGCs, at a ratio of around 200 DGCs per basket cell in rats (Seress and Pokorny, 1981). Basket cells are also asymmetrically distributed throughout the hippocampus, with higher concentrations in the caudal hippocampus and in the infrapyramidal blade (Seress and Pokorny, 1981).

1.3.1.3 Mossy cells

Mossy cells are the most common neuron type in the hilus (Amaral, 1978). Each mossy cell has three or more dendrites that project throughout the hilus, giving off a small number of side branches along the way. Most of the dendrites remain in the hilus, but a few extend as far as the molecular layer (Amaral, 1978). The proximal dendrites are covered in distinctive large spines called "thorny excrescences" which serve as termination sites for mossy fibers. Mossy cells are primarily glutamatergic, and form synapses with many neuron types, including DGCs, throughout all 3 layers of the dentate gyrus (Scharfman, 1995; Wenzel et al., 1997). Mossy cells are more abundant than basket cells, but are still about 50 fold less abundant than DGCs in rats (Amaral et al., 1990).

1.3.2 Major inputs to the dentate gyrus

The dentate gyrus receives its primary inputs from the ipsilateral entorhinal cortex via the perforant pathway. The fibers of the perforant pathway originate primarily from stellate cells in layer II of the entorhinal cortex (Hevner and Kinney, 1996; Steward and Scoville, 1976; van Groen et al., 2003; Witter et al., 1989). Projections from the entorhinal cortex to the dentate gyrus are topographically organized. Inputs to the dorsal dentate gyrus arise primarily from the lateral entorhinal cortex, whereas inputs to the ventral dentate gyrus arise primarily from the medial entorhinal cortex (van Groen et al., 2003). The fibers of the perforant pathway terminate primarily in the outer two thirds of the molecular layer of the dentate gyrus, where they form at least 85% of the synapses in this region (Matthews et al., 1976). The synapses formed by perforant pathway fibers are essentially all excitatory in nature (Leranth et al., 1990; Matthews et al., 1976; White et al., 1977).

While the major inputs to the dentate gyrus originate from the entorhinal cortex, other brain regions also project to the dentate gyrus. The dentate gyrus receives a combination of cholinergic and GABAergic inputs originating in the septal nuclei. The cholinergic inputs, which appear to form mostly excitatory synapses, primarily terminate on DGCs and mossy cells (Amaral and Kurz, 1985; Lubke et al., 1997). GABAergic neurons in the septal nucleus also project to the dentate gyrus, forming synapses primarily with inhibitory neurons (Amaral and Kurz, 1985; Bilkey and Goddard, 1985; Kohler et al., 1984). The overall effect of septal projections to the dentate gyrus is increased flow of information due to a net increase in excitatory signaling.

The dentate gyrus receives hypothalamic inputs originating primarily in the supramammillary nucleus. Fibers originating primarily from the supramammillary nucleus terminate in the molecular and granule cell layers, synapsing on the somata and proximal dendrites of DGCs (Dent et al., 1983; Magloczky et al., 1994; Vertes, 1992). These inputs originate in the bilateral supramammillary nuclei, although the ipsilateral nucleus contributes the majority of the fibers, and they terminate in the suprapyramidal blade about twice as often as the infrapyramidal blade (Kiss et al., 2000; Vertes, 1992; Wyss et al., 1979). Although the earliest work suggested these hypothalamo-hippocampal connections were inhibitory in nature (Segal, 1979), subsequent work has shown the synapses on DGCs are primarily excitatory and likely glutamatergic (Dent et al., 1983; Kiss et al., 2000; Magloczky et al., 1994). Activation of the supramammillary nucleus results in an increase in DGC activity and can influence the theta rhythm (Carre and Harley, 1991; Mizumori et al., 1989).

The final inputs to the dentate gyrus considered here originate from a few locations in the brainstem. The dentate gyrus receives noradrenergic input from the locus coeruleus and serotonergic input from the raphe nuclei. Noradrenergic fibers from the locus coeruleus form synapses on the somata and dendrites of DGCs and at interneurons in the hilus (Loughlin et al., 1986; Milner and Bacon, 1989; Pickel et al., 1974). The locus coeruleus fibers primarily form excitatory synapses with DGCs, whereas they form roughly equal number of excitatory and inhibitory synapses with interneurons (Milner and Bacon, 1989). Noradrenergic innervation enhances long-term potentiation (LTP) in the dentate gyrus, allowing the locus coeruleus to influence dentate gyrus function over time (Dahl and Sarvey, 1989; Izumi and Zorumski, 1999; Neuman and Harley, 1983; Walling and Harley, 2004).

Serotonergic inputs to the dentate gyrus originate primarily in the caudal portion of the dorsal raphe nucleus and peripheral part of the median raphe nucleus (Köhler and Steinbusch, 1982; McKenna and Vertes, 2001; Vertes et al., 1999). These fibers terminate throughout the molecular layer and hilus, especially targeting the subgranular zone, and account for nearly 75% of the serotonergic input to the hippocampus (Moore and Halaris, 1975). Increased serotonergic input suppresses the theta rhythm and inhibits LTP (Vertes et al., 1994). This disruption in activity may serve to prevent encoding of irrelevant information into memory (Vertes and Kocsis, 1997).

1.3.3 Major outputs from the dentate gyrus

The dentate gyrus projects primarily to CA3 via the mossy fibers, which are composed of the unmyelinated axons of the DGCs. Mossy fibers give rise to several collateral branches in the hilus before extending through CA3, terminating on pyramidal

cells (Blackstad et al., 1970; Claiborne et al., 1986). Mossy fibers form three different types of presynaptic terminals. The synapses onto CA3 pyramidal neurons are made through large mossy terminals, but filopodial extensions can also form from the axon end bouton that synapse onto interneurons through smaller terminals (Acsády et al., 1998; Claiborne et al., 1986). Mossy fibers can also form en passant varicosities that synapse with interneurons through small terminals (Acsády et al., 1998; Amaral, 1979; Frotscher et al., 1991). The smaller filopodial and en passant terminals, which typically form synapses with inhibitory neurons, are far more numerous than the large terminals, so that the majority of mossy fiber synapses form on inhibitory neurons (Acsády et al., 1998).

Mossy fibers synapse directly onto CA3 pyramidal neurons in addition to nearby inhibitory interneurons which project to the same pyramidal neurons or back to DGCs (Gulyas et al., 1993). This increases the complexity of the circuit between the DGC and CA3 pyramidal neurons since the same fibers drive excitatory and inhibitory input. Single stimulations from the mossy fiber activate AMPA receptors at glutamatergic synapses while simultaneously triggering GABA release at the GABAergic synapse via activation of the inhibitory interneuron (Larson and Munkacsy, 2015). The resulting excitatory post-synaptic current (EPSC) and inhibitory post-synaptic current (IPSC) partially offset each other, curtailing the resulting depolarization in the CA1 pyramidal cell. However, GABA released at the GABAergic synapse activates presynaptic $GABA_B$ autoinhibitory receptors on the interneuron presynaptic terminal, inhibiting further release of GABA. Following repeated stimulation from the mossy fiber (which is typical during normal entorhinal cortex-driven DGC firing patterns), EPSCs strengthen while IPSCs diminish. With sufficient stimulation, long-term potentiation can occur, further strengthening the activity

at the glutamatergic synapse. This complexity plays an important role in shaping the flow of information from the dentate gyrus to CA3, improving precision of signals and contributing to memory formation (Henze et al., 2002; Jung and McNaughton, 1993; Torborg et al., 2010).

1.3.4 Local circuits in the dentate gyrus

Dentate granule cells exhibit a low overall level of activity due largely to the high degree of inhibitory signals they receive and their low resting potential. The high inhibitory tone results in the dentate gyrus forming a "gate" of information passing through the hippocampus and has major implications in the development epilepsy (see section 1.4.4). Ironically, this inhibitory tone relies heavily on glutamatergic signals from mossy cells and perforant pathway fibers, in conjunction with inhibitory neurons.

As previously discussed, mossy fibers have collateral branches that form frequent synapses with mossy cells and inhibitory interneurons in the hilus. These mossy cells in turn project back to the inner molecular layer, forming synapses with the interneurons and proximal dendrites of the DGCs (Buckmaster et al., 1992; Buckmaster et al., 1996; Frotscher et al., 1991; Scharfman, 1995; Wenzel et al., 1997). Mossy cells form monosynaptic connections directly to DGCs which are excitatory, but under normal conditions activation of mossy cells has an inhibitory effect on DGCs (Scharfman, 1995). The inhibitory effect is disynaptic and disappears in the presence of the $GABA_A$ antagonist bicuculline methiodide, indicating the involvement of an inhibitory interneuron. The result of this circuit is that increased DGC activity feeds back to inhibit DGCs. Interestingly, the inhibitory effect is relatively weak in DGCs at the same septotemporal level as the mossy cell, but becomes stronger in DGCs more distant along the septotemporal axis from the

mossy cell (Amaral and Witter, 1989). Therefore, DGC activity in one lamella of the dentate gyrus can influence DGC activity in nearby lamellae, allowing for local modulation of information as it passes through the dentate gyrus.

Mossy cell activity has a critical role in the function of inhibitory basket cells in the subgranular zone. Animal models of epilepsy will be discussed in more detail in section 1.4.5, but the effects of seizures on circuitry in the dentate gyrus reveal an important relationship between mossy cells, basket cells, and the tonic inhibitory input to DGCs. Mossy cells are highly sensitive to status epilepticus, whereas basket cells are relatively robust and more likely to survive (Sloviter, 1991). Despite the loss of glutamatergic neurons, mossy cell death is associated with increased DGC activity. Loss of mossy cells removes much of the excitatory drive to basket cells, causing them to fall dormant and removing inhibitory signals to DGCs (Sloviter et al., 2003).

1.4 Temporal Lobe Epilepsy

Epilepsy is one of the oldest known disorders and has been recognized in some form since ancient times (Wilson and Reynolds, 1990). Epilepsy affects around 1% of the global population, although incidence and prevalence vary significantly in different parts of the world (Asadi-Pooya et al., 2017; Hirtz et al., 2007; Thurman et al., 2011). Although epilepsy has historically been more common in children, in recent decades prevalence and incidence have been highest in adults older than 60 years (Sander, 2003; Sen et al., 2020). Epilepsy is not truly a single disorder, but rather refers to a spectrum of disorders characterized by recurrent seizures (Jensen, 2011). The minimum diagnostic criteria for epilepsy is the occurrence of 2 or more unprovoked seizures at least 24 hours apart (Fisher

et al., 2014). “Unprovoked” in this context is used to exclude seizures of known, discrete etiology that do not suggest a likelihood of recurrence, such as those associated with concussion, fever, or alcohol-withdrawal. An important exception are the seizures associated with reflex epilepsies that can be reliably provoked by specific sensory stimuli because these reflect a prolonged tendency toward seizures (Harding, 2004). This definition of epilepsy does not address the underlying etiology, however, so more information is needed to further classify any specific case of epilepsy.

1.4.1 Classification and description of seizures

Given epilepsy’s long history, it’s not surprising that the terminology used to describe seizures and epilepsy has changed over the years. Consistent classifications are crucial for proper care, leading the International League Against Epilepsy (ILAE) to release and revise its operational classifications of seizure types (Fisher et al., 2017). The ILAE defines a seizure as “a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain” (Fisher et al., 2005). The most recent classification system recognizes 3 categories of seizures defined by onset. Focal seizures, formerly called partial seizures, originate within networks of one hemisphere, potentially originating from subcortical structures. Generalized seizures originate from and rapidly engage networks bilaterally (Fisher et al., 2017). Seizures can further be classified as unknown-onset, but this should be viewed as a place holder classification until more information becomes available. Further classification based on seizure characteristics is optional but can provide a better description of the seizure.

Focal seizures can be further described based on awareness, defined as knowledge of self and environment. During focal seizures with intact awareness, formerly called

simple partial seizures, consciousness is maintained for the full duration of the seizure. If consciousness is lost for any duration during the seizure, it is classified as a focal seizure with impaired awareness, formerly called a complex partial seizure (Fisher et al., 2017). In addition to describing awareness, focal seizures are often described in terms of associated motor or non-motor symptoms. Motor descriptors include automatism (repetitive, coordinated movement usually associated with impaired awareness), atonic, clonic, epileptic spasm (sudden flexion, extension, or mixed extension-flexion of proximal and truncal muscles, lasting around 1 second; formerly called an infantile spasm), hyperkinetic, myoclonic, and tonic (Fisher et al., 2017). Non-motor descriptors include autonomic, behavioral arrest (lasting the entire seizure), cognitive (involving déjà vu, jamais vu or hallucinations, formerly called psychic), emotional, and sensory. Finally, the classification should describe whether the focal seizure evolves into a bilateral, tonic-clonic seizure.

Classification of generalized seizures follows similar conventions as focal seizures. Awareness is normally impaired during generalized seizures and therefore does not need to be specified. Generalized motor seizures can be described with some of the same descriptors as focal seizures (clonic, tonic, myoclonic, atonic, epileptic spasms). Motor involvement associated with generalized seizures can also develop through distinct patterns of motor involvement, including tonic-clonic (formerly called grand mal), myoclonic-tonic-clonic, and myoclonic-atonic (formerly called myoclonic-astatic). Generalized non-motor seizures (also called generalized absence, formerly called petit mal) usually consist of sudden interruption of ongoing activities accompanied by unresponsiveness lasting up to a half minute with rapid recover (Fisher et al., 2017). Generalized absence seizures are further classified as typical or atypical based on EEG findings. Generalized absence

seizures can also involve rhythmic movements of the upper limbs (myoclonic absence seizure) or eyelid myoclonus.

1.4.2 Temporal lobe epilepsy

Epilepsy is generally classified as focal or general depending on where the seizures originate. This classification is important in considering clinical management (discussed in section 1.4.3). Approximately 60% of epilepsy cases are focal in nature (Pascual, 2007; Wiebe, 2000). Cases of epilepsy are further classified into cases of primary epilepsy with idiopathic or cryptogenic etiology and cases of secondary epilepsy with known etiology (ILAE, 1989). Idiopathic epilepsy is often assumed to have a genetic etiology, whereas secondary epilepsy is associated with a CNS injury or disorder. Cryptogenic epilepsy is typically presumed to also be attributable to some injury, but does not have an identifiable etiology.

The primary focus of this work will be focal epilepsy affecting the temporal lobe. Temporal lobe epilepsy (TLE) is the most common type of focal epilepsy (Manford et al., 1992; Wiebe, 2000). Since TLE is highly focal in nature and has a high tendency to be resistant to medical management, cases are often referred for surgery (Engel et al., 2012; Quarato et al., 2005). As a result, the etiology of drug-resistant TLE is frequently identified. Hippocampal sclerosis is the most common histopathological finding, in addition to neoplasm, focal malformation, and necrosis (Helmstaedter et al., 2014; Salanova et al., 2004; Zentner et al., 1995). Many of the patients in these studies became seizure free or at least had significant seizure reduction (>75%), suggesting these pathologies are closely associated with the epileptic focus (Salanova et al., 2004).

1.4.3 General clinical management of epilepsy

The primary goal of epilepsy treatment is reducing/preventing occurrence of seizures. Epilepsy is considered "resolved" if an individual remains seizure free for at least 10 years and off medication for at least five years or has past the applicable age for age-dependent epilepsy syndromes (Fisher et al., 2014). The first line therapy for epilepsy is typically medical management with antiseizure medications (ASMs). Many studies use the term "antiepileptic drug (AED)" rather than ASM to refer to medications used to manage epilepsy. While these drugs are often very effective at controlling seizures, they should be viewed as symptom management rather disease modification. Calling them antiepileptic drugs is therefore inaccurate, leading some to call for a change in terminology (French and Perucca, 2020). Despite significant advances in ASMs, about one third of cases remain refractory to pharmacological management and require further intervention, often surgical resection of the epileptic focus (Cockerell et al., 1995; Engel, 1993; Kwan and Brodie, 2000). Management of epilepsy, especially drug resistant cases, remains a significant global socioeconomic burden (Ali, 2018; Begley et al., 2000).

1.4.3.1 Medical management of epilepsy

Pharmacotherapy is effective for many patients with epilepsy, and about half of patients achieve seizure control with the first ASM (Kwan and Brodie, 2000). In general, monotherapy is preferred to polytherapy. Polytherapy increases the risks of adverse effects while usually providing only minor improvement to seizure control (Carpay et al., 2005; Liu et al., 2017a; St Louis et al., 2009). It is generally recommended, therefore, to exhaust options for monotherapy with first line agents before initiating polytherapy. Early seizure

control after diagnosis is associated with a better long-term prognosis (Elwes et al., 1984; Ettore Beghi et al., 1992; Kwan and Brodie, 2000).

ASMs generally suppress seizures by inhibiting neuronal ion channels or increasing GABAergic signaling (White et al., 2007). Most ASMs routinely used for management of seizures block voltage-gated sodium (phenytoin, carbamazepine, topiramate, lamotrigine, valproic acid) or calcium (ethosuximide, valproic acid, gabapentin) channels, though many exhibit more than one mechanism of action. One notable exception which is commonly used as a first line therapy is levetiracetam, which suppresses seizures by modulating synaptic transmission via inhibition of SV2A (Lambeng et al., 2005). Benzodiazepines (diazepam, lorazepam, midazolam), which are agonists of the $GABA_A$ receptor, are recommended as first line therapy for cases of status epilepticus (Sirven and Waterhouse, 2003).

Most ASMs in use today have not undergone placebo-controlled studies due to the ethical concerns of withholding treatment. However, non-inferiority trials comparing several common ASMs have largely found comparable efficacy rates for different ASMs (Brodie et al., 2007; Costa et al., 2011; de Silva et al., 1996; Vazquez, 2004). Lamotrigine and valproic acid are strongly recommended as the first line therapy for newly diagnosed focal epilepsy and generalized epilepsy, respectively (Marson et al., 2021; Marson et al., 2007a, b). Lamotrigine and levetiracetam are acceptable alternatives to valproic acid for treating generalized seizures in women who are or may become pregnant (Nevitt et al., 2017). Ethosuximide and valproic acid demonstrate the best efficacy in treatment of generalized absence epilepsy in children, but ethosuximide is recommended due to its reduced rate of adverse effects (Brigo et al., 2021; Glauser et al., 2010).

Often, the selection of monotherapy ultimately depends on tolerability. The most common adverse effects associated with ASM therapy are fatigue/tiredness and memory impairment, although life threatening adverse events are possible with most ASMs (Brodie, 2017; Carpay et al., 2005). While all ASMs are associated with teratogenicity, the risk is highest with valproic acid and lowest with levetiracetam (Tomson et al., 2015). The goal in medical management of epilepsy should be to quickly find a monotherapy which provides adequate seizure control with tolerable side effects. While newer ASMs are often associated with more favorable adverse event profiles, the trials discussed comparing efficacy of older and newer ASMs have largely found them to be equally effective (Schmidt, 2011). This demonstrates a lack of major improvement in ASM efficacy and underscores the need for improved therapeutics, requiring better understanding on the mechanisms of epilepsy.

1.4.3.2 Surgical intervention in epilepsy

Although most cases of epilepsy respond to medical management with ASMs, about one third of cases, mostly TLE, remain refractory (Engel et al., 2012). Cases of TLE often respond well to surgical resection of the epileptic focus (Cersosimo et al., 2011; Engel et al., 2012; Engel et al., 2003; Salanova et al., 2004). Most patients who undergo resection experience significant reduction in seizures, and around half of patients remain seizure free 10 years after surgery (Cohen-Gadol et al., 2006; de Tisi et al., 2011; Mohan et al., 2018; Tellez-Zenteno et al., 2005). Early freedom from seizures predicts better long-term outcomes, with at least 75% of patients who are seizure free for the first two years remaining seizure free after 10 years (Cohen-Gadol et al., 2006; McIntosh et al., 2004). Presence of histopathology in the resected tissue, which may indicate a discrete epileptic

focus, is associated with higher probability of remaining seizure free (Zentner et al., 1995). In contrast, a history of tonic-clonic seizures, especially in the year preceding surgery, predicts a higher chance of seizure recurrence after resection (Asadi-Pooya et al., 2016).

1.4.3.3 Other treatment options

In addition to medical management and surgical resection, several additional therapeutic options are available. Given the epilepsy's long history, it is no surprise that numerous herbal remedies have been used. While some of these compounds have been shown to have anticonvulsant properties in animal studies, they have the potential to interact with common ASMs and have not been shown to be effective in clinical trials (Tyagi and Delanty, 2003). Limited clinical data is available for some treatments such as melatonin and acupuncture, but suggests these are not effective for treatment of seizures or epilepsy (Brigo and Del Felice, 2012; Cheuk and Wong, 2006). Despite these examples of ineffective treatments, some alternatives to mainstream ASM therapy and surgical resection have been found effective for managing epilepsy, particularly cases that remain refractory to other treatments. In particular, ketogenic diets and vagal nerve stimulation can provide effective seizure control despite failure of other options, and each will be briefly discussed here.

Several different types of ketogenic diets have been developed, but all consist of a diet that is primarily fat-based with little to no carbohydrates, which causes the body to begin producing ketone bodies from stored fat in a process called ketosis. The efficacy of the ketogenic diet for treating epilepsy was recognized in the 1920s (Barborka, 1928; Peterman, 1924) and recent trials have similarly found a ketogenic diet effective for treatment of pharmacoresistant epilepsy (Klein et al., 2010; Mady et al., 2003; Neal et al.,

2008). To promote adherence and reduce adverse effects, a modified Atkins diet with fewer restrictions was developed which has also been shown to be effective at treating epilepsy (Kossoff et al., 2006). The exact anti-epileptogenic mechanism of a ketogenic diet has not been identified, but several possibilities have been suggested. Ketone bodies seem to have a direct anticonvulsant effect (Keith, 1933; Rho et al., 2002). Some evidence suggests ketones promote an increase in production of GABA from glutamate (Dahlin et al., 2005; Yudkoff et al., 2005), while others have suggested direct modulation of ion channels to reduce neuronal excitability (Ma et al., 2007; Won et al., 2013). Additionally, many studies have suggested the ketogenic diet works through a variety of metabolic and bioenergetics mechanism. In particular, animals on a ketogenic diet exhibit resistance to oxidative stress, a process which is commonly upregulated in epilepsy (Kim et al., 2010; Maalouf et al., 2007; Milder et al., 2010). Finally, ketogenic diets result in significant metabolic changes including reduction in blood glucose, production of polyunsaturated fatty acids, and upregulation of the tricarboxylic acid cycle, which may contribute to the antiseizure potential of ketogenic diets (Fraser et al., 2003; Mantis et al., 2004; Willis et al., 2010). None of these individual mechanisms seems to fully account for the efficacy of ketogenic diets in treating refractory epilepsy, so it is likely that several of these mechanisms contribute to the overall antiseizure effect.

Vagal nerve stimulation (VNS) is a relatively new treatment that can be an effective adjunctive therapy for reducing seizure burden. The potential of VNS as an antiseizure therapy was recognized by the early 1990s (Clarke et al., 1991; Penry and Dean, 1990), and successful clinical trials resulted in FDA approval for treatment of refractory epilepsy in 1997 (Handforth et al., 1998; Morris and Mueller, 1999). Efficacy of VNS improves

over time (DeGiorgio et al., 2000; Morris et al., 2013), and patients undergoing VNS may be able to reduce their ASM dose while maintaining seizure control (Tatum et al., 2001). Several mechanisms are thought to contribute to the antiseizure effect on VNS. Stimulation of the vagus nerve induces changes in many brain areas, including the solitary nucleus, dorsal motor nucleus of the vagus, locus coeruleus, thalamus, hypothalamus, amygdala, and occipital, cingulate, and temporal cortex (Ko et al., 1996; Krahl et al., 1998; Naritoku et al., 1995), indicating the effects of VNS are not limited to the vagal system. Stimulation of vagal afferent fibers can modulate EEG activity in cortical and subcortical brain regions (Chase et al., 1967; Chase et al., 1966), which may disrupt seizures by preventing or disrupting synchronized activity. Further studies have found an increase in GABAergic inhibitory signaling after VNS which may suppress seizure development (Di Lazzaro et al., 2004; Marrosu et al., 2003). VNS is generally well-tolerated, although respiratory symptoms and vocal changes develop commonly after stimulation (Handforth et al., 1998). Given the significant reduction in seizure occurrence and improvement in quality of life reported by patients in the same study, these adverse events do not offset the value of VNS in treating refractory epilepsy. 1/2

1.4.4 Potential mechanisms of epileptogenesis

As the first step in the trisynaptic circuit of the hippocampus, the dentate gyrus plays an important role controlling the flow of information into the hippocampus. Since DGCs are normally not highly active, they effectively filter signals entering the hippocampus and are thought to protect hippocampal neurons downstream, forming a "dentate gate." The dentate gate normally prevents epileptiform bursts from the entorhinal cortex from propagating to CA3, but this gating breaks down in epileptic animals (Ang et

al., 2006; Behr et al., 1998). DGC gating is GABA-dependent and is mediated primarily by extrasynaptic $GABA_A$ receptors (Iijima et al., 1996; Maguire et al., 2005). Optogenetic restoration of DGC gating stops spontaneous seizures in epileptic mice, further supporting the role of the dentate gate in epilepsy (Krook-Magnuson et al., 2015).

Given the importance of GABA signals in the dentate gate, it is reasonable to expect a loss of $GABA_A$ receptors to accompany disrupted gating epileptic animals. However, numerous studies have found a paradoxical upregulation of $GABA_A$ receptors in TLE models (Nusser et al., 1998; Otis et al., 1994). Instead, a modulation of $GABA_A$ receptor composition occurs in DGCs from epileptic animals. The specific effects of these modulations have not been fully elucidated, though several important functional changes have been identified. DGCs from epileptic animals exhibit a downregulation in the δ-subunit of GABA receptors, which reduces the tonic $GABA_A$ current and may contribute to loss of gate function (Boychuk et al., 2016; Peng et al., 2004). Modulated $GABA_A$ receptors are also sensitive to inhibition by zinc, which is released in large amounts from sprouted mossy fibers (Assaf and Chung, 1984; Buhl et al., 1996; Cohen et al., 2003; Gibbs et al., 1997). While exogenously applied zinc effectively inhibits these $GABA_A$ receptors, it seems that zinc endogenously released at excitatory synapses is unable to reach and inhibit $GABA_A$ receptors, so an alternate explanation is needed to support zinc-induced inhibition of $GABA_A$ receptors (Molnar and Nadler, 2001). Finally, $GABA_A$ receptors in DGCs from epileptic animals show reduced sensitivity to benzodiazepine activation, further suggesting functional changes (Leroy et al., 2004). While the exact mechanism is still not clear, changes in $GABA_A$ receptors on DGC are likely a key aspect of epileptogenesis.

Other changes in excitatory and inhibitory signaling in the dentate gyrus have been identified which may further contribute to the loss of gating ability. Following epileptogenesis, animal models frequently develop abnormal sprouting of mossy fibers. Sprouted fibers aberrantly extend to the inner molecular layer and form synapses with nearby DGCs. These synapses create recurrent excitatory circuits which promote synchronization of aberrant activity within dentate gyrus (Bhaskaran and Smith, 2010; Cronin et al., 1992; Hunt et al., 2010; Lynch and Sutula, 2000; Patrylo and Dudek, 1998; Winokur et al., 2004; Wuarin and Dudek, 2001). In addition, several types of inhibitory neurons in the dentate gyrus are susceptible to death or dysfunction following epileptogenesis (Buckmaster and Dudek, 1997; Butler et al., 2017; Hunt et al., 2011; Lowenstein et al., 1992; Sloviter, 1991; Sloviter et al., 2003). Since many hilar inhibitory neurons receive excitatory inputs from mossy fibers, their activity is actually increased after mossy fiber sprouting (Halabisky et al., 2010; Hunt et al., 2011). While this likely represents a compensatory mechanism to prevent excessive DGC activity, chronic over activation of hilar interneurons may also negatively impact their survival and contribute to further loss of inhibitory signals to DGCs in epileptic brains. Overall, the changes in excitatory and inhibitory signaling observed in animal models of TLE, taken along with the changes in $GABA_A$ receptor function, create an environment that favors the development and propagation of seizures in epileptic brains.

1.4.5 Animal models to study seizures and epilepsy

Because epileptogenesis is a complex process affecting several interconnected neuron types, studying epilepsy requires complex models. Several animal models of seizure induction and/or acquired epilepsy have been developed for this purpose. Models

of seizure induction commonly used to study epileptogenesis can broadly be divided into chemically induced epilepsy, post-traumatic epilepsy (PTE), and kindling models. Electrical kindling is the classic model of induced seizures, involving repeated electrical stimulation of specific brain areas to induce seizures. While kindling has been replaced as the primary seizure model by newer, easier to implement models, it is still used in epilepsy research (Gorter et al., 2016). Epilepsy commonly develops as a result of traumatic brain injury, and several models mostly using rodents are commonly implemented to recapitulate aspects of PTE for study (D'Ambrosio et al., 2004; Hunt et al., 2009; Kharatishvili et al., 2006; McIntosh et al., 1989). The focus of the rest of this section will be models involving the use of chemoconvulsants to induce seizures. Models involving focal or systemic administration of chemoconvulsant agents can be used to study the process of seizure induction, or to induce status epilepticus in order to study epileptogenesis. Three chemoconvulsant agents (kainic acid, pilocarpine, and pentylenetetrazole) are particularly common in models of seizures/epilepsy, and each will be discussed in some detail here.

Kainic acid (KA), an analog of L-glutamate, was one of the earliest chemoconvulsant agents used to study the effects of seizures in rodents (Ben-Ari et al., 1979; Ben-Ari et al., 1980; Nadler et al., 1978). KA activates non-NMDA ionotropic receptors (AMPA and kainate) at a much higher affinity than glutamate (Sharma et al., 2007). Administration of KA and subsequent SE recapitulates the mossy fiber sprouting that is a key histopathological characteristic of TLE in human patients (Cronin and Dudek, 1988; Cronin et al., 1992; Sutula et al., 1989; Tauck and Nadler, 1985). When appropriate doses are used, systemic administration of KA reliably induces status epilepticus in rats and some strains of mouse without excessive mortality (Dudek and Staley, 2017), although

C57BL/6 mice in particular have been shown to exhibit resistance to this model (McKhann et al., 2003; McLin and Steward, 2006; Schauwecker and Steward, 1997). Focal administration of KA directly to the hippocampus (intrahippocampal kainate, IHK) or amygdala (intra-amygdala kainate, IAK) increases efficacy and avoids strain-resistance, with rates of SE approaching 100% in C57BL/6 mice (Welzel et al., 2020). IHK in C57BL6 mice reliably results in epileptogenesis and is associated with a short latent period to spontaneous seizures with EEG abnormalities apparent within days of treatment (Raedt et al., 2009; Welzel et al., 2020).

Pilocarpine is a muscarinic acetylcholine agonist that was another early chemoconvulsant agent used in rodents (Turski et al., 1984; Turski et al., 1983). Excessive muscarinic activation triggers seizures by creating an imbalance between excitatory and inhibitory signals (Priel and Albuquerque, 2002). Pilocarpine is frequently administered systemically via intraperitoneal injection. To prevent excessive systemic muscarinic activation, animals are typically pretreated with methylscopolamine, an anticholinergic agent that does not readily cross the blood-brain barrier. In rats, pretreatment with lithium reduces drastically reduces the dose of pilocarpine needed to induce seizures while also reducing mortality (Clifford et al., 1987; Honchar et al., 1983). Lithium has not frequently been used in pilocarpine-treated mice, but the limited data available suggest lithium does not potentiate pilocarpine-induced seizures in mice (Muller et al., 2009). Pilocarpine-induced status epilepticus induces mossy fiber sprouting in addition to widespread neuronal lesions (Turski et al., 1984; Turski et al., 1983). In contrast to systemic KA, C57BL/6 mice do not exhibit resistance to seizures induced by systemic pilocarpine (Shibley and Smith, 2002). Like kainic acid, pilocarpine can also be focally administered to the hippocampus,

resulting in reduced mortality while reliably inducing status epilepticus and epileptogenesis (Furtado Mde et al., 2002).

Pentylenetetrazole (PTZ) is a $GABA_A$ antagonist that has long been used to induce seizures in animal models (Squires et al., 1984). Unlike KA and pilocarpine, a single dose of PTZ typically triggers a single seizure. Repeated doses of PTZ gradually reduce the threshold required to induce seizures, making PTZ a good candidate for chemical kindling (Angelatou et al., 1991; Corda et al., 1991). While this makes PTZ more labor intensive in studies of epileptogenesis, PTZ is an ideal model for assessing seizure threshold and latency, and it is frequently used for this purpose (Garcia-Cabrero et al., 2013; Li et al., 2014; Roberson et al., 2011; Roberson et al., 2007; Van Erum et al., 2020).

1.4.6 Seizures and epilepsy associated with tauopathy

A growing body of evidence from human and animal studies suggests that tau may have a role in the development of seizures and epilepsy. Patients with AD have an increased likelihood to have unprovoked seizures, and many are diagnosed with epilepsy (Amatniek et al., 2006; Friedman et al., 2012; Hesdorffer et al., 1996; Horvath et al., 2018). These seizures are mostly non-motor focal seizures with intact awareness, making them easy to overlook without EEG (Horvath et al., 2018). Onset of seizures can occur at any point in the course of AD, but is most common early and frequently precedes AD diagnosis (Vossel et al., 2013). Earlier onset and severity of are correlated with a higher risk of seizures (Amatniek et al., 2006). Increased risk of new onset seizures is associated with AD and non-AD dementia, but may be more significant in cases of AD-dementia (Hesdorffer et al., 1996; Sherzai et al., 2014). Patients with AD but no confirmed history of seizures or

epilepsy also exhibit epileptiform abnormalities on EEG at a higher rate compared to healthy controls (Lam et al., 2020; Vossel et al., 2016).

Animal studies have supported and built upon findings from human studies. Mouse models expressing tau pathology commonly exhibit aberrant excitatory neuronal activity, particularly in the frontal cortex and hippocampus (Crimins et al., 2012; Crimins et al., 2011; Palop et al., 2007; Rocher et al., 2010; Yoshiyama et al., 2007). In tauopathic models, these changes are not correlated with development of tangle pathology, suggesting soluble rather than aggregated tau is responsible (Rocher et al., 2010; Yoshiyama et al., 2007). To further support this idea, hippocampal synaptic defects in a regulatable tauopathy model recover over time when pathological tau expression is suppressed despite persistence of tangles formed prior to tau suppression (Sydow et al., 2011). These observations are consistent with other studies showing that soluble tau oligomers, rather than insoluble tangles, are likely the primary toxic species (Berger et al., 2007; Flach et al., 2012; Lasagna-Reeves et al., 2012; Ondrejcak et al., 2018; Puangmalai et al., 2020; Spires et al., 2006). Given these observations, it is likely that soluble oligomers are also responsible for the synaptic dysfunction observed in tauopathic animal models.

Consistent with observations from human studies, animal models of tauopathy exhibit increased susceptibility to seizures. Mouse models of frontotemporal dementia exhibit lower thresholds to induced seizures in several seizure induction models (Garcia-Cabrero et al., 2013; Liu et al., 2017b; Van Erum et al., 2020). Increasing the expression of 4R tau, which increases the formation of oligomeric tau in htau mice, increases susceptibility to PTZ induced seizures (Schoch et al., 2016). In contrast, tau deficient mice have improved outcomes in some genetic epilepsy models (Gheyara et al., 2014; Holth et

al., 2013) and are resistant to induced seizures (DeVos et al., 2013; Liu et al., 2017b; Roberson et al., 2011; Roberson et al., 2007). Many animal models expressing other AD pathology also develop increased susceptibility to seizure induction (Chan et al., 2015; Miszczuk et al., 2016; Reyes-Marin and Nunez, 2017). Amyloid-β is associated with neuronal hyperexcitability, possibly due to impairment of inhibitory interneurons (Busche et al., 2008; Perez et al., 2016; Verret et al., 2012). Although neurofibrillary tangles are not generally found in amyloid-β animal models lacking tau modification, amyloid-β can induce tau hyperphosphorylation and soluble tau pathology including oligomers has been identified in some of these same models (Castillo-Carranza et al., 2015; Cohen et al., 2013a; Echeverria et al., 2004; Gotz et al., 2001b; Radde et al., 2006; Shipton et al., 2011; Sturchler-Pierrat et al., 1997; Takashima et al., 1996). Taken together with the observation that tau deficient animals are protected against amyloid-β induced seizure susceptibility and synaptic dysfunction (Roberson et al., 2011; Roberson et al., 2007; Shipton et al., 2011), it is likely that amyloid-β induced tau pathology is responsible for the pro-epileptic changes in these animal models.

Epilepsy appears to accelerate the development of tauopathy. Patients with AD and epilepsy experience more rapid cognitive decline compared to patients with AD but not epilepsy (Volicer et al., 1995; Vossel et al., 2013). Tau hyperphosphorylation is also correlated with cognitive decline in patients with TLE (Tai et al., 2016). One possible explanation for this observation in patients with diagnosed/treated epilepsy is that ASM therapy, rather than tau dysfunction, accelerates cognitive decline. Although studies of the effect of ASM therapy on cognitive decline are limited, the existing data do not strongly support this idea. One study reported accelerated cognitive decline in patients with AD

(Fleisher et al., 2011), but this effect has not been observed in other studies (Herrmann et al., 2007; Porsteinsson et al., 2001; Profenno et al., 2005; Tariot et al., 2011). Still, valproic acid is not recommended as first line therapy in patients with AD due to the high occurrence of other adverse events. In contrast, treatment with levetiracetam has been associated with reduced rate of cognitive decline (Cumbo and Ligori, 2010). Furthermore, occurrence of epileptiform activity in patients with AD but no history of epilepsy is likewise associated with more rapid cognitive decline (Vossel et al., 2016), suggesting ASM therapy is not responsible.

An alternate explanation for accelerated cognitive decline in patients with AD and epilepsy is that seizures promote tau pathology. Seizures have been associated with tau hyperphosphorylation in several human and animal models. Temporal lobe tissue resected as treatment for intractable epilepsy frequently exhibits tauopathy-like tau hyperphosphorylation decades earlier than in typical cases of tauopathy (Jones et al., 2018; Puvenna et al., 2016; Smith et al., 2019; Tai et al., 2016). Supporting this finding, hyperphosphorylated tau and increased activity of tau kinases are common findings after induced seizures in animal models (Alves et al., 2019; Bracey et al., 2009; Crespo-Biel et al., 2007; Jiang et al., 2005; Liang et al., 2009). The promotion of tau phosphorylation and aggregation associated with seizures may explain the accelerated cognitive decline observed in patients with AD and epilepsy.

1.5 Study Aims and Significance

This study focuses on tau's contribution to neuronal excitability in normal and disease conditions. One particular strength of the current study compared to previous work is the

use of the htau mouse. This model was chosen specifically to avoid the potential confounding effects of endogenous murine tau expressed in most other models of tauopathy. Endogenous murine tau does not develop pathology in the same manner as human tau, and may continue to function normally even after the human tau has become pathological and ceased its physiological function. Since other studies have shown deletion of tau ameliorates the hyperexcitability associated with other types of pathology, it is important to understand the contribution of tau pathology to excitability without endogenous tau.

The specific aims of this project are as follows:

1. **Determine the effect of tau modulation on neuronal excitability in the normally aging mice.** Expression of pathological mutant tau has been associated with hyperexcitability in mouse models of tauopathy. Furthermore, tau deletion appears to abrogate hyperexcitability associated with several models of AD. However, two key questions have not been answered. Does pathological tau promote hyperexcitability in the absence of normally functioning tau? How does loss of tau expression affect neuronal excitability in the absence of additional pathological processes? To address these questions, whole-cell patch-clamp electrophysiology was used to assess neuronal excitability in DGCs from tau$^{-/-}$, htau, and non-transgenic C57BL/6J mice at 1.5, 4, and 9 months of age.
2. **Determine the effect of tau modulation on induced seizures and epileptogenesis following intrahippocampal injection of kainic acid.** Tau deficient mice have been shown to be resistant to PTZ-induced seizures and exhibit improved survival in several genetic models of epilepsy. Conversely, tauopathic model exhibit

reduced seizure latency, increased seizure severity, and poorer survival in several seizure induction models. However, it is not known whether these differences in seizure development are associated with changes in epileptogenesis. Furthermore, the propensity of pathological tau to promote seizures has not been assessed in the absence of endogenous murine tau. To address these questions, $tau^{-/-}$, htau, and non-transgenic C57BL/6J mice underwent intrahippocampal injection of kainate. Initial seizure induction and development of spontaneous seizures were observed, including a small cohort monitored by video-electroencephalography. Neuronal excitability was assessed by whole-cell patch-clamp electrophysiology 1.5-2 months after IHK.

CHAPTER 2. MATERIALS AND METHODS

2.1 Animals

Transgenic B6.Cg-*Mapt*$^{tm1(EGFP)Klt}$Tg(MAPT)8cPdav/J mice (male and female; #005491) were produced in house from breeders obtained from The Jackson Laboratory (JAX; Bar Harbor, ME). This mouse line was generated previously by introducing a transgene encoding six isoforms of human tau without disease-associated mutations onto homozygous tau$^{-/-}$ mice (Andorfer et al., 2003). These mice lack any obvious disease phenotype at birth, but develop impairments in Morris Water Maze, spatial learning, and gross motor function (i.e. food burrowing) with age, particularly in mice older than 9 months (Geiszler et al., 2016; Phillips et al., 2011; Polydoro et al., 2009). This mouse strain was originally generated on a hybrid Swiss Webster/B6D2F1 hybrid background but has been backcrossed to C57BL/6J for more than 10 generations. Genetic analysis of SNPs performed by The Jackson Laboratory (a technique they frequently use to demonstrate cogenicity) was consistent with a pure C57BL/6J background, which served as the control strain. All breeding mice were homozygous for a deletion of the murine tau gene. One mouse in each breeding pair was hemizygous for a transgene expressing all six isoforms of non-mutant human tau protein. The offspring are therefore either full tau knockout (tau$^{-/-}$) or express only human tau (htau).

DNA was extracted from tail snips and genotype was confirmed via PCR according to the protocols supplied by Jackson labs. Disruption of the endogenous murine tau gene was confirmed using the primer pair 5'-CGTTGTGGCTGTTGTAGTTG-3' and 5'-TCGTGACCACCCTGACCTAC-3', which amplifies a fragment at 270 bp in both tau$^{-/-}$ and htau mice. Presence of the human-tau transgene was confirmed using the primer pair

5’-CGAAGTGATGGAAGATCACG-3’ and 5’-GTCTTGGTGCATGGTGTAGC-3’, which amplifies a fragment at 79 bp in htau mice. Protein expression was confirmed via western blot of hippocampal homogenate using the H150 antibody (1:2000; Santa Cruz Biotechnology). Age matched male C57BL/6J control mice were obtained from Jackson labs (Jax#000664) and allowed to acclimate after delivery for at least one week prior to any experiments. All mice were housed under a 14 hour light / 10 hour dark cycle in an Association for Assessment and Accreditation of Laboratory Animal Care Internal (AALAC) facility. Food and water were available *ad libitum*. The University of Kentucky Institutional Animal Care and Use Committee approved all procedures.

2.2 Intrahippocampal kainate (IHK) mouse model of epilepsy

All surgical procedures were performed under isoflurane general anesthesia with 0.05% bupivacaine local anesthesia. Kainic acid (100 nL, 20 mM in 0.9% saline, Tocris Bioscience) or saline (100 nL) was injected into the left dorsal hippocampus (2.0 mm posterior, 1.25 mm left, and 1.6 mm ventral to bregma) between 6 and 8 weeks of age (Krook-Magnuson et al., 2013). The injection rate was 20 nL/minute, and the needle was left in place for 5 minutes before and after injection. Buprenorphine (0.05 mg/kg) and carprofen (10 mg/kg) were administered subcutaneously after surgery. Mice were transferred to a heated cage for recovery and monitored for Racine scale seizures for 2 hours to assess development of status epilepticus (SE). SE was defined as the occurrence of at least 3 seizures of Racine scale 3 or higher during the observation period (Shibley and Smith, 2002). After 2 hours, diazepam (7.5 mg/kg) was administered intraperitoneally to terminate status epilepticus (SE). Video monitoring for spontaneous seizures began 2 weeks after the IHK surgery and continued for 6 weeks. Each animal underwent 7-8

recording sessions (average duration 7.8 hours), averaging 56 hours of monitoring per animal. The videos were reviewed by a reviewer blind to genotype and treatment.

A separate cohort of mice (n=2-4 per group) were fitted with wireless transmitters to allow video-electroencephalographic (vEEG) recording (Data Sciences International; DSI; St. Paul, MN). Kainic acid or saline was injected as described above. Screws were placed (1.0mm anterior and 1.0mm right to bregma and 3.0mm posterior and 3.0mm left to bregma) after injection. A wireless transmitter (ETA-F10, DSI) was implanted intraperitoneally and connected to the screws by subcutaneous wires. vEEG began immediately after surgery and continued for 2 hours until administration of diazepam as described above. EEG recordings were collected using Ponema (v6.42, DSI). Mice underwent a total of 5 sessions of 24 hour vEEG recording between 1 and 3 weeks after IHK. EEG recordings were manually reviewed for seizure-like activity, defined as rhythmic high amplitude (>3 fold larger than baseline), high frequency (>10Hz) activity lasting at least 20 seconds, using NeuroScore (v3.3.1, DSI). Potential seizures identified on EEG were confirmed by corresponding Racine behavior on video recorded in Open Broadcaster Software (v21.0.1) using a Logitech C270 HD camera. Seizure prevalence and average daily seizure frequency recorded via vEEG was calculated for each group.

2.3 Hippocampal slice preparation

Mice were deeply anesthetized via inhalation of isoflurane to effect (lack of tail pinch response) and decapitated while anesthetized. The brain was rapidly removed from the skull and immersed in ice-cold oxygenated (95% O_2/5% CO_2) cutting/holding artificial cerebrospinal fluid (aCSF). The cutting/holding aCSF contained (in mM): 85 NaCl, 75 sucrose, 2.5 KCl, 25 glucose, 1.25 $NaH_2PO_4 \cdot H_2O$, 4 $MgCl_2 \cdot 6H_2O$, 0.5 $CaCl_2 \cdot 2H_2O$, and

24 $NaHCO_3$ (pH 7.2-7.4). Coronal sections (300 μm) were cut on a vibrating microtome (Vibratome Series 1000; Technical Products International, St. Louis, MO). Each slice was divided with a midsagittal cut and hippocampi were isolated and transferred to a holding chamber with warmed (30-32° C), oxygenated cutting/holding aCSF and incubated for >1 hour before recordings. In a subset of animals, 5-8 hippocampal slices were set aside for protein analysis. Extrahippocampal tissue was removed from these slices. Approximately 30mg of hippocampal tissue was collected and flash frozen in liquid nitrogen. One slice at a time was transferred to a chamber mounted under an upright microscope (BX51WI; Olympus) and was superperfused with warmed (30-32° C) oxygenated recording aCSF. The recording aCSF contained (in mM): 124 NaCl, 3 KCl, 2 $CaCl_2$, 1.3 MgCl, 1.4 $NaHCO_3$, and 11 glucose (pH 7.2-7.4). Each slice was equilibrated in the microscope chamber for >10 min to allow the cutting aCSF to wash off before recording.

2.4 Electrophysiological recordings

Whole-cell patch-clamp recordings were obtained from hippocampal DGCs, identified by location and morphological characteristics. Recording pipettes were pulled from borosilicate glass (open tip resistance 3-5 MΩ; King Precision Glass Co.). The pipette recording solution contained (in mM): 126 K^+-gluconate, 4 KCl, 10 HEPES, 4 MgATP, 0.3 NaGTP, and 10 PO-creatine (pH 7.2). Electrophysiological recordings were performed using a Multiclamp 700B amplifier (Molecular Devices), low pass filtered at 2 kHz, digitized at 20 kHz (Digidata 1440A; Molecular Devices), and recorded onto a computer using pClamp 10.2 software (Molecular Devices). Seal resistance was typically 2-5 GΩ. Series resistance was <25 MΩ (mean: 10.8±0.1 MΩ) and was monitored periodically

during the recordings. Recordings were discontinued if series resistance changed by more than 20% during the recording.

Resting membrane potential and input resistance were measured in current clamp mode. Current steps (-100 pA to 400 pA in 50 pA steps) were injected to record membrane voltage response. The input resistance was calculated from the slope of the linear portion of the resulting current-voltage curve. The resting membrane potential was averaged from the portions of recorded traces between current steps. Spontaneous and electrically-evoked excitatory post-synaptic currents (i.e., sEPSCs and eEPSCs) were recorded in voltage-clamp mode at a holding potential of -70 mV. To elicit evoked EPSCs, a platinum-iridium concentric-bipolar electrode (125 μM diameter; FHC, Bowdoin, ME) was positioned on the lateral perforant pathway (Figure 2.1) and 30 pairs of current pulses (30-50 μA; 400 μs; interpulse interval 75 ms; 5 seconds between pulse pairs) were administered to evoke paired eEPSCs (i.e., paired pulse response; PPr). The stimulus intensity was adjusted so that responses occurred after >80% of pulses. Stimulus sweeps that failed to elicit a response with both stimuli were excluded from analysis.

2.5 Tissue Homogenization and Western Blot

The hippocampal tissue from each mouse was homogenized as described previously (Koren et al., 2019). Tissue samples were mechanically homogenized in RIPA lysis buffer (VWR) with phosphatase inhibitor cocktails 2 and 3 (Sigma), cOmplete protease inhibitor (Sigma), and PMSF (1 mM final concentration, Roche Diagnostics). Homogenates were centrifuged at 4°C at 13,000 rpm for 30 minutes. The fresh supernatant

from each sample was divided into aliquots and stored at -80°C. Protein concentration was quantified using the Pierce BCA kit (Thermo Fisher, 23225).

Sample protein concentrations were normalized with lysis buffer and denatured by boiling for 5 minutes in 4x Laemmli buffer (BioRad) plus 10% β-mercaptoethanol (Sigma). The samples were separated on a 10% tris-glycine gel (BioRad) and transferred to a polyvinylidene fluoride membrane (Millipore). Membranes were blocked in 6% non-fat dry milk (w/v) in 0.01M tris buffered saline (TBS). All antibodies were diluted in 6% non-fat milk in TBS. Primary antibodies used are as follows: AT8 (pS202/pT205 tau; 1:2000; ThermoFisher), H-150 (human tau; 1:2000; Santa Cruz), GAPDH (1:5000; Cell Signaling Technology). Goat anti-mouse and goat anti-rabbit secondary antibodies conjugated to horseradish peroxidase (Southern Biotech) used were diluted in 6% non-fat dry milk in TBS (w/v). Images were collected on an Amersham Imager 600 (General Electric).

2.6 Data Analysis

All electrophysiological measures (evoked action potential frequency, resting membrane potential, input resistance, spontaneous EPSC frequency, and paired pulse responses) were analyzed with MiniAnalysis (Synaptosoft, Fort Lee, NJ). Statistical measures were performed with Prism (GraphPad, San Diego, CA). Data were disaggregated by sex and no sex-dependent differences were detected for any measure, so sexes were combined for all analyses. All data were tested for normality with a Shapiro Wilk test and, except where noted, all data were found to be normally distributed. Specific statistical tests used for each comparison will be described in sections 3.2.5 and 4.2.5 as appropriate. Data are presented as mean ± SEM and statistical significance was set to $p<0.05$ for all tests.

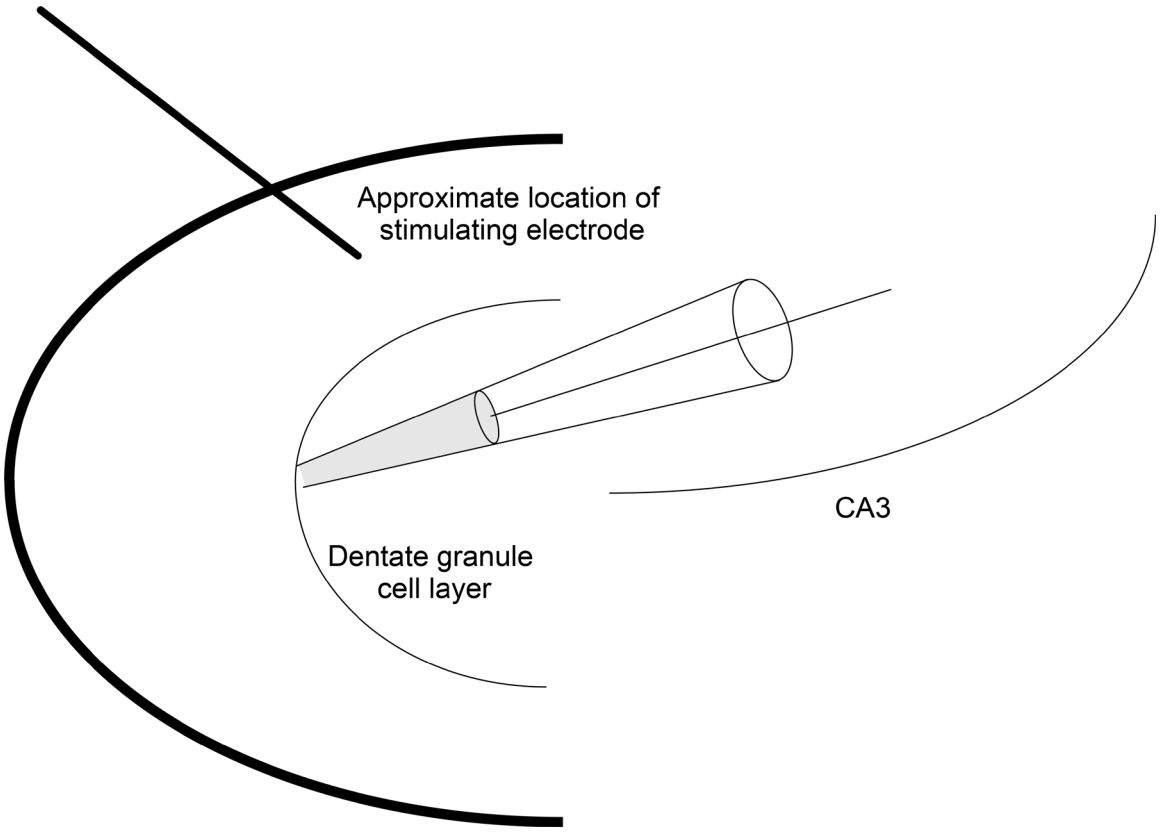

Figure 2.1 Approximate location of stimulating and recording electrodes
The stimulating electrode was positioned preferentially over the lateral perforant pathway to reduce stimulation of the medial perforant pathway. The stimulating electrode was repositioned as needed to ensure recorded cells were stimulated.

CHAPTER 3. EFFECTS OF ALTERED TAU EXPRESSION ON DENTATE GRANULE CELL EXCITABILITY IN MICE

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3.1 Introduction

Microtubule dynamics are critical for central nervous system (CNS) function. Disruptions in microtubule homeostasis are associated with a variety of CNS dysfunctions including Alzheimer's disease and other tauopathies, epilepsy, Parkinson's disease, congenital brain malformations, psychiatric disorders, and autism spectrum disorder (Chang et al., 2018; Gardiner and Marc, 2010; Goncalves et al., 2018; Marchisella et al., 2016; Pellegrini et al., 2017; Saha and Sen, 2019). The microtubule-associated protein tau (MAPT, tau) plays an important role in the assembly and stabilization of microtubules. To allow normal microtubule dynamics, phosphorylated tau has reduced affinity for microtubules, promoting their dismantling (Lindwall and Cole, 1984). The equilibrium of tau phosphorylation, which is tightly regulated under physiological conditions (Martin et al., 2013a; Martin et al., 2013b), becomes disrupted in Alzheimer's disease and other tauopathies, to allow accumulation and aggregation of hyperphosphorylated tau (pTau) (Castellani and Perry, 2019). Tau solubility decreases as hyperphosphorylation increases, eventually forming insoluble neurofibrillary tangles, which are a hallmark of Alzheimer's disease and other tauopathies (Castellani and Perry, 2019).

Hyperphosphorylated tau promotes increased neuronal excitability. In mouse models expressing mutant human tau protein, principal neurons in the frontal cortex and hippocampus exhibit depolarized neuronal resting membrane potentials (Crimins et al.,

2012; Crimins et al., 2011; Rocher et al., 2010), increased evoked action potential firing and spontaneous excitatory postsynaptic currents (sEPSCs) (Crimins et al., 2012; Crimins et al., 2011; Rocher et al., 2010), increased glutamate release and decreased glutamate reuptake (Decker et al., 2016; Hunsberger et al., 2015), and abnormal neuron morphology and synaptic organization (Crimins et al., 2012; Crimins et al., 2011; Rocher et al., 2010; Yoshiyama et al., 2007). Consistent with this increased neuronal excitability, mice expressing mutant human tau protein exhibit increased vulnerability to induced epilepsy (Garcia-Cabrero et al., 2013; Liu et al., 2017b), a finding consistent with studies demonstrating increased seizure prevalence in patients with Alzheimer's disease (Pandis and Scarmeas, 2012; Vossel et al., 2013; Vossel et al., 2016).

In addition to establishing a correlation between tau hyperphosphorylation and neuronal hyperactivity in animals, previous work has studied the effect of removing or reducing tau expression on seizures. Disruption of *Mapt* expression reduces seizure burden and improves survival in some genetic epilepsy models (Gheyara et al., 2014; Holth et al., 2013), confers resistance to chemically induced seizures (DeVos et al., 2013; Li et al., 2014), and prevents glutamate excitotoxicity in cultured neurons (Miyamoto et al., 2017). Furthermore, reducing tau phosphorylation by administration of the protein phosphatase 2A (PP2A) activator, sodium selenate, reduces seizure burden and promotes survival in multiple seizure models (Jones et al., 2012; Liu et al., 2016). Taken together, these studies suggest involvement of tau hyperphosphorylation in epilepsy due to its effects on neuronal excitability.

While previous studies have outlined an important role for tau in promoting neuronal hyperexcitability, the full impact of tau's effect on neuronal function remains

unknown. Age related functional changes in tau knockout mice may impact neuronal activity and animal survival at later ages. Furthermore, the effect of pTau on excitability has been studied in mouse models that express mutant human tau, without suppressing endogenous mouse tau. The presence of two different tau species could thus confound results, since the murine tau may remain functional despite the presence of pathologic human tau. It is unclear whether the presence of wild-type murine tau in most tauopathy models modulates the pathological functions conferred by hyperphosphorylation. This study will address these gaps in current knowledge by measuring neuronal excitability in $tau^{-/-}$ mice and in the htau mouse model, which expresses six isoforms of non-mutant human tau with a concurrent deletion of endogenous murine tau (Andorfer et al., 2003). The htau model recapitulates features of tau pathology and cognitive deficits present in Alzheimer's disease, which include appearance of hyperphosphorylated tau species as early as 1.5mo, deposition of late-stage tangle pathology at 9mo, and presentation of substantial cognitive deficits by 12mo. Given these considerations and previous data demonstrating profound hippocampal deficits in tauopathy models (Abisambra et al., 2010; Abisambra et al., 2013; Fontaine et al., 2017), we tested the hypotheses that pTau promotes hyperexcitability of dentate gyrus granule cells (DGCs) in the absence of functional endogenous tau, and that complete tau ablation reduces neuronal excitability throughout the life of the animal. We chose to study DGCs because they contribute to epileptogenesis and tauopathy-associated cognitive decline (Alcantara-Gonzalez et al., 2021; Boychuk et al., 2016; Hunt et al., 2010; Lee et al., 2012; Martin-Belmonte et al., 2020), and epilepsy-related changes in the dentate gyrus were ameliorated by tau deletion (Gheyara et al 2014).

Despite their importance in cognition and disease processes, the electrophysiological effects of modifying tau expression have not been studied extensively in DGCs.

3.2 Materials and Methods

3.2.1 Animals

Transgenic B6.Cg-*Mapt*$^{tm1(EGFP)Klt}$Tg(MAPT)8cPdav/J mice (male and female) were produced in house from breeders obtained from The Jackson Laboratory (Jax# 005491; Bar Harbor, ME). This mouse line was generated previously by introducing a transgene encoding six isoforms of human tau without disease-assocaited mutations onto homozygous tau$^{-/-}$ mice (Andorfer et al., 2003). These mice lack any obvious disease phenotype at birth, but develop impairments in Morris Water Maze, spatial learning, and food burrowing with age (Geiszler et al., 2016; Phillips et al., 2011; Polydoro et al., 2009). This mouse strain was originally generated on a hybrid Swiss Webster/B6D2F1 hybrid background but has been backcrossed to C57BL/6J for more than 10 generations. SNP analysis performed by The Jackson Laboratory was consistent with a pure C57BL/6J background, which served as the control strain. All breeding mice were homozygous for a disruption of the murine tau gene. One mouse in each breeding pair was hemizygous for a transgene expressing all six isoforms of non-mutant human tau protein. The offspring are therefore either full tau knockout (tau$^{-/-}$) or express only human tau (htau).

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and htau mice. Presence of the human-tau transgene was confirmed using the primer pair 5'-CGAAGTGATGGAAGATCACG-3' and 5'-GTCTTGGTGCATGGTGTAGC-3', which amplifies a fragment at 79 bp in htau mice. Protein expression was confirmed via western blot of hippocampal homogenate using the H150 antibody (Figure 3.1; 1:2000; Santa Cruz Biotechnology). Age matched male C57BL/6J control mice were obtained from Jackson labs (Jax#000664) and allowed to acclimate after delivery for at least one week prior to any experiments. All mice were housed under a 14 hour light / 10 hour dark cycle in an Association for Assessment and Accreditation of Laboratory Animal Care Internal (AALAC) facility. Food and water were available *ad libitum*. The University of Kentucky Institutional Animal Care and Use Committee approved all procedures.

3.2.2 Hippocampal slice preparation

Mice were deeply anesthetized via inhalation of isoflurane to effect (lack of tail pinch response) and decapitated while anesthetized. The brain was rapidly removed from the skull and immersed in ice-cold oxygenated (95% O_2/5% CO_2) cutting/holding artificial cerebrospinal fluid (aCSF). The cutting/holding aCSF contained (in mM): 85 NaCl, 75 sucrose, 2.5 KCl, 25 glucose, 1.25 $NaH_2PO_4 \cdot H_2O$, 4 $MgCl_2 \cdot 6H_2O$, 0.5 $CaCl_2 \cdot 2H_2O$, and 24 $NaHCO_3$ (pH 7.2-7.4). Coronal sections (300 μm) were cut on a vibrating microtome (Vibratome Series 1000; Technical Products International, St. Louis, MO). Each slice was divided with a midsagittal cut and hippocampi were isolated and transferred to a holding chamber with warmed (30-32° C), oxygenated cutting/holding aCSF and incubated for at least 1 hour before recordings. In a subset of animals, 5-8 hippocampal slices were set aside for protein analysis. Extrahippocampal tissue was removed from these slices. Approximately 30mg of hippocampal tissue was collected and flash frozen in liquid nitrogen. One slice at a time was transferred to a chamber mounted under an upright

microscope (BX51WI; Olympus) and was superperfused with warmed (30-32° C) oxygenated recording aCSF. The recording aCSF contained (in mM): 124 NaCl, 3 KCl, 2 $CaCl_2$, 1.3 MgCl, 1.4 $NaHCO_3$, and 11 glucose (pH 7.2-7.4). Each slice was equilibrated in the microscope chamber for >10 minutes before recording.

3.2.3 Electrophysiological recordings

Whole-cell patch-clamp recording were obtained from hippocampal DGCs, identified by location and morphological characteristics. Recording pipettes were pulled from borosilicate glass (open tip resistance 3-5 MΩ; King Precision Glass Co.). The pipette recording solution contained (in mM): 126 K^+-gluconate, 4 KCl, 10 HEPES, 4 MgATP, 0.3 NaGTP, and 10 PO-creatine (pH 7.2). Electrophysiological recordings were performed using a Multiclamp 700B amplifier (Molecular Devices), low pass filtered at 2 kHz, digitized at 20 kHz (Digidata 1440A; Molecular Devices), and recorded onto a computer using pClamp 10.2 software (Molecular Devices). Seal resistance was typically 2-5 GΩ. Series resistance was <25 MΩ (mean: 11.4 MΩ) and was monitored periodically during the recordings. Recordings were discontinued if series resistance changed by more than 20% during the recording.

Spontaneous excitatory post-synaptic currents (sEPSCs) were recorded in voltage-clamp mode at a holding potential of -70 mV. Resting membrane potential and input resistance were measured in current clamp mode. Current steps (-100 pA to 400 pA in 50 pA steps) were injected to record membrane voltage response. The input resistance was calculated from the slope of the linear portion of the resulting current-voltage curve. The resting membrane potential was averaged from the portions of recorded traces between current steps. A platinum-iridium concentric-bipolar electrode (125 μM diameter; FHC)

was positioned on the lateral perforant pathway of each slice. A series of 30 pairs of current pulses (10-60 μA; 400 μs; interpulse interval 75 ms; 5 seconds between pulse pairs) were administered to evoke EPSCs. The stimulus intensity was adjusted so that responses occurred after >80% of pulses. Stimulus sweeps that failed to elicit a response with both stimuli were excluded from analysis.

3.2.4 Tissue Homogenization and Western Blot

The hippocampal tissue from each mouse was homogenized as described previously (Koren et al., 2019). Tissue samples were mechanically homogenized in RIPA lysis buffer (VWR) with phosphatase inhibitor cocktails 2 and 3 (Sigma), cOmplete protease inhibitor (Sigma), and PMSF (1 mM final concentration, Roche Diagnostics). Homogenates were centrifuged at 4°C at 13,000 rpm for 30 minutes. The fresh supernatant from each sample was divided into aliquots and stored at -80°C. Protein concentration was quantified using the Pierce BCA kit (Thermo Fisher, 23225).

Sample protein concentrations were normalized with lysis buffer and denatured by boiling for 5 minutes in 4x Laemmli buffer (BioRad) plus 10% β-mercaptoethanol (Sigma). The samples were separated on a 10% tris-glycine gel (BioRad) and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore). Membranes were blocked in 6% non-fat dry milk (w/v) in 0.01M tris buffered saline (TBS). All antibodies were diluted in 6% non-fat milk in TBS. Primary antibodies used are as follows: AT8 (pS202/pT205 tau; 1:2000; ThermoFisher), H-150 (human tau; 1:2000; Santa Cruz), GAPDH (1:5000; Cell Signaling Technology). Goat anti-mouse and goat anti-rabbit secondary antibodies conjugated to horseradish peroxidase (Southern Biotech) used were diluted in 6% non-fat

dry milk in TBS (w/v). Images were collected on an Amersham Imager 600 (General Electric).

3.2.5 Data Analysis

All electrophysiological measures (evoked action potential frequency, resting membrane potential, input resistance, spontaneous EPSC frequency, and paired pulse responses) were analyzed with MiniAnalysis (Synaptosoft, Fort Lee, NJ). Statistical measures were performed with Prism (GraphPad, San Diego, CA). Data were tested for normality by a Shapiro-Wilk test and log-transformed where appropriate. Data were normally distributed except where noted. A two-way ANOVA with age and genotype as factors was used to compare mean values for each electrophysiological value. All data for each electrophysiological measurement were analyzed together. Some control data are presented in multiple figures for presentation clarity. Data were disaggregated by sex and no sex-dependent differences were detected for any measure, so sexes were combined for all analyses. Total cell counts for each electrophysiological measurement are summarized in Table 3.1. Data are presented as mean ± SEM and statistical significance was set to $p<0.05$ for all tests.

3.3 Results

3.3.1 Resting membrane potential and input resistance in tau$^{-/-}$ and htau mice

Overexpression of hyperphosphorylated tau is associated with a depolarized resting membrane potential in neocortical pyramidal neurons of Tg4510 mutant mice (Crimins et al., 2012; Crimins et al., 2011; Rocher et al., 2010), but the electrophysiological effects of modifying tau expression in DGCs have not been studied in detail. Additionally, the insertion of pathologic human tau in other models (e.g., Tg4510) may compete with

Table 3.1 Summary of Replicates used for Electrophysiological Measurements

Age	Genotype	N (cells)		
		Induced Action Potential Frequency	Spontaneous EPSC Frequency	Paired Pulse Ratio
1.5 months	$tau^{-/-}$	26	20	21
	htau	25	16	26
	control	25	13	22
4 months	$tau^{-/-}$	24	15	17
	htau	19	11	17
	control	35	21	16
9 months	$tau^{-/-}$	28	23	23
	htau	18	16	12
	control	30	21	19

endogenous mouse tau to regulate microtubule assembly and function (Alonso Adel et al., 2006). To better understand the contribution of tau to intrinsic properties, resting membrane potential and input resistance were measured in DGCs from non-transgenic control, tau$^{-/-}$, and htau mice at 1.5, 4, and 9 months of age, which represent time points in which early pTau species are detected, mid-stage pathological processes occur, and insoluble tangle pathology is evident, respectively (Andorfer et al., 2003).

Differences in resting membrane potential and input resistance were analyzed between control, tau$^{-/-}$, and htau mice at each age by a two-way ANOVA with age and genotype as factors. In DGCs from tau$^{-/-}$ mice, the resting membrane potential was depolarized compared to those from age-matched control mice at 1.5 months of age ($F=11.83$; $p<0.05$; Table 3.2). No difference in resting membrane potential was detected at 4 months or 9 months of age ($p>0.05$; Table 3.2). No difference in resting membrane potential was detected between htau and control mice at any age ($p>0.05$). Input resistance was also not different between DGCs from control mice and either tau$^{-/-}$ or htau mice at any age ($p>0.05$; Table 3.2). With the exception of resting membrane potential differences in young tau$^{-/-}$ mice, differences in passive membrane properties were not detected across genotypes at any age.

The effect of age on resting membrane potential and input resistance was determined within each genotype. Resting membrane potential was hyperpolarized in DGCs from 9 month old tau$^{-/-}$ mice, relative to 1.5 and 4 month old tau$^{-/-}$ mice ($F=11.83$; $p<0.05$). Resting membrane potential was also hyperpolarized in DGCs from 9 month old control mice compared to 4 month old control mice ($F=11.83$; $p<0.05$). Resting membrane potential did not change with age in DGCs from htau mice ($p>0.05$), and no age-related

Table 3.2 Summary of Resting Membrane Potential and Input Resistance

Age	Genotype	N (cells)	Membrane potential Mean (mV)	Input Reseistance Mean (MΩ)
1.5 months	tau$^{-/-}$	25	-68.8±1.8*	278.7±28.4MΩ
	htau	26	-71.1±1.8	288.5±27.8MΩ
	control	25	-72.1±2.1	265.6±33.1MΩ
4 months	tau$^{-/-}$	35	-70.1±1.9	287.4±22.5MΩ
	htau	24	-70.8±1.7	278.4±32.4MΩ
	control	19	-70.3±2.1	276.9±22.1MΩ
9 months	tau$^{-/-}$	30	-73.9±1.3†ǂ	236.4±15.3MΩ †ǂ
	htau	28	-73.2±1.7	227.1±17.4MΩ †ǂ
	control	18	-73.8±1.7ǂ	234.1±21.5MΩ

*significant difference versus control.

† significant difference versus 1.5 month.

ǂ significant difference versus 4 month.

difference in input resistance was detected in control mice ($p>0.05$). Input resistance in DGCs from 9 month old tau$^{-/-}$ mice, however, was lower compared to those from 1.5 or 4 month old tau$^{-/-}$ mice ($F=14.11$; $p<0.05$). Similarly, input resistance was lower in DGCs from 9 month old htau mice compared to 1.5 or 4 month old htau mice ($F=14.11$; $p<0.05$). Thus, input resistance was lower at the oldest ages examined for DGCs in both tau$^{-/-}$ and htau mice. Input resistance was not different between DGCs from tau$^{-/-}$ and htau mice at any age ($p>0.05$).

3.3.2 Lower action potential firing frequency in young tau$^{-/-}$ and htau mice

At 4 and 8-9 months of age, neocortical pyramidal cells from the Tg4510 tau mouse model exhibit a higher frequency of evoked action potentials in response to depolarizing current injection, relative to control mice (Crimins et al., 2012; Crimins et al., 2011; Rocher et al., 2010), but lower action potential threshold and reduced action potential frequency was reported in CA1 pyramidal cells from the same mouse strain (Hatch et al., 2017), and age-related effects of tau expression on intrinsic excitability of DGCs has not been assessed. To better understand the role of tau in determining evoked action potential frequency, depolarizing and hyperpolarizing currents were injected into DGCs from non-transgenic control, tau$^{-/-}$, and htau mice at 1.5, 4, and 9 months of age.

Dentate granule cells from 1.5 month old tau$^{-/-}$ mice had a lower peak action potential frequency relative to cells from age-matched control mice ($F=7.216$; $p<0.05$; Figure 3.1B). Action potential frequency response at any current injection magnitude did not differ between DGCs from tau$^{-/-}$ and control mice at 4 or 9 months ($p>0.05$; Figure 3.1C&D). DGCs from 1.5 month old htau mice also had a lower peak frequency of evoked action

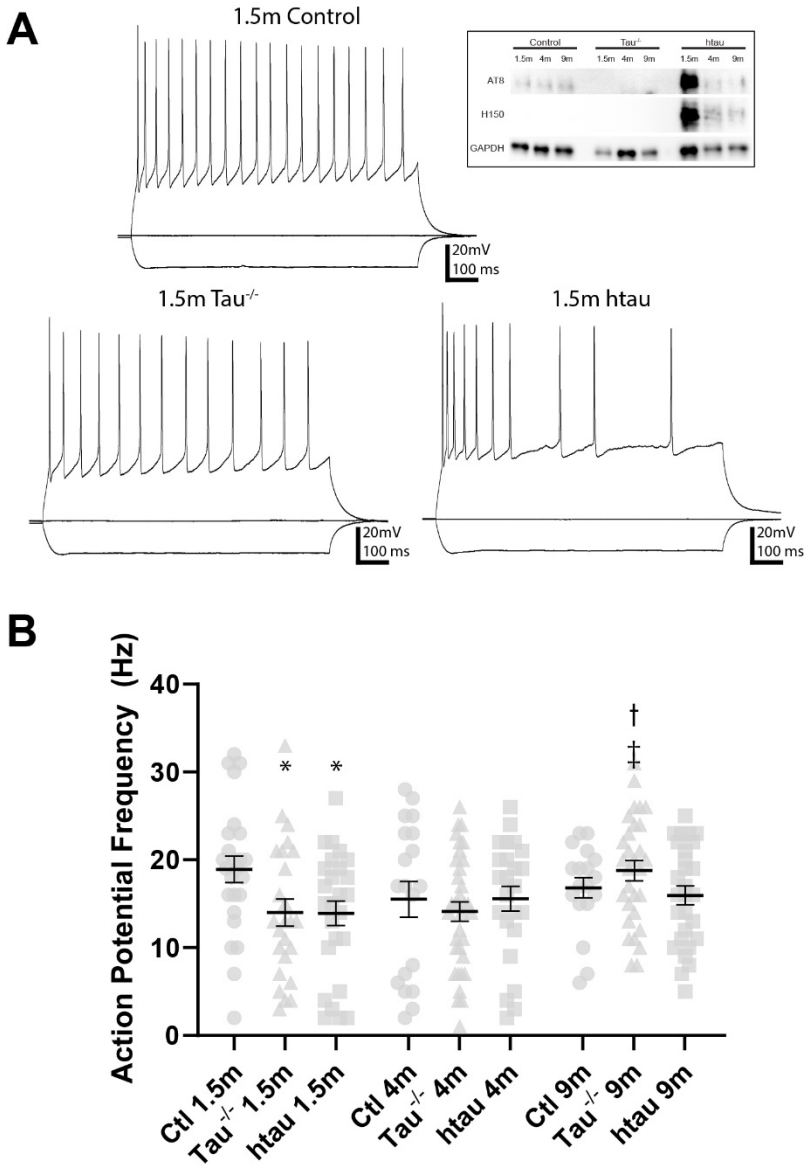


Figure 3.1 Membrane voltage response to injected current in DGCs from tau$^{-/-}$ and htau mice compared to non-transgenic control mice.
(A) Sample recordings showing voltage response from -100, 0, and 200pA current steps in DGCs from 1.5 month old tau$^{-/-}$, htau, and control mice. Inset: representative Western blot showing absence of tau protein in tau$^{-/-}$ and presence of human tau protein in htau mice. (B) Comparison of evoked action potential frequency in response to injection of 200pA current. At 1.5 months, DGCs from control mice fire more actions potentials than those from tau$^{-/-}$ and htau mice. In tau$^{-/-}$ mice, DGCs from 9 month old mice fire more action potentials than at 1.5 or 4 months. *: Different from 1.5 month control ($p<0.05$) †: Different from 1.5 month tau$^{-/-}$ ($p<0.05$) ǂ: Different from 4 month tau$^{-/-}$ ($p<0.05$). Error bars indicate SEM. Statistical comparisons were made between all groups by two-way ANOVA with age and genotype as factors.

potentials to cells from control mice ($F=7.216$; $p<0.05$; Figure 3.1B). Action potential frequency in these neurons did not differ in response to any current injection between htau and control mice at 4 or 9 months ($p>0.05$). Thus, versus age-matched controls, lower action potential frequency was observed in DGCs at 1.5 months of age in both tau$^{-/-}$ and htau mice, but not at older ages. No differences in action potential frequency were detected between DGCs from tau$^{-/-}$ and htau mice at any age ($p>0.05$).

Membrane voltage response to injected current was analyzed at different time points to assess the effect of aging within each genotype. In DGCs from control mice, cells from 4 month old mice had a lower peak frequency of evoked action potentials compared to 1.5 month old mice ($p<0.05$; Figure 3.2A). DGCs from both 9 month old tau$^{-/-}$ and htau mice had a higher peak frequency of evoked action potentials compared to 1.5 or 4 month old mice ($F=7.216$; $p<0.05$; Figure 3.2B,C). Thus, higher peak action potential frequency emerged at 9 months of age in both htau and tau$^{-/-}$ mice, but this was not evident in control mice. Together, these results suggest intrinsic properties in both tau$^{-/-}$ and htau mice develop age-related changes that are not observed in control mice. DGCs from both transgenic strains exhibit reduced cellular excitability compared to control early in life, but these differences are abrogated with age. Furthermore, the intrinsic properties of DGCs from tau$^{-/-}$ and htau mice change with age in a similar fashion.

3.3.3 Spontaneous EPSC frequency was not impacted by tau

Excitatory synaptic input to DGCs is thought to contribute to network excitability and seizure generation in several models of acquired epilepsy (Butler et al., 2015; Hunt et al., 2010; Winokur et al., 2004), and changes in sEPSC frequency related to changes in tau expression could contribute to the changes in seizure susceptibility observed by others (Garcia-Cabrero et al., 2013; Gheyara et al., 2014; Holth et al., 2013). To determine if tau

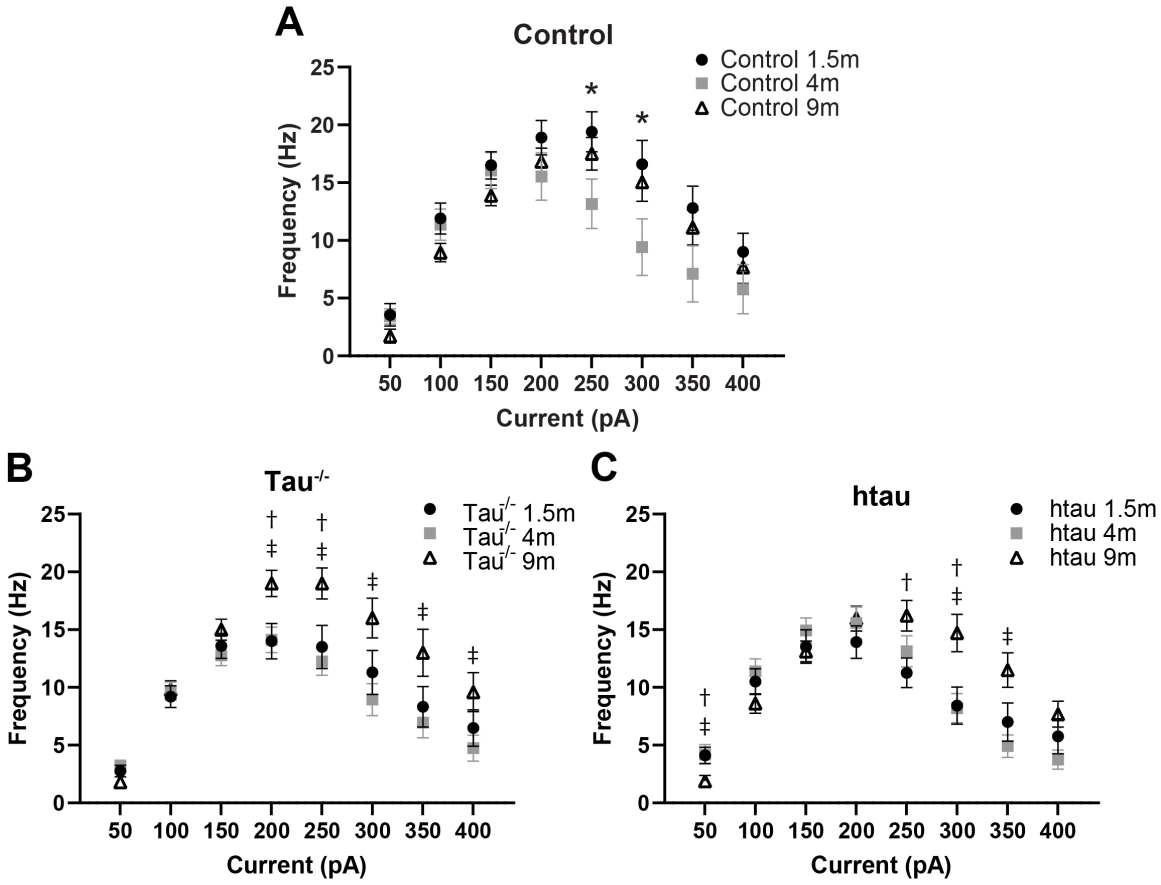


Figure 3.2 Membrane voltage responses change with age in DGCs.
Same data from figure 1 re-presented to show age-related changes in evoked action potential frequency. (A) DGCs from 4 month old non-transgenic control mice fire fewer action potentials compared to DGCs from 1.5 month old control mice in response to current injection of 250 and 300pA. No differences in firing pattern were found between DGCs from 1.5 and 9 month old control mice. (B) DGCs from 9 month old tau$^{-/-}$ mice fire more action potentials compared to DGCs from 1.5 month old tau$^{-/-}$ mice in response to current injections of 200 and 250pA. DGCs from 9 month old tau$^{-/-}$ mice also fire more action potentials compared to DGCs from 4 month old tau$^{-/-}$ mice in response to current injection of 200pA and higher. No differences in firing pattern were found between DGCs from 1.5 and 4 month old tau$^{-/-}$ mice. (C) DGCs from 9 month old htau mice fire more action potentials compared to DGCs from 1.5 month old htau mice in response to current injections of 250 and 300pA. DGCs from 9 month old htau mice also fire more action potentials compared to DGCs from 4 month old htau mice in response to current injection of 300 and 350pA. No differences in firing pattern were found between DGCs from 1.5 and 4 month old htau mice. Error bars indicate SEM. *: 1.5 month different from 4 month ($p<0.05$) †: 1.5 month different from 9 month ($p<0.05$) ǂ: 4 month different from 9 month ($p<0.05$).

expression influences overall excitatory synaptic input to DGCs, sEPSC frequency was measured in DGCs from tau$^{-/-}$, htau, and control mice. A Shapiro-Wilk test found the data were not normally distributed but were positively skewed, so a log transformation was performed on the raw data. Log-transformed sEPSC frequencies were compared with a two-way ANOVA with age and genotype as factors and did not differ between groups at any ages ($p>0.05$; Figure 3.3A). Overall, there was not a strong influence of age or strain on sEPSC frequency.

3.3.1 Paired pulse facilitation is enhanced in young tau$^{-/-}$ and htau mice

Altered probability of glutamate release can also influence DGC activity and may be susceptible to tau hyperphosphorylation or mutation (Yoshiyama et al., 2007). To determine effects of tau expression on paired pulse facilitation in the dentate gyrus, pairs of stimuli were administered to the lateral perforant path and evoked EPSCs were recorded in DGCs from tau$^{-/-}$, htau, and control mice. The paired pulse ratio, which is calculated as the amplitude of the second evoked EPSC divided by the amplitude of the first evoked EPSC, is inversely proportional to the probability of neurotransmitter release from the presynaptic terminal (i.e. a higher paired pulse ratio indicates a lower probability of neurotransmitter release; Graziane and Dong, 2016). The paired pulse ratio of evoked EPSCs was compared between all groups with a two-way ANOVA with age and genotype as factors. The paired pulse ratio of evoked EPSCs was significantly greater in DGCs from tau$^{-/-}$ mice compared to age-matched control mice at 1.5 months (control: 1.27±0.10; tau$^{-/-}$: 1.56±0.16; $F=6.480$; $p=0.002$) but did not differ at 4 or 9 months ($p>0.05$; Figure 3.4A-C). The paired pulse ratio was lower in DGCs from 9 month old tau$^{-/-}$ mice compared to 1.5 month old tau$^{-/-}$ mice (1.5 month tau$^{-/-}$: 1.56±0.16, 9 month tau$^{-/-}$: 1.28±0.09, $F=8.924$;

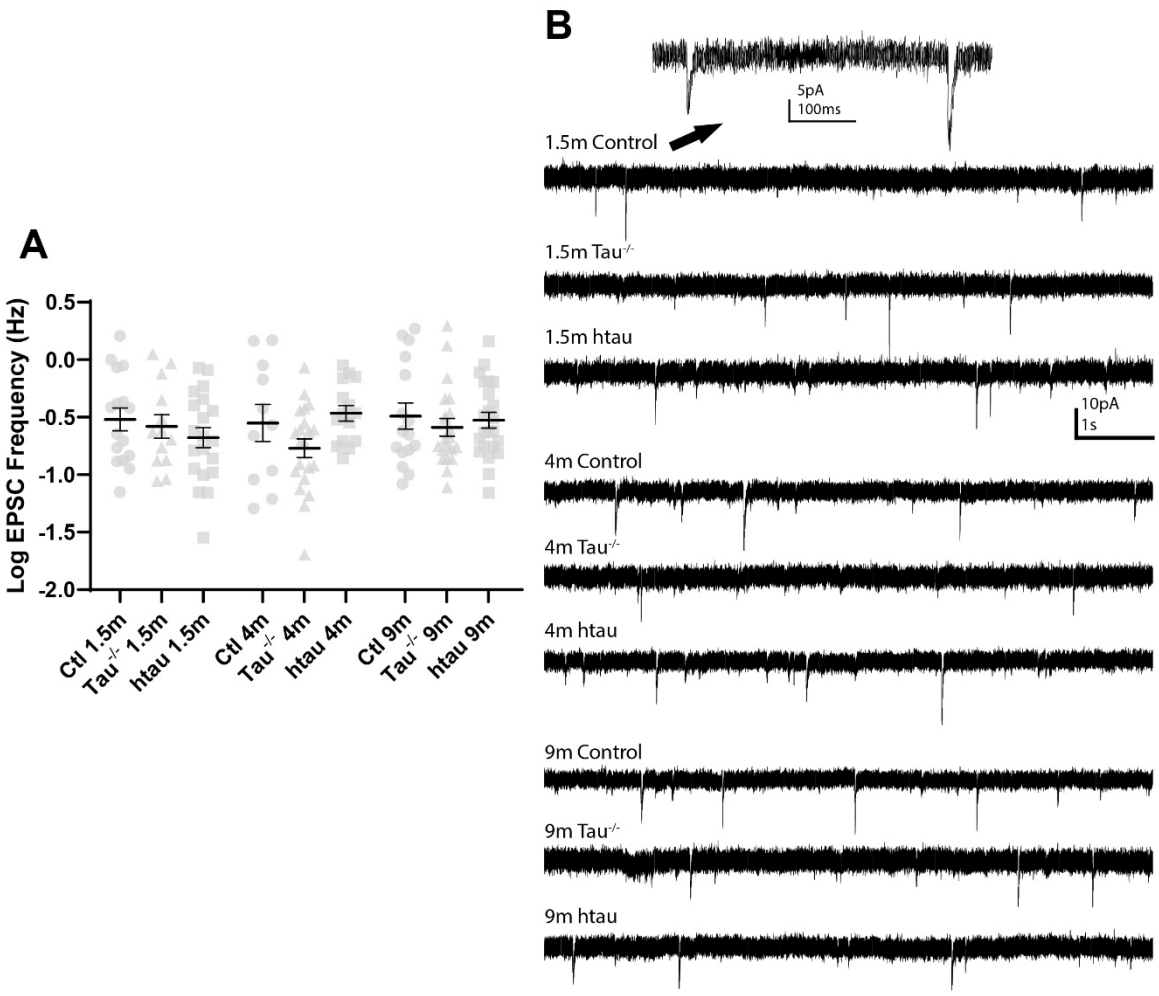


Figure 3.3 Average spontaneous EPSC frequency in tau$^{-/-}$, htau, and non-transgenic control mice.
(A) No differences sEPSC frequency were found between DGCs from tau$^{-/-}$ or htau mice and control mice at any age. sEPSC frequency did not change with age in DGCs from tau$^{-/-}$, htau, or control mice ($p>0.05$). A Shapiro-Wilk test found the data are not normally distributed, so raw data were log transformed prior to further analysis. Statistical comparisons were made between all groups by two-way ANOVA with age and genotype as factors. (B) Representative traces of sEPSCs in DGCs from tau$^{-/-}$, htau, or control mice at 1.5, 4, and 9 months. Error bars indicate SEM.

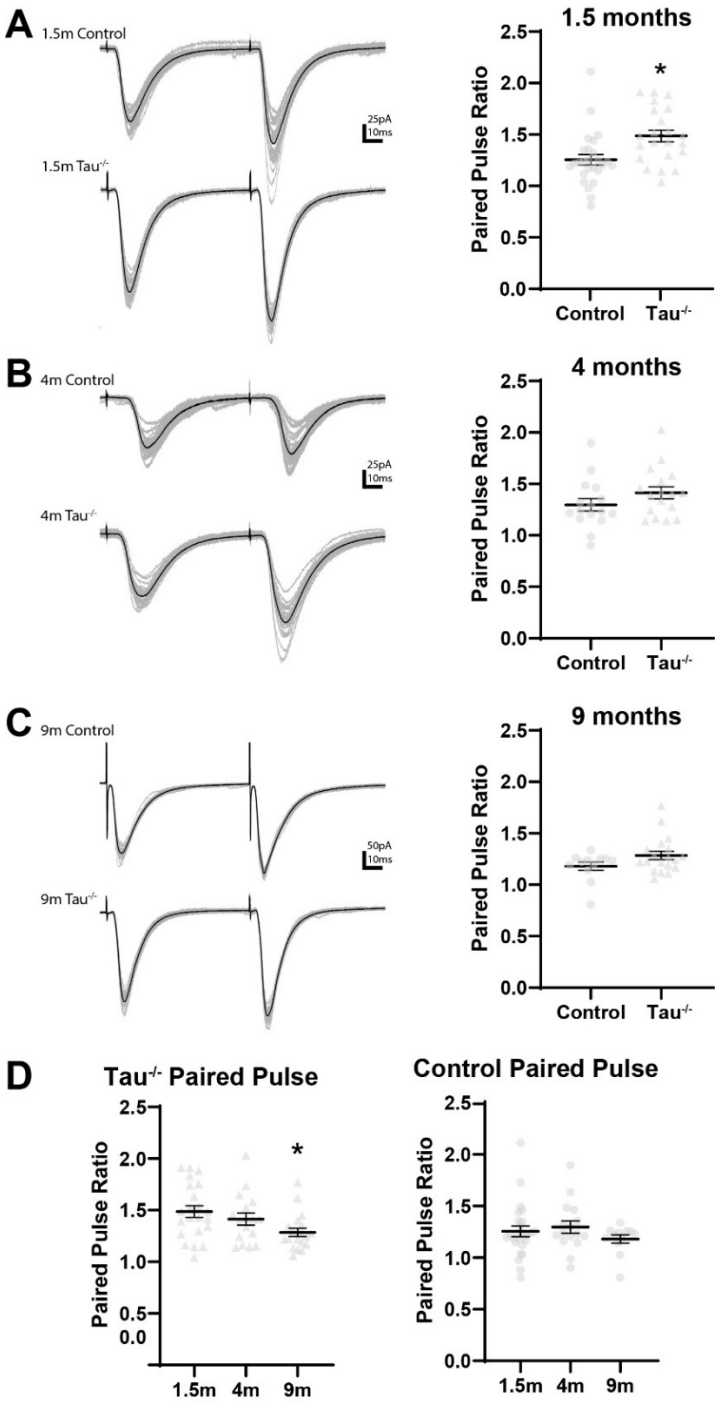


Figure 3.4 Paired pulse ratio in tau$^{-/-}$ mice compared to non-transgenic control mice.
(A) The paired pulse ratio is higher in DGCs from 1.5 month old tau$^{-/-}$ mice compared to 1.5 month old control mice. *: $p<0.05$ (B, C) No differences in paired pulse ratio were found between DGCs from tau$^{-/-}$ and control mice at 4 (B) or 9 (C) months old. (D) The paired pulse ratio is lower in DGCs from 9 month old tau$^{-/-}$ mice compared to 1.5 month old tau$^{-/-}$ mice. The paired pulse ratio did not change with age in DGCs from control mice. Statistical comparisons were made between all groups by two-way ANOVA with age and genotype as factors. *: different from 1.5 month ($p<0.05$) Error bars indicate SEM.

Figure 3.4D). Similar to results from tau$^{-/-}$ mice, the paired pulse ratio was greater in DGCs from htau mice compared to control mice at 1.5 months (control: 1.27±0.10; htau: 1.43±0.09; F=6.480; p=0.023) but did not differ at 4 or 9 months of age (p>0.05; Figure 3.5A-C). The paired pulse ratio did not change with age in DGCs from htau mice (p>0.05; Figure 3.5D). Thus, in both transgenic mouse strains, the paired pulse ratio in DGCs after stimulation of the lateral perforant pathway was greater compared to those from non-transgenic control mice at 1.5 months of age, but not later in life. The paired pulse ratio in DGCs after stimulation of the perforant pathway did not differ between tau$^{-/-}$ and htau animals at any age (p>0.05). The paired pulse ratio decreased with age in tau$^{-/-}$ mice. This contrasted with results from htau mice and non-transgenic control mice, in which age-related changes in paired pulse ratio were not detected (p>0.05). The amplitudes of evoked EPSCs varied significantly across all cells but were not different between any groups (data not shown).

3.4 Discussion

Tau pathology is associated with greater excitability and increased susceptibility to seizures in epilepsy models (Garcia-Cabrero et al., 2013; Liu et al., 2017b). Similarly, loss of tau through genetic deletion or suppression with antisense oligonucleotides is associated with decreased mossy fiber sprouting in the dentate gyrus, reduced seizure burden, and improved cognition and survival in models of epilepsy (DeVos et al., 2013; Gheyara et al., 2014; Holth et al., 2013) and ameliorates the increased susceptibility to seizures associated with some models of Alzheimer's disease (Ittner et al., 2010; Roberson et al., 2007). This study measured several intrinsic and synaptic membrane properties in tau$^{-/-}$ and htau mice to provide better understanding of tau's role in neuronal excitability, which has been

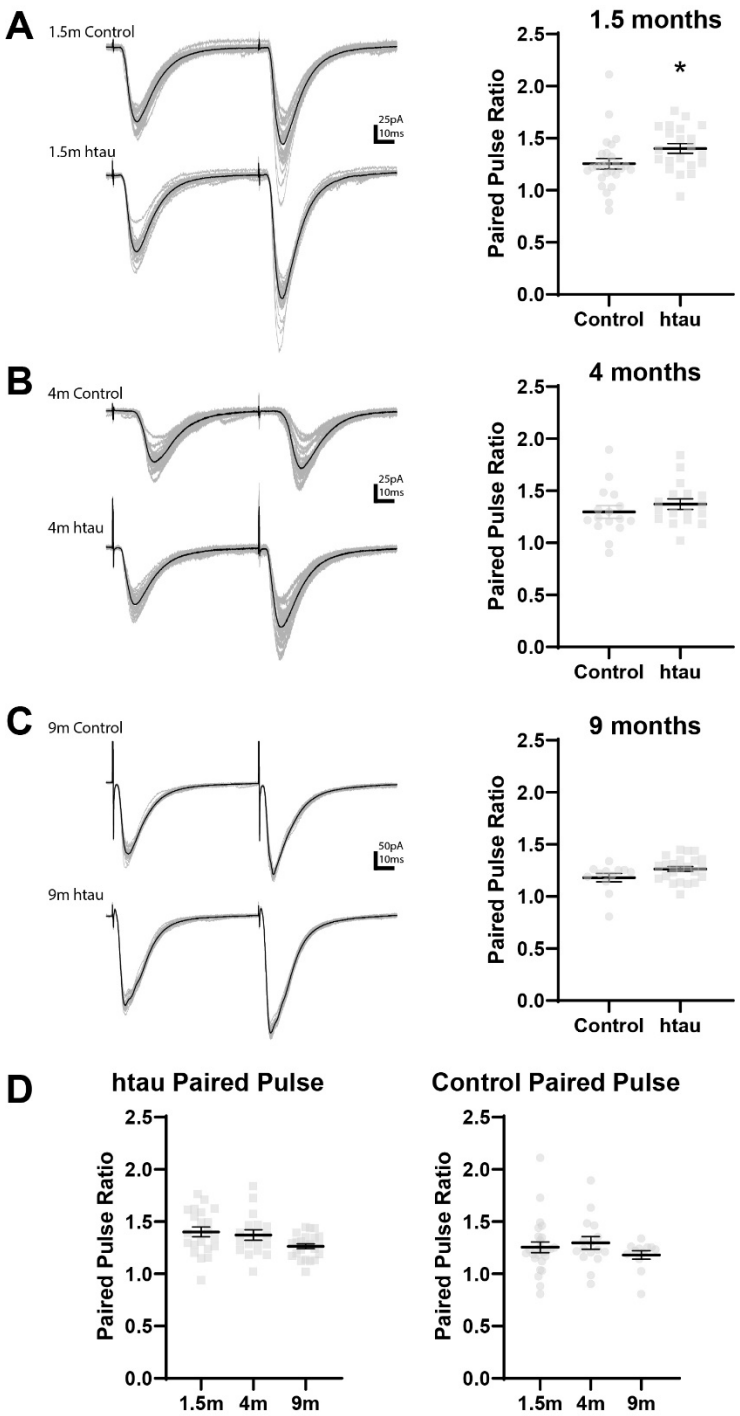


Figure 3.5 Paired pulse ratio in htau mice compared to non-transgenic control mice.
(A) The paired pulse ratio is higher in DGCs from 1.5 month old htau mice compared to 1.5 month old control mice. *: $p<0.05$ (B, C) No differences in paired pulse ratio were found between DGCs from htau and control mice at 4 (B) or 9 (C) months old. (D) The paired pulse ratio did not change with age in DGCs from htau or control mice. Statistical comparisons were made between all groups by two-way ANOVA with age and genotype as factors.*: different from 1.5 month ($p<0.05$) Error bars indicate SEM

hypothesized to play a role in genetic epilepsies and, possibly, seizure disorders associated with neurodegeneration (Decker et al., 2016; Garcia-Cabrero et al., 2013; Gheyara et al., 2014; Holth et al., 2013; Ittner et al., 2010; Roberson et al., 2011; Roberson et al., 2007).

Although other studies have measured the influence of tau expression and hyperphosphorylation on neuronal properties, most have used forms of tau with disease-related mutations such as P301L (Crimins et al., 2012; Crimins et al., 2011; Hatch et al., 2017; Hunsberger et al., 2015; Liu et al., 2017b; Rocher et al., 2010), P301S (Yoshiyama et al., 2007), and A152T (Decker et al., 2016). In contrast, the htau mouse used in this study expresses six isoforms of human tau without any disease-related mutations (Andorfer et al., 2003). This model was selected for two reasons. First, the non-mutated human tau expressed in this model results in slower development of pathology that more reasonably resembles human aging than in more aggressive pathologic tau models. The htau mouse exhibits progressive tau pathology, developing pTau by 1.5 months, somatodendritic redistribution of tau around 3 months, and neurofibrillary tangles around 9 months (Andorfer et al., 2003). Additionally, the endogenous murine tau gene is deleted in the htau mouse, setting it apart from other commonly used tauopathy models. The deletion of endogenous murine tau is important because tau hyperphosphorylation leads to concurrent loss of normal tau function and consequently increased pathology due to the increase in soluble pTau. Tau hyperphosphorylation reduces the pool of functional tau available to stabilize microtubules, resulting in a loss of physiologic function (Lindwall and Cole, 1984). At the same time, soluble pTau itself is neurotoxic through several mechanisms, including mislocalization of pathologic tau, inhibition of protein translation, and abnormal interaction with other cellular components (Flach et al., 2012; Fulga et al., 2007; Hoover

et al., 2010; Koren et al., 2019; Meier et al., 2015; Meier et al., 2016; Thies and Mandelkow, 2007; Tian et al., 2013). Because tauopathy results in loss of function as well as development of neurotoxicity, the presence of normal murine tau expression in other tau mouse models confounds the interpretation of outcomes based on tau hyperphosphorylation. Some inconsistency between the results across previous studies in different tau models, along with the similarities found between the htau and tau$^{-/-}$ animals in the current study, demonstrate the importance of separating the effects of removing tau and adding hyperphosphorylated tau in interpreting electrophysiological outcomes.

While previous work suggests an important role for tau in the electrophysiological function of the neuron, the direct effects of tau deletion on neuronal excitability have not been extensively studied without the presence of additional pathology. Previous studies on the electrophysiological effects of hyperphosphorylated tau have primarily used the rTg4510 transgenic mouse, which expresses 4R0N human tau with the pathogenic P301L mutation with endogenous murine tau expression intact (Santacruz et al., 2005). The major effects on the intrinsic properties of frontal cortex pyramidal neurons in these studies were depolarized resting membrane potential and increased frequency of evoked action potentials (Crimins et al., 2012; Crimins et al., 2011; Rocher et al., 2010). A decrease in paired pulse facilitation in the hippocampus has also been reported in other transgenic tau models (Maeda et al., 2016; Roberson et al., 2011; Sydow et al., 2011; Yoshiyama et al., 2007). Based on these studies, we hypothesized that similar results would be detected in DGCs from htau mice, but this hypothesis was not supported. Instead we found no changes in resting membrane potential in the htau mice, and reduced evoked action potential firing and increased paired pulse facilitation was detected in both htau and tau$^{-/-}$ mice, but only at

1.5 months of age. Thus, deletion of murine tau tended to reduce intrinsic and synaptic excitability of DGCs early, but not later in life, and replacing murine tau with human tau did not reinstate normal excitability.

Our results in htau mice might be linked to pathogenic mechanisms of Alzheimer's disease. Unlike rTg4510 mice, which overexpress 13x more human mutant 4R0N P301L tau in the forebrain, htau mice express all six splice variants of non-mutant human tau (Andorfer et al., 2003; Santacruz et al., 2005). The P301L mutation is associated with fronto-temporal dementia (Hutton et al., 1998); meanwhile, tau pathology in Alzheimer's brains has accumulation of the six splice variants of non-mutant human tau. The difference in tau pathogenicity between the htau and rTg4510 mice may explain why no change in resting membrane potential was detected in DGCs from htau mice. Other factors, including the endogenous properties of the different neuron types studied (i.e., pyramidal cells versus DGCs) or the continued expression of endogenous mouse tau in the rTg4510 mice, could also contribute substantially to these outcomes. However, the differences in tau species, or even cell type examined, do not adequately explain the patterns of evoked action potentials or paired pulse facilitation observed in each mouse model: A difference in relative pathogenicity could result in a smaller magnitude of difference between these measures, but it does not fully explain the opposite direction of effects (i.e., action potential frequency decreased in htau but increased in rTg4510 and paired pulse ratio increased in htau but decreased in rTg4510).

Because the htau mice lack endogenous tau, physiological tau function is likely reduced because the transgenically expressed human tau may not function normally. This notion is supported by the similar patterns of evoked action potentials observed in dentate

granule cells from htau and tau$^{-/-}$ mice early in life, when human tau may not function normally in the htau mouse. Similar to studies demonstrating that the pro-excitatory effects of amyloid-beta rely on the presence of functional tau (Roberson et al., 2011; Shipton et al., 2011), our results suggest that excitatory effects associated with pathologic tau in other models requires a pool of functional tau as well, which is not expressed in either strain used here. Any potential pro-excitatory effects may therefore have been abrogated by the absence of functional tau.

This study found important similarities between dentate granule cell responses in tau$^{-/-}$ and htau mice, consistent with the hypothesis that the substitution of tau species in the htau mouse contributes to the loss of normal tau function. In both tau$^{-/-}$ and htau mice, peak evoked action potential frequency in DGCs was reduced relative to age-matched controls early in life, but not at later ages. Similarly, peak evoked action potential frequency in DGCs increased with age in both tau$^{-/-}$ and htau mice, becoming comparable to that seen in controls. These results together indicate that absence of functional tau, due to hyperphosphorylation and/or gene deletion, has an effect on intrinsic neuronal function early in life that is not evident as the animals age. Tau deletion reduces excitability in both young tau$^{-/-}$ and htau mice, but this does not persist in older mice.

Tau's ability to affect synaptic function, either on its own or in conjunction with other Alzheimer's disease pathology, has been established in several transgenic animal models. The major effect of pathologic tau on synaptic function is impairment of long term potentiation (LTP) and a decrease in the paired pulse ratio at several hippocampal synapses (Decker et al., 2016; Maeda et al., 2016; Roberson et al., 2011; Shipton et al., 2011; Sydow et al., 2011; Yoshiyama et al., 2007). The impairment of LTP likely contributes to deficits

in learning and memory in these models. The decrease in paired pulse ratio suggests an increased probability of neurotransmitter release and may at least partially explain the hyperexcitability and susceptibility to seizures observed in these models. Previous studies have found that removal of tau rescues synaptic deficits associated with Alzheimer's disease, particularly amyloid-β induced impairment of LTP (Roberson et al., 2011; Shipton et al., 2011). These studies reported minimal differences between tau$^{-/-}$ and non-transgenic mice in the absence of additional pathology. In the current study we measured the frequency of spontaneous excitatory post-synaptic currents (sEPSCs) and found no differences between genotypes at any age. Conversely, changes in presynaptic release associated with absence of functional murine tau protein were observed in both transgenic strains used herein. We measured the dentate granule cell response after stimulation of the lateral perforant path to assess synaptic release probability, based on paired pulse response ratios. DGCs from both tau$^{-/-}$ and htau mice exhibited greater paired pulse facilitation compared to age-matched, non-transgenic control mice early in life, but the paired pulse ratio was not different from controls in either tau$^{-/-}$ or htau mice later in life. The paired pulse ratio decreased significantly with age in tau$^{-/-}$ mice, whereas it did not change with age in htau or non-transgenic mice. Our study found similar presynaptic neurotransmitter release in both transgenic strains, suggesting a loss of tau function in both groups.

Taken together, the reduced frequency of evoked action potentials and increased paired pulse facilitation in young tau$^{-/-}$ mice provide a basis for the seizure resistance in genetic epilepsy models observed in tau$^{-/-}$ mice at similar ages (Gheyara et al., 2014; Holth et al., 2013). One limitation of those studies is the relatively young age at which the mice were, necessarily, assessed. The results of the present study suggest that the anti-epileptic

and anti-SUDEP effect of tau deletion evident in mutant mice, which express channelopathies that underlie seizures, may not fully translate to epilepsies with a later age of onset. These effects may be lost or reduced with age as excitability increases. Previous work found changes in expression of other microtubule-associated proteins in $tau^{-/-}$ mice (Harada et al., 1994; Ma et al., 2014). More work is required to understand how these changes in expression might affect electrophysiological function in $tau^{-/-}$ mice.

3.5 Conclusions

This study sheds new light on the role of tau in promoting neuronal excitability. We found that DGCs from mice lacking tau protein or in which endogenous mouse tau is replaced by human tau, which has previously been shown to become hyperphosphorylated early in life (Andorfer et al., 2003), exhibited reduced measures of excitability in the form of reduced peak evoked action frequency and increased paired pulse facilitation that were significant in young animals but were abrogated with age. The loss of excitability in the htau mouse contrasts with previous work showing a pro-excitatory role of pathologic tau in pyramidal neurons from other tauopathy models, but this may be due to differences in tau or other pathogenicity, endogenous tau expression in these models, or in the characteristics of the types of neurons and networks examined. Finally, our results suggest that, while early changes in tau expression may influence neuronal excitability and seizures in young mice, other compensatory mechanisms may participate in stabilizing neuronal circuits later in life. Identifying additional links between tau phosphorylation and neuronal network function may help resolve the influence of tauopathy on disease progression.

CHAPTER 4. LOSS OF TAU MODIFIES BUT DOES NOT PREVENT EPILEPTOGENESIS AFTER INTRAHIPPOCAMPAL KAINATE TREATMENT IN MICE

A similar version of this chapter was submitted to Experimental Neurology for peer review with Rafael Roberts and Bret N. Smith as additional authors.

4.1 Introduction

Temporal lobe epilepsy (TLE) is the most common focal epilepsy and accounts for the majority of drug-resistant epilepsy cases (Asadi-Pooya et al., 2017; Pascual, 2007; Reynolds, 2000; Semah et al., 1998; Wiebe, 2000). Although drug resistant TLE with an identifiable focus often responds well to surgical resection (Engel, 1996; Engel et al., 2012; Engel et al., 2003), development of additional treatment options remains essential to reduce the burden of care and improve quality of life for patients with TLE. A better understanding of the mechanisms that drive development of TLE is crucial to developing new, specific therapies. One potential target that has received attention in recent years is the microtubule-associated protein, tau.

Hyperphosphorylated tau plays a key disease-promoting role in tauopathies including Alzheimer's disease (AD) and has been associated with epilepsy in human and animal studies (Gheyara et al., 2014; Holth et al., 2013; Puvenna et al., 2016; Tai et al., 2016). The relationship between tau pathology and epilepsy is complex and bidirectional. Brain tissue resected as treatment for intractable epilepsy shows advanced tau pathology much earlier in life than typical tauopathies (Gourmaud et al., 2020; Puvenna et al., 2016; Smith et al., 2019), and animal studies have demonstrated an increase in tau phosphorylation after seizure induction (Jones et al., 2012; Liu et al., 2016). Current evidence suggests pathological tau maybe involved in seizure susceptibility. Animal

models of tauopathy that express mutant forms of tau that are associated with human disease display increased susceptibility to seizure induction (Garcia-Cabrero et al., 2013; Liu et al., 2017b), and human patients with Alzheimer's disease have an increased risk of having seizures and developing epilepsy (Lam et al., 2020; Pandis and Scarmeas, 2012; Tabuas-Pereira et al., 2019; Vossel et al., 2013). Subclinical epileptiform activity is more common in patients with AD but no history of clinical seizures, and predicts faster progression of cognitive impairment (Vossel et al., 2016). Reducing tau phosphorylation confers resistance to seizure induction in animal models (Jones et al., 2012; Liu et al., 2016). Similar seizure resistance is observed in animals lacking tau expression due to genetic deletion or suppression with antisense oligonucleotides (DeVos et al., 2013; Li et al., 2014). Furthermore, loss of tau expression reduces seizure burden and improves survival in genetic models of epilepsy (Gheyara et al., 2014; Holth et al., 2013). The relationship between tau phosphorylation and seizures and epilepsy suggests that tau could be a target for novel treatments to modify epileptogenesis or treat seizures.

Although many studies have demonstrated seizure resistance in animals lacking tau, whether this translates to resistance to epileptogenesis is not clear. Furthermore, the degree to which tau promotes seizures and epileptogenesis in the absence of additional pathology has not been adequately studied. The current experiment seeks to address these issues using the htau mouse model of tauopathy, which produces littermate mice that express either no tau of any type (i.e., $tau^{-/-}$ mice) or non-mutant human tau that becomes hyperphosphorylated by 1.5 months of age (Andorfer et al., 2003), but no murine tau (i.e., htau mice) . We previously showed that both htau and $tau^{-/-}$ mice exhibit lower neuronal excitability in dentate gyrus granule cells (DGCs) compared to non-transgenic control mice

at 1.5 months of age (Cloyd et al., 2021). In this study, we determined whether the reduced excitability associates with seizure resistance, modification of epilepsy development, or altered neuronal excitability in the intrahippocampal kainate (IHK) model of TLE. We tested the hypothesis that loss of native tau function confers protection against IHK induced epilepsy as assessed behaviorally and via 24-hour video-electroencephalogram (vEEG). We also assessed neuronal excitability in DGCs using whole cell patch-clamp electrophysiology 6-8 weeks after IHK treatment. Determining how tau expression influences cellular and behavioral correlates of acquired TLE provides important insight relevant to epileptogenesis, especially in the context of tauopathy related dementias.

4.2 Materials and Methods

4.2.1 Animals

Transgenic B6.Cg-*Mapt*$^{tm1(EGFP)Klt}$Tg(MAPT)8cPdav/J mice (male and female; #005491) were produced in house from breeders obtained from The Jackson Laboratory (JAX; Bar Harbor, ME). These mice lack any obvious disease phenotype at birth, but develop impairments in Morris Water Maze, spatial learning, and food burrowing with age, particularly in mice older than 9 months (Geiszler et al., 2016; Phillips et al., 2011; Polydoro et al., 2009). The human tau becomes hyperphosphorylated by 1.5 months of age (Andorfer et al., 2003). This mouse strain was originally generated on a hybrid Swiss Webster/B6D2F1 hybrid background but has been backcrossed to C57BL/6J for more than 10 generations. Single nucleotide polymorphism (SNP) analyses performed by JAX were consistent with a pure C57BL/6J background, which served as the control strain. All breeding mice were homozygous for a deletion of the murine tau gene. One mouse in each breeding pair was hemizygous for a transgene expressing all six isoforms of non-mutant,

human tau protein. The offspring are therefore either full tau knockout ($tau^{-/-}$) or express only human tau (htau), and genotypes and protein expression phenotypes were confirmed in our recent report (Cloyd et al., 2021).

DNA was extracted from tail snips and genotype was confirmed via PCR according to the protocols supplied by JAX. Disruption of the endogenous murine tau gene was confirmed using the primer pair 5'-CGTTGTGGCTGTTGTAGTTG-3' and 5'-TCGTGACCACCCTGACCTAC-3', which amplifies a fragment at 270 bp in $tau^{-/-}$ and htau mice. Presence of the human-tau transgene was confirmed using the primer pair 5'-CGAAGTGATGGAAGATCACG-3' and 5'-GTCTTGGTGCATGGTGTAGC-3', which amplifies a fragment at 79 bp in htau mice. Age matched male C57BL/6J control mice were bred in house from breeders obtained from JAX (#000664). All mice were housed under a 14 hr light / 10 hr dark cycle in an Association for Assessment and Accreditation of Laboratory Animal Care Internal (AALAC) approved facility. Food and water were available *ad libitum*. The University of Kentucky Institutional Animal Care and Use Committee approved all procedures.

4.2.2 Intrahippocampal kainate (IHK) mouse model of temporal lobe epilepsy

All surgical procedures were performed under isoflurane general anesthesia with 0.05% bupivacaine local anesthesia. Kainic acid (IHK; 100 nL, 20 mM in 0.9% saline, Tocris Bioscience; Minneapolis, MN) or saline (sham; 100 nL) was injected into the left dorsal hippocampus (2.0 mm posterior, 1.25 mm left, and 1.6 mm ventral to bregma) at 6-8 weeks of age (Krook-Magnuson et al., 2013). The injection rate was 20 nL/minute, and the needle was left in place for 5 minutes before and after injection. Buprenorphine (0.05 mg/kg) and carprofen (10 mg/kg) were administered subcutaneously after surgery. Mice were transferred to a heated cage for recovery and monitored for seizures for 2 hours to

assess development of status epilepticus (SE). SE was defined as the occurrence of at least 3 seizures of Racine scale 3 or higher during the observation period (Racine, 1972; Shibley and Smith, 2002). After 2 hours, diazepam (7.5 mg/kg) was administered intraperitoneally to terminate SE. Video monitoring for spontaneous seizures began 2 weeks after the IHK surgery and continued for 6 weeks. Each animal underwent 7-8 recording sessions (average duration 7.8 hours), averaging 56 hours of monitoring per animal. The videos were reviewed at 3-4x speed by an investigator blind to genotype and treatment.

A separate cohort of mice (n=2-4 per group) were fitted with wireless transmitters to allow video-electroencephalographic (vEEG) recording (Data Sciences International; DSI; St. Paul, MN). Kainic acid or saline was injected as described above. Screws were placed (1.0mm anterior and 1.0mm right to bregma and 3.0mm posterior and 3.0mm left to bregma) after injection. A wireless transmitter (ETA-F10, DSI) was implanted intraperitoneally and connected to the screws by subcutaneous wires. vEEG began immediately after surgery and continued for 2 hours until administration of diazepam as described above. EEG recordings were collected using Ponema (v6.42, DSI). Mice underwent a total of 5 sessions of 24 hour vEEG recording between 1 and 3 weeks after IHK. EEG recordings were manually reviewed for seizure-like activity, defined as rhythmic high amplitude (>3 fold larger than baseline), high frequency (>10Hz) activity lasting at least 20 seconds, using NeuroScore (v3.3.1, DSI). Potential seizures identified on EEG were confirmed by corresponding behavior on video recorded in Open Broadcaster Software (v21.0.1) with the Snaz file add-on to allow for 24 hour long recordings using a Logitech C270 HD camera. Seizure prevalence and average daily seizure frequency recorded via vEEG was calculated for each group.

4.2.3 Hippocampal slice preparation

Mice were sacrificed for electrophysiology 6-8 weeks after IHK or sham (i.e., saline injection) surgery. Mice were deeply anesthetized via inhalation of isoflurane to effect (lack of tail pinch response) and decapitated while anesthetized. The brain was rapidly removed from the skull and immersed in ice-cold oxygenated (95% O_2/5% CO_2) cutting/holding artificial cerebrospinal fluid (aCSF). The cutting/holding aCSF contained (in mM): 85 NaCl, 75 sucrose, 2.5 KCl, 25 glucose, 1.25 $NaH_2PO_4 \cdot H_2O$, 4 $MgCl_2 \cdot 6H_2O$, 0.5 $CaCl_2 \cdot 2H_2O$, and 24 $NaHCO_3$ (pH 7.2-7.4). Coronal sections (300 μm) were cut on a vibrating microtome (Vibratome Series 1000; Technical Products International, St. Louis, MO). Each slice was divided with a midsagittal cut and hippocampi were isolated and transferred to a holding chamber with warmed (30-32° C), oxygenated cutting/holding aCSF and incubated for at least 1 hour before recordings. One slice at a time was transferred to a chamber mounted under an upright microscope (BX51WI; Olympus) and was superfused with warmed (30-32° C) oxygenated recording aCSF. The recording aCSF contained (in mM): 124 NaCl, 3 KCl, 2 $CaCl_2$, 1.3 MgCl, 1.4 $NaHCO_3$, and 11 glucose (pH 7.2-7.4). Each slice was perfused with recording aCSF in the microscope chamber for >10 minutes to allow the cutting aCSF to wash off before recording.

4.2.4 Electrophysiological recordings

Whole-cell patch-clamp recordings were obtained from hippocampal DGCs identified by location and morphological characteristics. Recording pipettes were pulled from borosilicate glass (open tip resistance 3-5 MΩ; King Precision Glass Co. Claremont, CA). The pipette recording solution contained (in mM): 126 K^+-gluconate, 4 KCl, 10 HEPES, 4 MgATP, 0.3 NaGTP, and 10 PO-creatine (pH 7.2). Electrophysiological recordings were performed using a Multiclamp 700B amplifier (Molecular Devices, San

Jose, CA), low pass filtered at 2 kHz, digitized at 20 kHz (Digidata 1440A; Molecular Devices), and recorded onto a computer using pClamp 10.2 software (Molecular Devices). Seal resistance was typically 2-5 GΩ. Series resistance was <25 MΩ (mean: 10.3±0.1 MΩ) and was monitored periodically during the recordings. Recordings were discontinued if series resistance changed by more than 20% during the recording. Membrane voltage was not adjusted for liquid junction potential, calculated to be -15 mV.

Resting membrane potential and input resistance were measured in current clamp mode. Current steps (-100 pA to 400 pA in 50 pA steps) were injected to record membrane voltage response. The input resistance was calculated from the slope of the linear portion of the resulting current-voltage curve. The resting membrane potential was averaged from the portions of recorded traces between current steps. Spontaneous and electrically-evoked excitatory post-synaptic currents (i.e., sEPSCs and eEPSCs) were recorded in voltage-clamp mode at a holding potential of -70 mV. To elicit eEPSCs, a platinum-iridium concentric-bipolar electrode (125 μM diameter; FHC, Bowdoin, ME) was positioned on the lateral perforant pathway and 30 pairs of current pulses (30-50 μA; 400 μs; interpulse interval 75 ms; 5 seconds between pulse pairs) were administered to evoke paired eEPSCs (i.e., paired pulse response; PPr). The stimulus intensity was adjusted so that responses occurred after >80% of pulses. Stimulus sweeps that failed to elicit a response with both stimuli were excluded from analysis.

4.2.5 Data Analysis

All electrophysiological measures (evoked action potential frequency, resting membrane potential, input resistance, sEPSC frequency, and PPr) were analyzed with MiniAnalysis (Synaptosoft, Fort Lee, NJ). Statistical measures were performed with Prism

(GraphPad, San Diego, CA). Data were disaggregated by sex and no sex-dependent differences were detected for any measure, so sexes were combined for all analyses. Data were tested for normality by a Shapiro-Wilk test and non-parametric tests were used where appropriate. Seizure frequencies from tau$^{-/-}$, htau, and control mice were compared by one-way ANOVA. Survival curves were compared by Mantel-Cox logrank test and hazard ratios (HRs) were calculated between each transgenic strain and control. A 2-way ANOVA was used to compare action potential frequency in response to current injection at the current step which resulted in the highest frequency of action potentials (i.e. 200pA). An unpaired t-test was used to compare mean values for input resistance, RMP, and PPr from sham- and IHK-treated animals of each genotype. A Mann-Whitney test was used to compare mean values for sEPSC frequency from sham and IHK treated animals of each genotype. Summary data and cell counts for each electrophysiological measurement are presented in Table 4.1. Data are presented as mean ± SEM and statistical significance was set to $p<0.05$ for all tests.

4.3 Results

4.3.1 Seizure induction after IHK differs in tau$^{-/-}$ mice

We previously determined that at least 3 convulsive seizures (Racine seizure scale 3-5; Racine, 1972) after intraperitoneal pilocarpine injection constituted status epilepticus (SE), resulting in epileptogenesis in mice (Shibley and Smith, 2002; Winokur et al., 2004). Non-transgenic C57BL/6J mice reliably develop SE after IHK (Kang et al., 2021; Welzel et al., 2020). In the current study, 70% (7/10) of C57BL/6J mice developed SE after IHK, compared to 75% (9/12) of htau mice. No tau$^{-/-}$ mice (0/8) met the behavioral definition for SE. Although the tau$^{-/-}$ mice exhibited non-convulsive seizure-like behavior after IHK, they

Table 4.1 Summary of electrophysiological measurements

			Point Estimate ± SEM (n)			
			RMP (mV)	R_{In} (MΩ)	sEPSC Frequency (Hz)	Paired Pulse Ratio
C57BL/6J	Ipsilateral	Sham	-72.4 ± 1.6 (9)	291.2 ± 22.1 (9)	0.133 ± 0.045 (8)	1.51 ± 0.04 (8)
		IHK	-70.2 ± 1.7 (13)	271.2 ± 13.4 (13)	0.802 ± 0.246 (11)*	1.13 ± 0.04 (11)*
	Contralateral	Sham	-71.4 ± 1.5 (8)	257.3 ± 14.2 (8)	0.152 ± 0.064 (7)	1.63 ± 0.11 (8)
		IHK	-72.9 ± 0.9 (13)	217.0 ± 15.8 (13)	0.565 ± 0.117 (10)*	1.29 ± 0.07 (13)*
$tau^{-/-}$	Ipsilateral	Sham	-74.9 ± 0.9 (11)	230.8 ± 10.7 (11)	0.265 ± 0.106 (11)	1.41 ± 0.06 (12)
		IHK	-67.2 ± 1.6 (16)*	294.2 ± 18.8 (16)*	0.356 ± 0.084 (13)	1.42 ± 0.07 (16)
	Contralateral	Sham	-73.9 ± 1.4 (12)	253.6 ± 19.1 (12)	0.163 ± 0.079 (10)	1.54 ± 0.10 (12)
		IHK	-69.5 ± 1.2 (18)*	254.1 ± 13.5 (18)	0.607 ± 0.239 (11)*	1.34 ± 0.10 (13)
htau	Ipsilateral	Sham	-72.7 ± 1.6 (9)	250.8 ± 12.1 (9)	0.167 ± 0.056 (7)	1.35 ± 0.10 (7)
		IHK	-71.0 ± 1.0 (8)	205.3 ± 19.0 (8)	0.654 ± 0.230 (7)*	1.10 ± 0.03 (8)
	Contralateral	Sham	-73.5 ± 1.2 (8)	251.3 ± 17.5 (8)	0.084 ± 0.017 (7)	1.43 ± 0.16 (8)
		IHK	-74.0 ± 1.7 (10)	239.1 ± 11.9 (10)	0.130 ± 0.072 (7)	1.33 ± 0.09 (7)

*: different from sham control

did not reliably develop convulsive seizures.

Video EEG recordings from tau$^{-/-}$, htau, and C57BL/6J mice during the 2 hour period after treatment showed extensive electrographic activity in all IHK-treated mice. No abnormal electrographic activity was observed in any saline-treated mice. Discrete periods of electrographic seizure-like activity occurred throughout the induction period in all IHK-treated mice, regardless of whether convulsive seizure behavior was observed.

4.3.2 Prevalence and frequency of spontaneous seizures in tau$^{-/-}$ and htau mice

All mice used for electrophysiology experiments underwent an average of 56 hours of video-recorded behavioral observation for spontaneous seizures during the 8 week period between the IHK treatment and electrophysiological recordings in order to assess the effectiveness of IHK in inducing epileptogenesis. During these limited observations, convulsive seizures (Racine scale 3-5) were observed in 0% (0/7) of tau$^{-/-}$ mice, 50% (3/6) of htau mice, and 22% (2/9) of C57BL/6J mice.

Video-EEG was performed on a subset of mice from one to three weeks (total of 5 separate 24 hour recordings) after IHK or saline treatment. Spontaneous seizures were detected in mice of each genotype within 21 days of IHK treatment. Average daily seizure frequencies were calculated (C57BL/6J: 1.6±1.0, range 0.2-2.6; Tau$^{-/-}$: 0.8±0.5, range 0-2.2; htau: 0.6±0.4, range 0.2-1.0). Convulsive spontaneous seizure prevalence in mice that underwent vEEG recording was 50% (2/4) in tau$^{-/-}$ mice, and 100% (4/4 each) in htau and C57BL/6J mice. Additionally, EEG spiking was observed in all IHK treated animals of each genotype (Figure 4.1B). No EEG spiking or seizures were observed in any sham-control mice (n=6). These results confirmed that IHK treatment induced epileptogenesis in all strains of mouse used here.

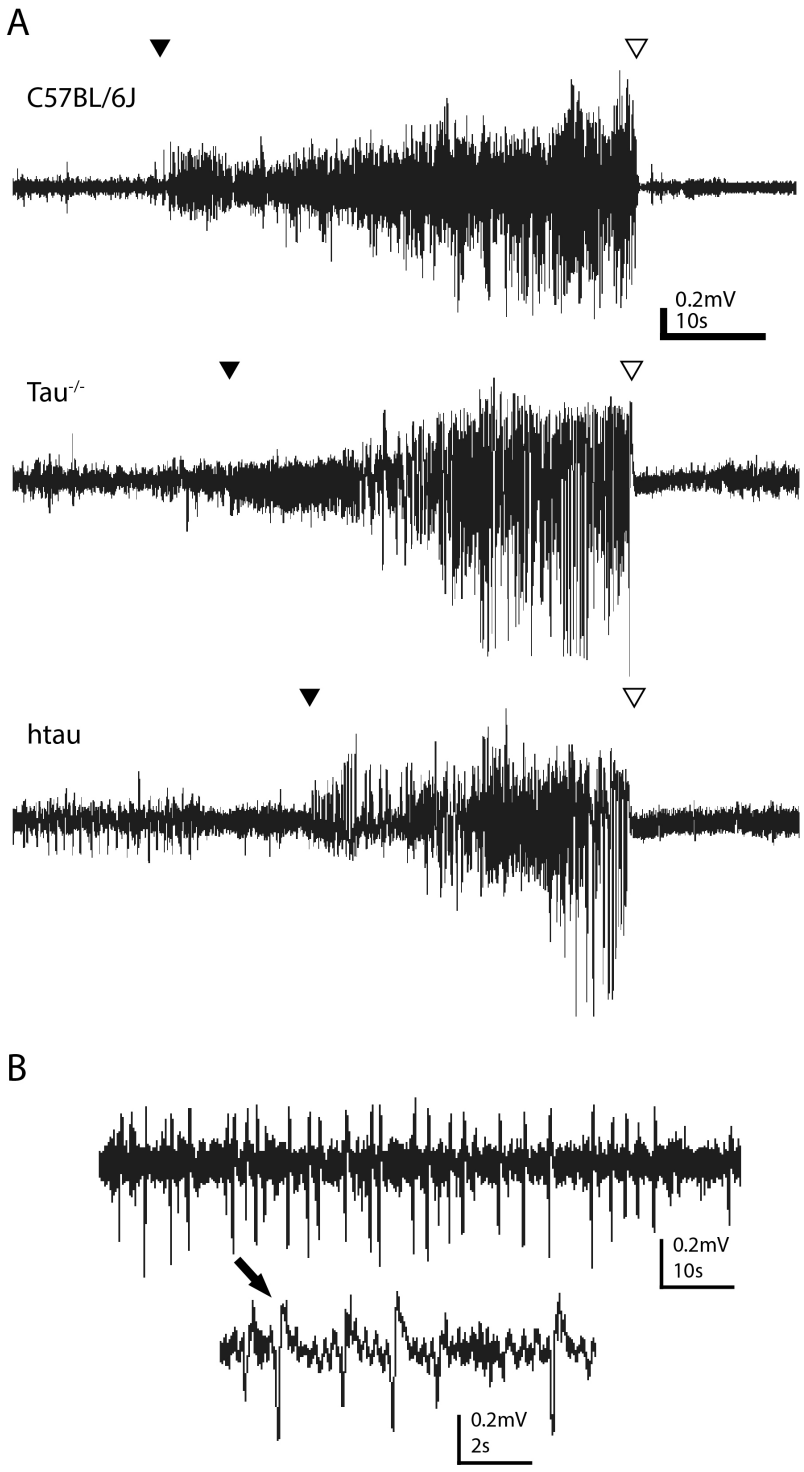


Figure 4.1 Daily seizure frequency recorded by vEEG
(A) Electrographic seizures detected in C57BL/6J, tau$^{-/-}$, and mice in the 21 days following IHK treatment. Each seizure began at the time indicated by the closed arrowhead and was followed by a period of post-ictal suppression (open arrowhead). (B) Interictal spiking was detected within the first 21 days after IHK treatment. Similar activity was detected in all IHK-treated mice regardless of genotype. Neither seizures not interictal spiking were detected in sham control mice of any genotype.

4.3.1 All-cause mortality is greater in htau mice

Reduction or deletion of tau is associated with improved survival in some genetic models of epilepsy (Gheyara et al., 2014; Holth et al., 2013), whereas pathological tau is associated with increased mortality after peripheral chemoconvulsant administration (Garcia-Cabrero et al., 2013). In the current study, overall 40 day survival was calculated for tau$^{-/-}$, htau, and non-transgenic C57BL/6J mice after IHK (Figure 4.2). Survival did not differ between tau$^{-/-}$ and C57BL/6J mice (HR=0.2, 95% CI 0.003 to 8.5; $p>0.05$). In contrast, mortality was significantly increased in the htau mice compared to C57BL/6J mice (HR=4.4, 95% CI 1.1 to 17.9; $p<0.05$). No sham-treated mice of any genotype died prematurely during the course of experiments.

4.3.2 Intrinsic membrane properties after IHK

We previously identified differences in intrinsic membrane properties in DGCs from 1.5 month old tau$^{-/-}$ and htau mice compared to age-matched non-transgenic C57BL/6J mice (Cloyd et al., 2021). To determine whether intrinsic membrane properties are altered after IHK, resting membrane potential (RMP), input resistance, and induced action potential firing were measured in DGCs from tau$^{-/-}$, htau, and C57BL/6J mice at 6-8 weeks after IHK or saline treatment. The RMP of DGCs from IHK-treated tau-/- mice was depolarized compared to that of DGCs from sham treated tau-/- mice, and this effect was observed both ipsilateral and contralateral to the injection site (Figure 4.3A; $p<0.05$ for both comparisons). No differences in RMP were observed in DGCs from IHK-treated htau or C57BL/6J mice compared to sham ($p>0.05$). In tau$^{-/-}$ mice, DGCs ipsilateral to the IHK injection also had a higher input resistance compared to DGCs from sham-treated tau$^{-/-}$ mice (Figure 4.3B; $p<0.05$), but no difference in input resistance was observed in DGCs

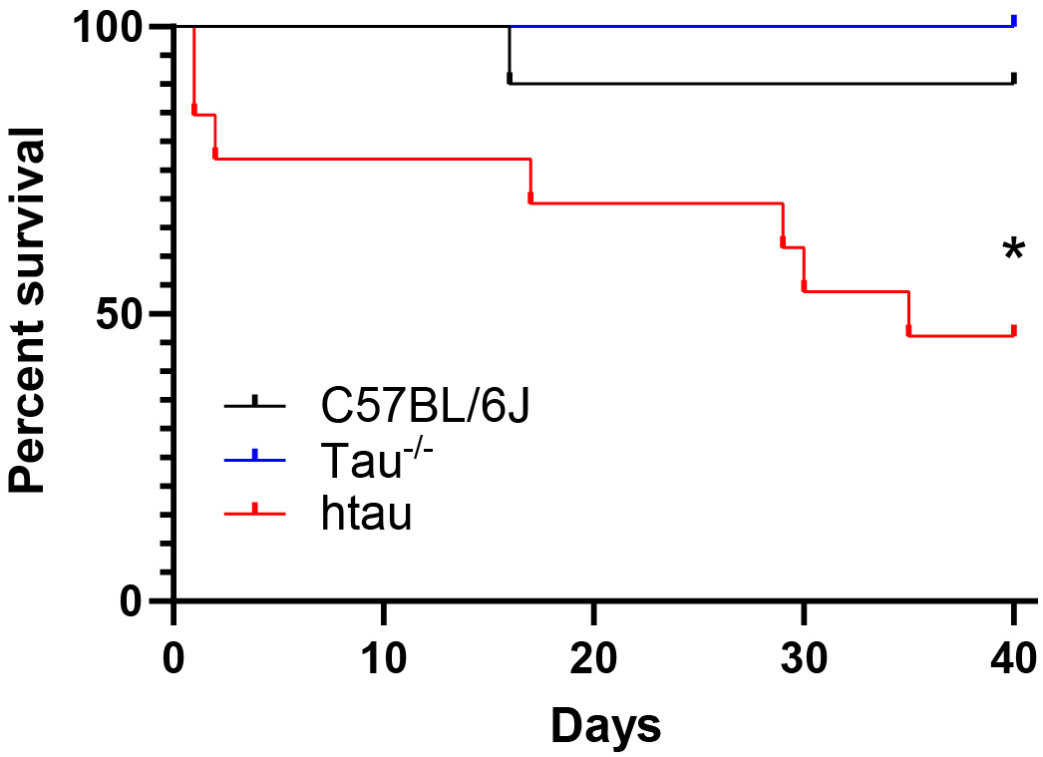


Figure 4.2 Survival after IHK in tau$^{-/-}$, htau, and non-transgenic mice
All-cause mortality within 40 days after IHK is increased in htau mice (7/13, 54%) compared to tau$^{-/-}$ (0/8, 0%) or non-transgenic mice (1/10, 10%; $p<0.05$).

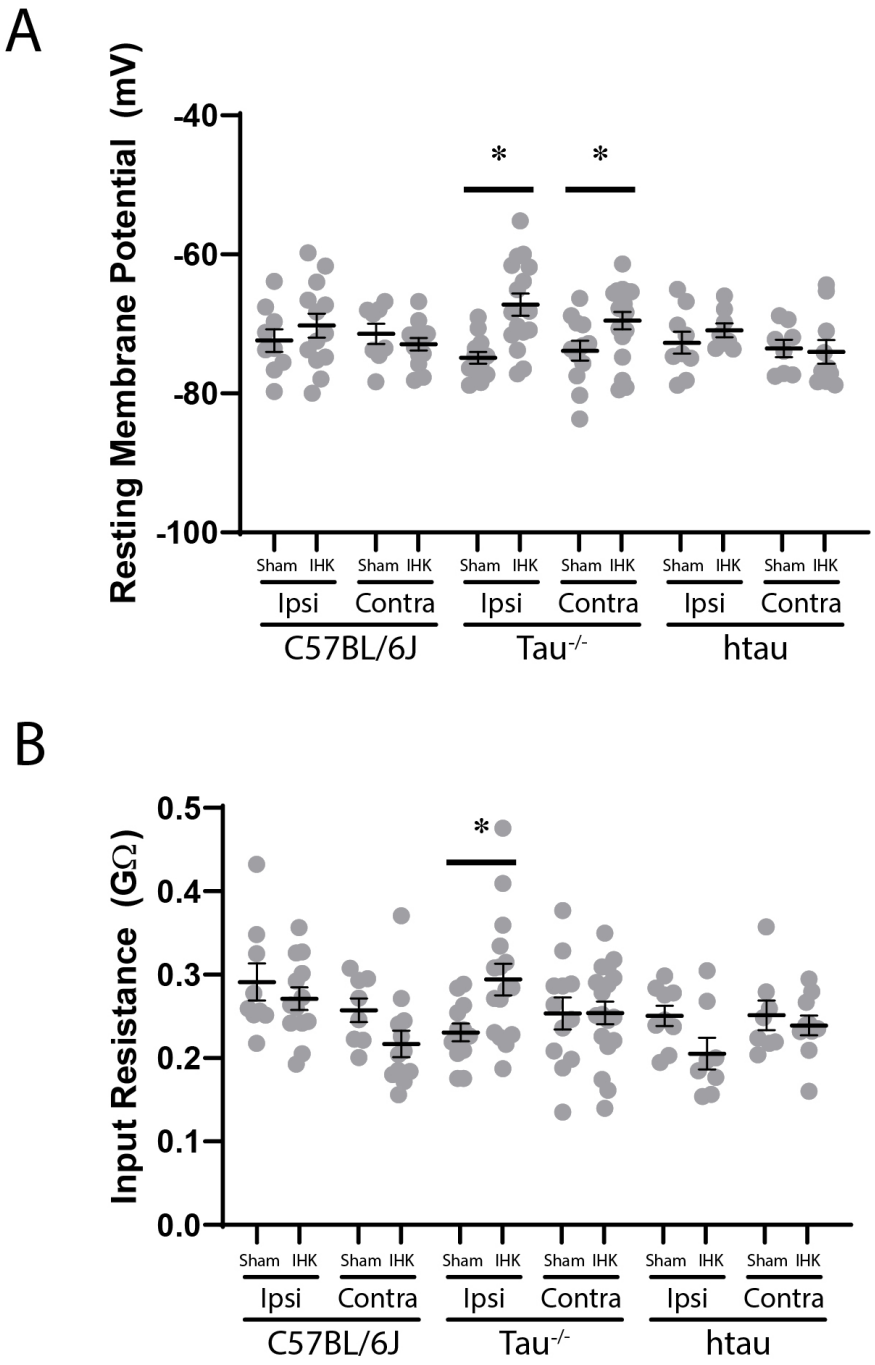


Figure 4.3 Effect of IHK on resting membrane potential and input resistance in DGCs from tau$^{-/-}$, htau, and non-transgenic mice

(A) DGCs from tau$^{-/-}$ mice developed a depolarized resting membrane potential ipsilateral and contralateral to the injection site 6-8 weeks after IHK. No changes in resting membrane potential were observed in DGCs from htau or non-transgenic mice. (B) DGCs ipsilateral to the injection site developed an increased input resistance after IHK. No changes were observed in DGCs contralateral to the injection site in tau$^{-/-}$ mice or in DGCs from htau or non-transgenic mice after IHK. *: $p<0.05$. Error bars indicate SEM.

contralateral to the injection site in tau$^{-/-}$ mice ($p>0.05$). Input resistance was unaffected by IHK in DGCs from htau or C57BL/6J mice ($p>0.05$).

To determine how tau expression impacts epilepsy-related changes in action potential firing, membrane voltage responses to current injection were measured in DGCs from tau$^{-/-}$, htau, and C57BL/6J mice (Figure 4.4). No changes in peak action potential frequency were detected ipsilateral to the injection site in C57BL/6J, tau$^{-/-}$, or htau mice (Figure 4.4B; $p<0.05$). In non-transgenic C57BL/6J mice, IHK resulted in an increased peak frequency of action potentials in DGCs contralateral to the injection site relative to sham-treated mice (Figure 4.4C; $p<0.05$). Peak action potential in DGCs contralateral to the injection site in IHK-treated C57BL/6J mice was also increased relative to IHK-treated tau$^{-/-}$ and htau mice (Figure 4.4C; $p<0.05$). Overall, the effect of IHK on intrinsic properties in DGCs was relatively minor, affecting RMP and input resistance in DGCs from tau$^{-/-}$ mice. The impact on action potential firing was most significant contralateral to the IHK site in C57BL/6J mice.

4.3.1 IHK-related changes in synaptic function are abrogated in tau$^{-/-}$ and htau mice

Tau ablation is associated with protection against epilepsy-related excitability in some genetic models of epilepsy (Garcia-Cabrero et al., 2013; Gheyara et al., 2014), and altered synaptic excitability in the dentate gyrus is associated with TLE development (Cronin et al., 1992; Patrylo and Dudek, 1998; Winokur et al., 2004; Wuarin and Dudek, 2001). To better understand how tau affects synaptic function in epileptogenesis, we measured sEPSCs 6-8 weeks after IHK or saline injection in DGCs from tau$^{-/-}$, htau, and C57BL/6J mice. In IHK-treated C57BL/6J mice, sEPSC frequency in DGCs ipsilateral and

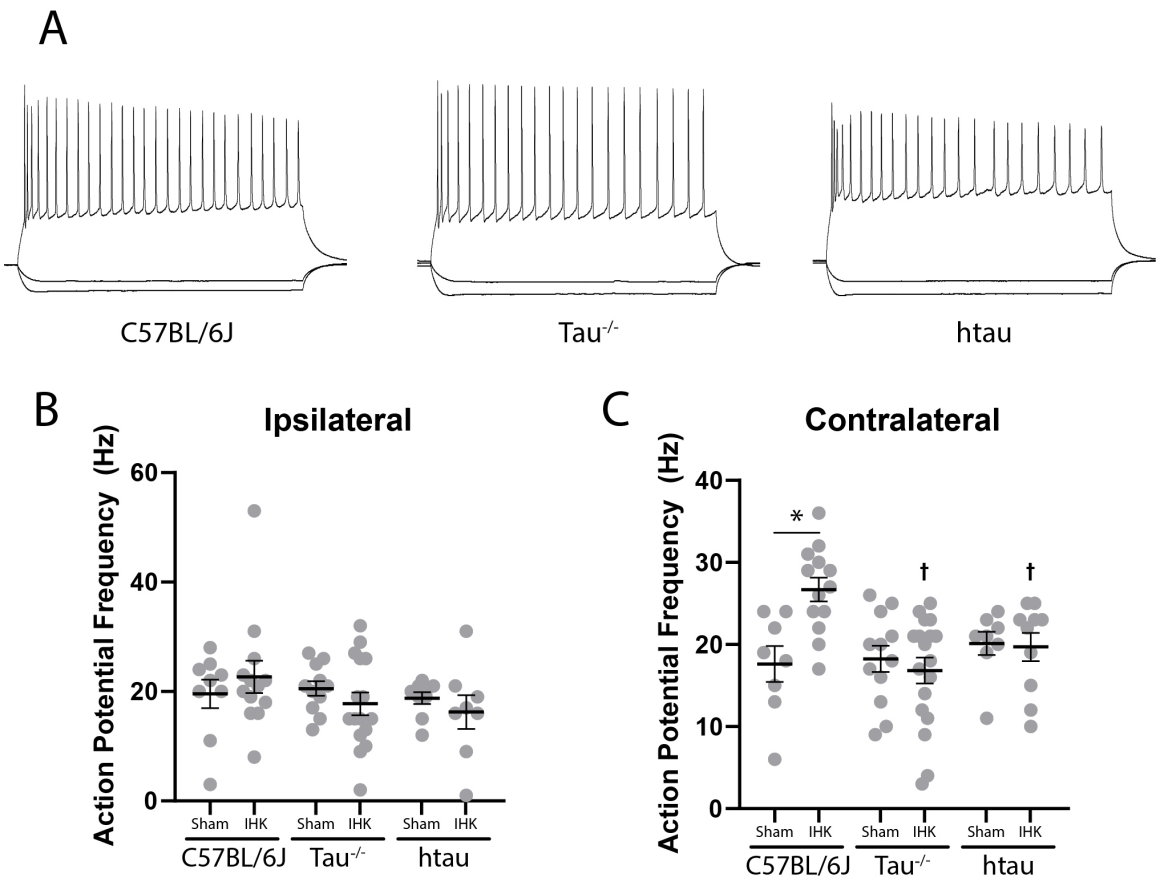


Figure 4.4 Effect of IHK on membrane voltage response in DGCs from tau$^{-/-}$, htau, and non-transgenic mice

(A) Sample membrane voltage responses in response to injection of -100, -50, and +200pA currents in DGCs contralateral to IHK injection. (B&C) Average peak frequency of action potentials in response to current injection in DGCs ipsilateral (B) and contralateral (C) to IHK injection. Action potential frequency was not affected by IHK in DGCs ipsilateral to the injection site in any genotype, but was increased in DGCs contralateral to the injection site in C57BL/6J mice. *: difference between sham and IHK ($p<0.05$). †: different from C57BL/6J IHK ($p<0.05$). Error bars indicate SEM.

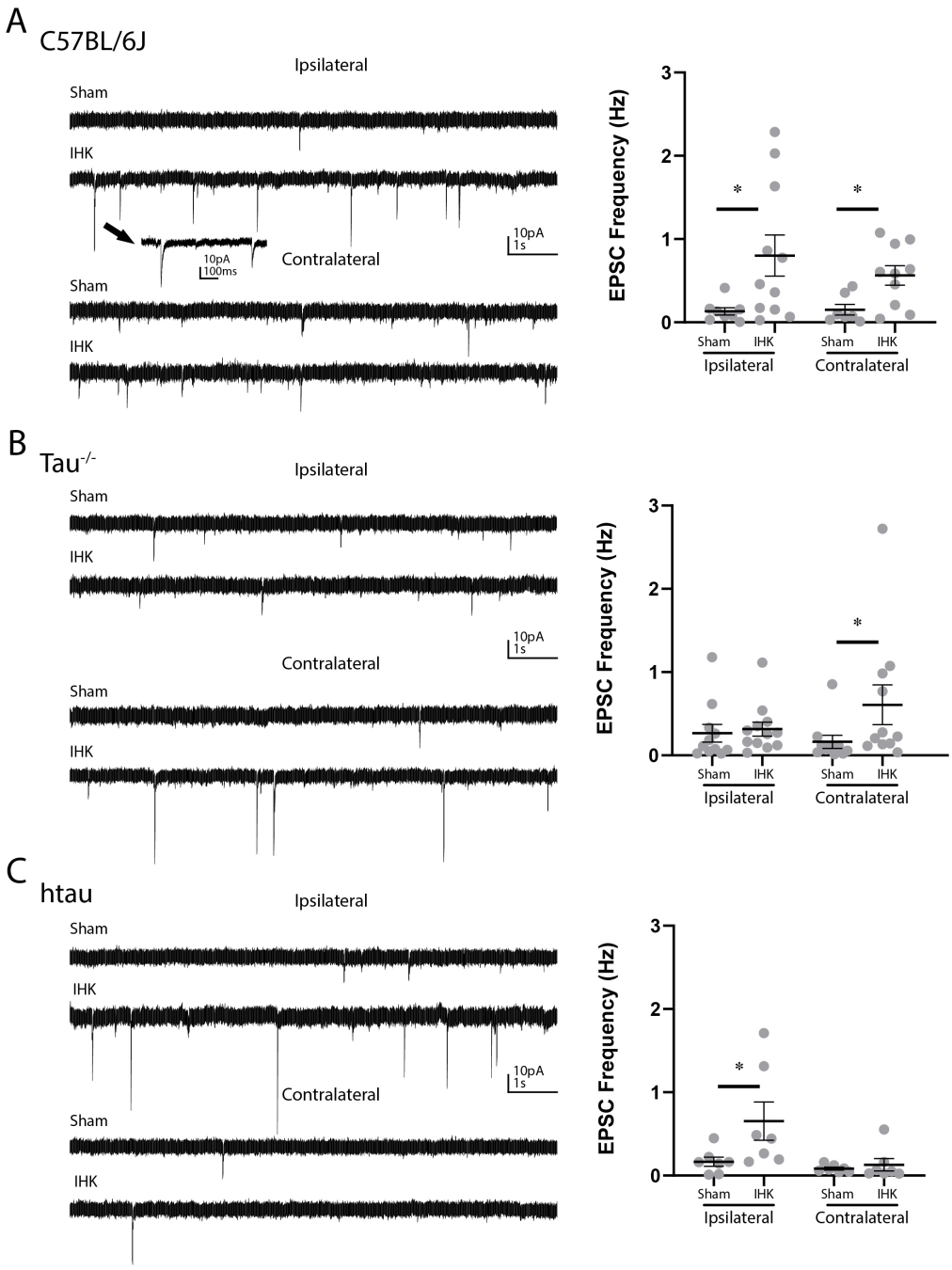


Figure 4.5 Effect of IHK on spontaneous EPSC frequency in DGCs from tau$^{-/-}$, htau, and non-transgenic mice

Sample traces and sEPSC frequencies in DGCs from non-transgenic (A), tau$^{-/-}$ (B), and htau (C) mice. (A) DGCs ipsilateral and contralateral to the injection site from non-transgenic mice receive more sEPSCs after IHK. (B) DGCs contralateral to the injection site from tau$^{-/-}$ mice receive more sEPSCs after IHK. No change in sEPSC frequency was observed ipsilateral to the injection. (C) DGCs ipsilateral to the injection site from tau$^{-/-}$ mice receive more sEPSCs after IHK. No change in sEPSC frequency was observed contralateral to the injection. *: $p<0.05$. Error bars indicate SEM.

contralateral to the injection site was significantly greater than in sham-treated mice (Figure 4.5A; $p<0.05$). In IHK-treated tau$^{-/-}$ mice, DGCs contralateral to the injection site received more sEPSCs compared to sham-treated mice (Figure 4.5B; $p<0.05$), but no difference in sEPSC frequency was observed in DGCs ipsilateral to the injection site ($p>0.05$). In IHK-treated htau mice, DGCs ipsilateral to the injection site received more sEPSCs compared to sham-treated mice (Figure 4.5C; $p<0.05$), but no difference in sEPSC frequency was observed in DGCs contralateral to the injection site ($p>0.05$).

To assess changes in presynaptic neurotransmitter release, we measured the DGC response to pairs of stimuli applied to the lateral perforant pathway and calculated the paired pulse ratio (PPr) of evoked EPSC amplitudes. In IHK-treated C57BL/6J mice, the PPr was lower in DGCs both ipsilateral and contralateral to the injection site compared to sham-treated mice (Figure 4.6A; $p<0.05$). No differences in PPr were detected in DGCs from IHK-treated tau$^{-/-}$ mice compared to sham-treated control (Figure 4.6B; $p>0.05$). In IHK-treated htau mice, the PPr was lower in DGCs ipsilateral to the injection site compared to sham-treated mice (Figure 4.6C; $p>0.05$), but no difference in PPr was detected in DGCs contralateral to the injection site ($p>0.05$). DGCs from both transgenic strains exhibited resistance to IHK-induced changes in synaptic function compared to non-transgenic, C57BL/6J mice.

4.4 Discussion

This study measured the effects of IHK on seizures, epileptogenesis, and intrinsic neuronal properties and synaptic function the DGCs from tau$^{-/-}$ and htau mice compared to non-transgenic C57BL/6J controls. A lack of tau expression was associated with a

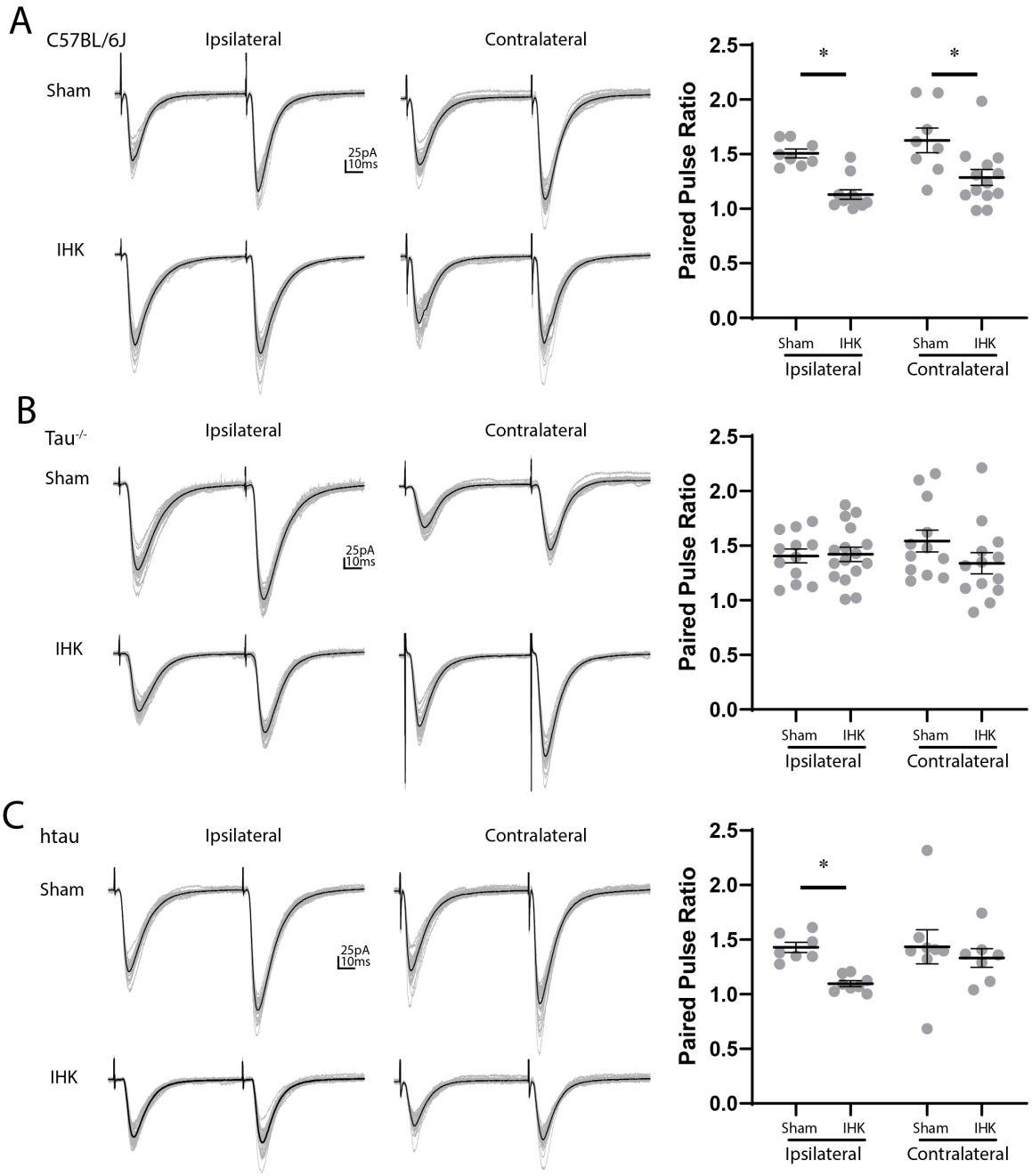


Figure 4.6 Effect of IHK on paired pulse ratio in DGCs from tau$^{-/-}$, htau, and non-transgenic mice

Sample traces and paired pulse ratio in DGCs from non-transgenic (A), tau$^{-/-}$ (B), and htau (C) mice after IHK. (A) PPr was reduced in DGCs ipsilateral and contralateral to the injection site in non-transgenic mice. (B) No change in PPr was observed in DGCs from tau$^{-/-}$ mice. (C) PPr was reduced in DGCs ipsilateral to the injection site in htau mice. No change in PPr was observed in DGCs contralateral to the injection site in htau mice. *: $p<0.05$. Error bars indicate SEM.

significant reduction in network excitability, mortality, and seizure burden in genetic models of epilepsy (Gheyara et al., 2014; Holth et al., 2013) and resistance to chemically induced seizures (DeVos et al., 2013; Li et al., 2014). Reduced tau expression also contributed to an abrogation of the increased susceptibility to induced seizures or the reduction in frequency of spontaneous seizures that is associated with some animal models of AD (Roberson et al., 2011; Roberson et al., 2007). We previously reported that presynaptic neurotransmitter release is inhibited by the loss of murine tau early in life in DGCs from tau$^{-/-}$ and htau mice (Cloyd et al., 2021). We hypothesized that the similarities we found in tau$^{-/-}$ and htau mice were attributable to the fact that neither strain expressed native, functional tau. Here we tested the hypothesis that the loss of native tau, through deletion in tau$^{-/-}$ mice or htau mice, would confer protection from seizure induction and epileptogenesis after IHK. This hypothesis was partially supported and the results presented here further our understanding of tau's role in epileptogenesis.

We found a striking difference in the immediate seizure-inducing effects of IHK on tau$^{-/-}$ mice compared to htau mice or non-transgenic, C57BL/6J mice. C57BL/6J and htau mice reliably displayed convulsive seizures immediately after IHK and most (70-75%) developed SE during the defined two hour observation period. In contrast, very few tau$^{-/-}$ mice exhibited any behavioral seizures and none met the definition for SE that results in epileptogenesis after systemic pilocarpine treatment (Shibley and Smith, 2002). However, EEG analysis revealed extensive electrographic activity in all mice after IHK, regardless of genotype, which was consistent with electrographic activity during convulsive SE reported by others (Haussler et al., 2012). Additionally, all htau and C57BL/6J mice, and two of four tau$^{-/-}$ mice, developed epilepsy with spontaneous seizures within 3 weeks of

IHK treatment. Thus, partial seizures that are not associated with generalized convulsive behavior at the time of IHK treatment may be sufficient to induce epileptogenesis within three weeks in this model. All IHK-treated mice also exhibited EEG spiking, a finding previously described in a posttraumatic epilepsy model (Statler et al., 2009). These results address an important gap in understanding of tau's effect on epileptogenesis. Although numerous studies have shown seizure resistance in various tau$^{-/-}$ models (DeVos et al., 2013; Li et al., 2014; Pallo et al., 2016; Roberson et al., 2011; Roberson et al., 2007; Tan et al., 2018), little is currently known about how this resistance affects the process of epileptogenesis. The results of the current study suggest tau deletion does not prevent development of acquired epilepsy, but may instead alter the process or rate of epileptogenesis after IHK treatment. Notably, EEG monitoring was not continuous during the observation period and seizures frequently occur in clusters (Lim et al., 2018; Williams et al., 2009). Furthermore, the frequency of spontaneous seizures detected in the individual tau$^{-/-}$ mice that developed epilepsy was similar to the seizure frequency in C57BL/6J mice. Taken together with the observation of EEG spiking in all IHK-treated animals, we cannot exclude the possibility that epileptogenesis occurred, even in cases where spontaneous behavioral seizures were not documented. Compared to mice monitored by vEEG, spontaneous seizure prevalence in mice that underwent monitoring for behavioral seizures without EEG was lower for all genotypes. It should be noted, however, that all of the behavioral observations occurred during the day. Approximately 75% of spontaneous convulsive seizures recorded by vEEG occurred at night, so these behavioral observations likely underestimated the actual prevalence of spontaneous seizures in the mice used for electrophysiology. Furthermore, electrophysiological data collected from mice that were

observed to have spontaneous convulsive seizures did not obviously differ from those that were not observed to have spontaneous convulsive seizures.

Loss of tau expression is associated with reduced mortality in genetic epilepsy and induced seizure models (Gheyara et al., 2014; Holth et al., 2013; Roberson et al., 2011; Roberson et al., 2007), whereas expression of disease-related mutant tau is associated with increased mortality after pentylenetetrazole (PTZ) induced seizures (Garcia-Cabrero et al., 2013) and may increase epilepsy-related death in some models of AD (Maeda et al., 2016). The results of the current study were largely consistent with previous findings. Overall survival in htau mice was significantly lower than in tau$^{-/-}$ and C57BL/6J mice. The human tau expressed by htau mice lacks disease related mutations common in other transgenic tau mice, but total tau expression and phosphorylation is elevated (Andorfer et al., 2003), a finding we previously confirmed (Cloyd et al., 2021). Our results indicate that disease related mutations are not required for tau to exacerbate epilepsy-related mortality. Rather, accumulation of hyperphosphorylated tau, which occurs in human patients with epilepsy (Puvenna et al., 2016; Tai et al., 2016), may be associated with epilepsy-related mortality. We did not detect a difference in survival between tau$^{-/-}$ and non-transgenic C57BL/6J mice. Previous work using IHK in C57BL/6J mice found a low rate of mortality in this model (Kang et al., 2021; Welzel et al., 2020), similar to our results. Due to the low mortality rate in both groups, it is not possible from the current data to determine whether tau deletion improves survival after IHK in tau$^{-/-}$ mice compared to C57BL/6J mice.

Temporal lobe epilepsy results in increased and recurrent excitatory signaling in the dentate gyrus (Buckmaster et al., 2002; Cronin et al., 1992; Hunt et al., 2010; Winokur et al., 2004; Wuarin and Dudek, 2001). The current study found evidence of increased

excitability in DGCs from IHK-treated C57BL/6J mice that was less or absent in DGCs from tau$^{-/-}$ and htau mice. The effects on synaptic function were most apparent. DGCs from IHK treated C57BL/6J mice received more sEPSCs and the probability of neurotransmitter release after perforant pathway stimulation was also increased compared to DGCs from sham-control C57BL/6J mice. These effects were observed in DGCs both ipsilateral and contralateral to the IHK injection site, indicating widespread changes in DGC excitability. These effects on excitatory synaptic connectivity were partially abrogated in DGCs from both tau$^{-/-}$ and htau mice.

DGCs contralateral, but not ipsilateral, to the IHK site exhibited an increased peak frequency of induced action potentials in response to current injection compared to sham-control in C57BL/6J mice (Figure 4.4). This finding suggests that commissural inputs to the dentate gyrus, or the secondary generalization of seizure activity that involves both hemispheres, may play important roles in mediating changes in intrinsic excitability over time, possibly more so than local circuitry. The DGCs recorded in this study were located in the ventral dentate gyrus, relatively distant from the IHK site in the dorsal CA1, further implicating commissural connections in the induction of altered intrinsic excitability in DGCs. The absence of changes in peak action potential firing in IHK treated tau$^{-/-}$ and htau mice suggests that generalization of seizures may be impaired in mice lacking functional tau, which may be related to the protection against IHK-induced synaptic dysfunction we detected.

Although increased susceptibility to seizure induced cellular excitability has been demonstrated in other animal models of tauopathy (Decker et al., 2016; Garcia-Cabrero et al., 2013; Liu et al., 2017b; Maeda et al., 2016), we found the opposite in DGCs from htau

mice. The htau mouse was selected for this study because, unlike other transgenic tau models, it lacks murine tau. We previously showed that DGCs from htau mice exhibit reduced excitability compared to non-transgenic mice at 1.5 months of age, the age at which mice were treated with IHK in the current study, suggesting that the pro-excitatory effects of hyperphosphorylated tau require the presence of normal murine tau (Cloyd et al., 2021). The abrogation of IHK-induced changes in DGC excitability observed in htau animals further supports this notion. Interestingly, survival after IHK was significantly poorer in htau mice, which is more consistent with previous studies of the seizure-related survival in animal models of tauopathy (Garcia-Cabrero et al., 2013) and AD (Chan et al., 2015), which develop hyperphosphorylated tau despite lacking neurofibrillary tangles (Castillo-Carranza et al., 2015). This suggests that tau impacts animal survival and cellular excitability by separate mechanisms after IHK treatment.

4.5 Conclusions

Our results are inconsistent with the hypothesis that loss of tau prevents epileptogenesis, though we did find evidence that seizure expression, and possibly the process of epileptogenesis, are altered in mice lacking native tau. $Tau^{-/-}$ mice did not develop SE as conventionally defined by the occurrence of convulsive seizures, but exhibited electrographic activity consistent with subconvulsive seizures. Spontaneous generalized seizures and EEG spiking were documented 1-3 weeks after IHK treatment in all strains, but was not present in sham-treated animals. Although eliminating native tau did not prevent epilepsy in $tau^{-/-}$ or htau mice, it abrogated several of the IHK-related changes in DGC electrophysiology identified in C57BL/6J mice. The specific mechanisms

by which tau and/or hyperphosphorylation of tau influences epileptogenesis and epilepsy related mortality warrants further study.

CHAPTER 5. DISCUSSION

5.1 Summary of findings

This dissertation focused on studying the effects of tau expression on DGC electrophysiology in normally aging and IHK-treated tau$^{-/-}$ and htau mice. The principal findings of this work are as follows: 1) DGCs from both tau$^{-/-}$ and htau mice are intrinsically less excitable compared to non-transgenic control early in life, but this effect is lost with age, 2) presynaptic neurotransmitter release from perforant pathway fibers is inhibited in both tau$^{-/-}$ and htau mice, 3) aging-related changes in neuronal function depend more upon loss of tau expression than by development of hyperphosphorylated tau, 4) tau$^{-/-}$ mice display an altered behavioral phenotype during IHK-SE compared to htau or C57BL/6J mice, but reliably experience SE as measured electrographically after IHK, 5) survival after IHK is significantly impaired in htau mice, but appears unaffected or improved in tau$^{-/-}$ mice, 6) changes in tau expression may not significantly impact the development of spontaneous seizures after IHK, and 7) modulation of tau expression reduces synaptic dysfunction and may limit spread of seizure. This dissertation is the first direct comparison of the effects of complete removal of tau and introduction of mild tau pathology in the absence of endogenous murine tau on electrophysiological function in DGCs. This dissertation is also the first work to perform IHK in htau mice and compare survival, epileptogenesis, and DGC function in epileptic tau$^{-/-}$ and htau mice.

5.2 DGC function tau$^{-/-}$ and htau mice

5.2.1 Synaptic function in DGCs from tau$^{-/-}$ and htau mice

Several studies have examined synaptic function in tau$^{-/-}$ mice (Ahmed et al., 2014; Biundo et al., 2018; Kimura et al., 2014) or in mouse models expressing pathological tau (Hoover et al., 2010; Sydow et al., 2011; Tracy et al., 2016; Yoshiyama et al., 2007). Several studies have also examined on the effect of tau deletion on hyperexcitability associated with AD pathology (Ittner et al., 2010; Roberson et al., 2011; Shipton et al., 2011). Introduction of soluble tau oligomers (exogenously or via intracellular recording solution during a whole cell recording) can induce impairments in LTP (Hill et al., 2019; Ondrejcak et al., 2018). The majority of previous work has studied the synapse between Schaffer collateral fibers and CA1 pyramidal neurons, but some work has focused on the synapses between the perforant pathway and DGCs and between the mossy fibers and CA3 pyramidal neurons.

Tau$^{-/-}$ mice and mice expressing pathological tau both exhibit deficits in synaptic plasticity, particularly impairment of LTP. Impairments in LTP may be attributable in part to derangements in glutamate signaling and post-synaptic receptor function. Tau pathology promotes glutamate release and inhibits its reuptake, promoting excitotoxicity which can be abrogated by stimulating astrocytic reuptake of glutamate (Decker et al., 2016; Hunsberger et al., 2015). Tau also contributes to the organization and function of the post-synaptic density, primarily through interactions with the Src-kinase Fyn (Ittner et al., 2010; Lopes et al., 2016; Miyamoto et al., 2017; Mondragon-Rodriguez et al., 2012). Although most evidence implicates pathological tau in NMDA receptor function, it has also been shown to inhibit AMPA receptor trafficking (Tracy et al., 2016). The impairment of LTP

associated with different tau models could therefore be due to a combination of derangements of post-synaptic organization and excessive glutamate signaling resulting in excitotoxic neuron death, which may explain why tau$^{-/-}$ and pathological tau models frequently exhibit similar deficits in synaptic plasticity.

It is not entirely clear from the studies discussed why loss of tau expression improves synaptic plasticity in models expressing Aβ pathology. One explanation is that Aβ induces synaptic dysfunction through induction of tau pathology. This explanation is consistent with observations of Aβ-associated hyperexcitability (Busche et al., 2008). Aβ-associated changes of pre- and post-synaptic function are largely consistent to those observed in pathological tau models (Roberson et al., 2011; Shipton et al., 2011). Abrogation of excitotoxicity after tau loss in these models is straightforward given pathological tau's association with excessive glutamate release. However, the improvement in LTP after tau deletion in several studies of Aβ models is counterintuitive (Ittner et al., 2010; Roberson et al., 2011; Shipton et al., 2011) given that tau deficient mice develop LTP deficits. One explanation is that Aβ is not inherently detrimental to synaptic function. Supporting this idea, low dose (picomolar) infusion of Aβ enhances LTP in CA1 pyramidal neuron (Puzzo et al., 2008). With this in mind, it is possible that in tau$^{-/-}$ animals with increased Aβ expression, there is actually an improvement in LTP. However, this hypothesis is largely untested and mostly speculative. The idea that Aβ-induced pathology is mainly tau-mediated is not novel and is supported by studies of synaptic function in tau$^{-/-}$ expressing Aβ pathology, but more work is needed to elucidate specific mechanisms involved.

In contrast to synaptic plasticity, which is affected similarly by tau deficiency or pathology, presynaptic function in the trisynaptic circuit is affected differently in tau$^{-/-}$ mice and mice which express pathological tau. Neurotransmitter release probability is generally increased in pathological tau models (Sydow et al., 2011; Yoshiyama et al., 2007), but is appears to be unaffected in tau$^{-/-}$ models (Ahmed et al., 2014; Kimura et al., 2014). Consistent with these observations, other studies have suggested tau does not have a major role in physiological presynaptic function, although the evidence is relatively sparse (McInnes et al., 2018; Zhou et al., 2017). The increased neurotransmitter release in pathological tau models is consistent with the increased glutamate release previously described, though the specific mechanism remains to be determined. Although the limited evidence of pathological tau's role in presynaptic function suggests it impairs fusion of synaptic vesicles with the plasma membrane (McInnes et al., 2018; Zhou et al., 2017), these studies were conducted in the neuromuscular junction of *Drosophila* which expressed human 0N4R tau. While vesicle fusion is largely assumed to be conserved across different synapses and species, the body of evidence summarized here suggests the same cannot be said of tau's role in vesicle fusion. The tau species expressed does not represent tau expression in human (mixture of all 6 isoforms) or mouse (3 isoforms of 4R tau). Therefore, the results from these studies may not be generalizable to tau's pathologic pre-synaptic function. Still, taken along with studies demonstrating little effect of tau deletion on presynaptic function in tau$^{-/-}$, these studies suggest tau does not play a major physiologic role in the presynaptic space.

Despite several studies examining tau's role in synaptic function, only one study was found which studied synaptic function specifically in the htau mouse. This study found

normal function at the synapse between Schaffer collateral fibers and CA1 pyramidal neurons from 4 month old htau (Polydoro et al., 2009). Synaptic function was impaired at 1 year of age, consisting of reduced presynaptic neurotransmitter release and impaired LTP in response to high frequency stimulation. LTP induced by theta-burst stimulation functioned normally, suggesting the high-frequency LTP impairment may be related to the presynaptic deficit (i.e. the Schaffer collateral fibers could not adequately release neurotransmitter in response to high frequency stimulation).

Most previous work into the effects of tau on synaptic function studied synapses onto CA1 and CA3 pyramidal neurons. In contrast, the current work studied DGCs and is the first to study DGC function in the htau mouse model. Despite studying different cell populations, the results of this study are largely consistent with previous studies. No difference in paired pulse ratio was detected in mice at 4 months of age and older. However, previous studies did not measure paired pulse ratio in younger mice. In this study, the paired pulse ratio was increased in both $tau^{-/-}$ and htau mice at 1.5 months of age compared to C57BL/6J mice, indicating reduced neurotransmitter release from perforant pathway fibers to DGCs. This reduction in presynaptic function suggests tau deletion reduces excitatory signaling, consistent with previous work (Decker et al., 2016; Hunsberger et al., 2015). Furthermore, the effect waning with age is consistent with observations of tau's role in neuronal development. $Tau^{-/-}$ neurons display impairments in development that seem to be partially compensated by other MAPs (Dawson et al., 2001; Harada et al., 1994). Tau isoform expression also varies throughout development, transitioning from 0N3R tau in the fetal brain to all six isoforms in the adult brain (Goedert and Jakes, 1990; Goedert et al., 1989a; Kosik et al., 1989). No difference in presynaptic neurotransmitter release was

detected at 9 months of age, the latest age studied. This may indicate a difference in the neurons studied, suggesting DGC function is less affected by age compared to CA1 pyramidal neurons. Alternatively, it may suggest that function declines between 9 and 12 months. Since this is the general age range where NFTs become increasingly prominent, this may suggest increased development of oligomeric tau in this timeframe (discussed more below).

A notable finding from this study was the overwhelming similarity between synaptic function measured in tau$^{-/-}$ and htau mice at all ages. Based on previous studies, significant differences in excitability were expected between the two models. Perforant pathway-DGC synaptic excitability in the tau$^{-/-}$ mice largely met expectations, but this synapse in htau mice was less excitable compared to C57BL/6J mice early in life. DGCs from tau$^{-/-}$ and htau mice displayed few differences in synaptic function at any age. A couple of important factors may explain the difference in excitability in htau mice compared to tau models. The htau mouse was chosen in part because it lacks *MAPT* mutations commonly expressed in other tau models. These mutations are valuable in better understanding tau's behavior in many pathologic contexts. However, they are relatively rare in the human population so findings in these models may not be widely generalizable. In contrast, the htau mouse expresses human tau lacking additional mutations. Mouse models that express non-mutant human tau are more resistant to tau pathology than models expressing mutant tau. For example, the first transgenic mouse model, which expressed 2N4R tau, developed somatodendritic distribution of hyperphosphorylated tau but lacked significant NFT formation, neurodegeneration, and functional impairment (Gotz et al., 1995). When the P301L mutation was introduced to the same model, significant pathology

associated with tauopathies developed (Gotz et al., 2001a). The absence of disease-related mutations in the htau mouse therefore likely reduces the severity of the tau pathology and lack of obvious synaptic deficits. Although the lack of pathogenic mutations may contribute to the reduced synaptic excitability in htau mice, this explanation does not fully account for the findings. Despite lacking disease related mutations, overall tau expression is increased in htau mice compared to C57BL/6J mice (Andorfer et al., 2003). Overexpression of endogenous murine tau alone has been shown to induce pathology including hyperphosphorylation and aggregation similar to that observed in the htau model, demonstrating that mutations or additional sources of pathology are not necessary for tau pathogenesis (Adams et al., 2009). Furthermore, htau mice develop tau aggregation (i.e. oligomers) by 2 months of age, and exhibit mature tangles by 9-12 months of age (Andorfer et al., 2003). Since soluble tau oligomers are likely the primary toxic tau species (Tian et al., 2013), and readily form from hyperphosphorylated tau without pathogenic mutations during the process of aggregation (Maeda et al., 2007; Tepper et al., 2014), the htau mice should have the same pathogenic potential as other models. Therefore, the lack of pathogenic mutations is unlikely to be the sole explanation for the current findings.

The htau mouse model has a second important characteristic that sets it apart from most tau mouse models. Whereas most transgenic models express additional forms of tau with murine tau expression intact, the htau model expresses a homozygous deletion of murine tau in addition to the human transgene so that they only express human tau. Both 3R and 4R tau are highly expressed in htau mice, though 3R tau is more abundant (Andorfer et al., 2003). In contrast, since murine tau is 4R only, intact murine tau expression greatly increases the ratio of 4R to 3R tau. Increasing relative 4R expression promotes aggregation

of tau (Schoch et al., 2016; von Bergen et al., 2000). Therefore, htau mice likely develop tau oligomers at a lower rate than transgenic models which co-express human and murine tau, especially compared to models that express pro-aggregation mutations such as P301L (Barghorn et al., 2000). Tau oligomer development has not been thoroughly studied in htau mice or directly compared between to other models, so the exact difference in oligomer burden is not known. Furthermore, because synaptic function has not been thoroughly studied in htau mice, it's not clear how much these potential changes impact function. The limited data suggest some impairment to LTP develops late in life, but even then LTP in response the theta burst was intact (Polydoro et al., 2009)

5.2.2 Intrinsic neuronal properties in DGCs from tau$^{-/-}$ and htau mice

Compared to studies of synaptic function, which typically involve extracellular field recordings, relatively few studies have examined the tau's role in intrinsic neuronal properties. Most work has been conducted in the rTg4510 mouse, in frontal cortical pyramidal neurons (Crimins et al., 2012; Crimins et al., 2011; Rocher et al., 2010) or CA1 pyramidal neurons (Hatch et al., 2017). These studies found largely different results, possibly reflecting the different neuron populations examined. Overall, frontal cortical pyramidal neurons from rTg4510 mice were more excitable than those non-transgenic control, exhibiting resting membrane potentials that were depolarized and firing action potentials at higher frequency in response to current injection (Crimins et al., 2012; Crimins et al., 2011; Rocher et al., 2010). The action potential firing appeared to be a consequence of the depolarized membrane potential, because no difference in firing was observed when the neurons were clamped at the same voltage.

In contrast to studies in frontal cortical pyramidal neurons, CA1 pyramidal neurons in rTg4510 mice are less excitable compared to those from non-transgenic control, exhibiting decreased action potential firing frequency in response to current injection (Hatch et al., 2017). In addition, action potential threshold and rheobase were increased, and action potential amplitude was decreased. Comparable responses were elicited after administering soluble tau oligomers via intracellular patch-pipet to the soma of CA1 pyramidal neurons in C57BL/6J mice (Hill et al., 2019).

No studies were found that measured the effects of tau pathology in DGCs. Furthermore, no studies could be found which reported whole-cell patch-clamp data from $tau^{-/-}$ animals. Therefore, this study presents the first examination of intrinsic DGC function in both $tau^{-/-}$ and htau mice. Overall, the effects were relatively minor in both models. Resting membrane potential was slightly depolarized in DGCs from $tau^{-/-}$ mice at 1.5 months, but this effect was not observed at later ages, and no other changes in resting membrane potential were observed. Peak action potential firing frequency was decreased in DGCs from $tau^{-/-}$ and htau mice compared to those from C57BL/6J at 1.5 months, and peak frequency increased with age in both models. Consistent with other studies of hippocampal neurons, the current study suggests tau does not have a major role in intrinsic neuronal properties. This conclusion is overall in line with tau's normal distribution and function, suggesting tau's contribution to neuronal function is focused primarily at the synapse. However, given the relatively sparse data available, it is possible that tau plays subtle roles in intrinsic function that have not be discovered.

5.2.3 Future directions

While this study has provided new insight into tau's role in neuronal excitability in tau$^{-/-}$ and htau mice, some key questions remain to be answered. Previous studies have identified a significant role for tau in the post-synaptic response, specifically in the processes of LTP. This study did not measure LTP or any other measure of synaptic plasticity. Future work should assess LTP at the major hippocampal synapses to better understand post-synaptic function in the htau mouse. Based on existing literature, it's reasonable to expect the htau mice would exhibit deficits in LTP. However, the noted differences between tau and the models previously studied, particularly the potential difference in oligomeric tau, could prevent LTP deficits in the htau mice. Assessing the rate of tau oligomerization in htau mice compared other tau mice would also help in understanding tau's role in synaptic function, especially the potential role of murine tau expression in tauopathy models. A side-by-side comparison between htau and 8c mice would clarify many questions since these models differ primarily only by the expression of murine tau. A clear understanding of the potential confounding influences of murine tau expression in models of tau pathology is crucial to interpreting existing and future studies.

5.3 Epileptogenesis in tau$^{-/-}$ and htau mice

5.3.1 Induction of SE by IHK

Tau's role in seizure development has been the focus of several studies involving tau$^{-/-}$ and tauopathy models. Tau$^{-/-}$ mice exhibit resistance (characterized by delayed onset and reduced severity) to seizures induced by intraperitoneal injection of PTZ (Li et al., 2014; Roberson et al., 2007; Tan et al., 2018) and KA (Pallo et al., 2016; Roberson et al., 2007). Comparable resistance to picrotoxin-induced seizures was achieved after ASO-

meditated inhibition of tau expression in adult mice (DeVos et al., 2013). Tau deletion also abrogates the increased susceptibility to PTZ-induced seizures associated with AD pathology (Palop et al., 2007; Roberson et al., 2011; Roberson et al., 2007). Interestingly, the seizure resistance is not limited to a single model, but has been demonstrated in several distinct tau$^{-/-}$ models (Dawson et al., 2001; Tan et al., 2018; Tucker et al., 2001), including several which had been crossed with other mouse models (Gotz et al., 2001a; Mucke et al., 2000; Sturchler-Pierrat et al., 1997), demonstrating the effect is not unique to any single model. Furthermore, reducing tau phosphorylation through activation of PP2A in rats with normal tau expression similarly confers resistance to PTZ-induced seizures (Jones et al., 2012). In contrast to tau$^{-/-}$ mice, mice expressing pathologic tau exhibit increased susceptibility to PTZ-induced seizures (Garcia-Cabrero et al., 2013).

A few studies have investigated tau's influence on kindling or SE models. One study found rTg4510 mice are more susceptible to amygdala kindling, but found no difference in kindling response between tau$^{-/-}$ and C57BL/6J mice (Liu et al., 2017b). Similarly, Tg2576 mice, an Aβ model which develops oligomeric tau pathology, showed similar susceptibility to kindling (Castillo-Carranza et al., 2015; Chan et al., 2015). Consistent with previous work, reducing tau phosphorylation by activating PP2A in rats with normal tau expression confers resistance to amygdala kindling and SE induced by repeated low dose KA (Liu et al., 2016).

In the current work, tau$^{-/-}$ and htau mice underwent IHK treatment to determine how tau influences development of SE. Although htau mice developed generalized convulsive seizures after IHK in a manner largely similar to C57BL/6J mice, tau$^{-/-}$ mice did not exhibit convulsive seizures. This seemed to suggest that tau deficiency prevented IHK-induced

SE. However, EEG monitoring of tau$^{-/-}$ mice after IHK found extensive non-convulsive seizure activity consistent with that observed in htau and C57BL/6J mice as well as other reports of IHK-induced SE (Haussler et al., 2012). This study showed that tau deficiency modifies IHK-SE, but does not prevent it outright. This finding has important ramifications for any study inducing seizures or SE in tau$^{-/-}$ animals. Considering the shift from convulsive to non-convulsive seizures, apparent absence of seizures should be confirmed via EEG before concluding that tau deficiency confers protection in these models.

5.3.2 Survival after SE

Consistent with the effect on seizure induction, tau expression is correlated with survival in seizure and epilepsy models. Expression of pathological tau is associated with poorer survival following PTZ-induced seizures (Garcia-Cabrero et al., 2013) and electrical kindling (Chan et al., 2015). In contrast, tau$^{-/-}$ animals exhibit improved survival in several models of seizure induction and epilepsy. Genetic models of epilepsy are often associated with sudden unexpected death in epilepsy (SUDEP). Tau deficient mice exhibit a dose dependent (tau$^{-/-}$ > tau$^{+/-}$ > tau $^{+/+}$) reduction in mortality associated with some genetic models of epilepsy (Gheyara et al., 2014; Holth et al., 2013).

The results of the current study are consistent with previous work. Mortality in htau mice (55% mortality overall, 35% SUDEP) was increased compared to C57BL/6J mice (14% SUDEP). Tau$^{-/-}$ mice may have exhibited reduced mortality compared to C57BL/6J (8% SUDEP), but the sample size was insufficient to draw this conclusion. Overall, the results of this study confirmed previous results demonstrating a correlation between tau expression and seizure/epilepsy related death. Impaired survival in htau mice demonstrates that the reduced synaptic excitability previously described at a similar age did not confer

protection against mortality. The increased rate of SUDEP may indicate involvement of extrahippocampal circuits, particularly the nucleus tractus solitarius (NTS) which has been implicated in SUDEP after pilocarpine-induced SE (Derera et al., 2017; Derera et al., 2019).

5.3.3 Development of spontaneous seizures after SE

Relatively few studies have examined tau's role in spontaneous seizures. Tau deletion reduces the frequency of spontaneous seizures in some genetic models of epilepsy (Gheyara et al., 2014; Holth et al., 2013). Similarly, tau deletion improves the epileptic phenotype associated with at least one AD mouse model. The hAPPJ9/Fyn mouse double transgenic model overexpresses Fyn alongside human amyloid precursor protein with two disease-related mutations, and develops spontaneous convulsive seizures (Roberson et al., 2011). Tau reduction resulted in a dose-dependent reduction in spontaneous epileptiform activity and shifted seizure phenotype to less severe, nonconvulsive seizures. Targeting tau phosphorylation by activation of PP2A in rats reduced the frequency of spontaneous seizures after amygdala kindling, fluid percussion injury, and KA-induced SE (Liu et al., 2017b).

The results of this study suggest a reduction in prevalence and frequency of spontaneous seizures, but lacked the statistical power to find a difference. C57BL/6J mice monitored by vEEG (two 24 hour recordings per week) all developed spontaneous convulsive seizures 1-3 weeks after IHK, mostly at a higher frequency than $tau^{-/-}$ or htau mice. Two out of four $tau^{-/-}$ mice were not observed to have a convulsive seizure or vEEG correlate, but seizure frequency in the other $tau^{-/-}$ mice was similar to C57BL/6J mice. Interestingly, although all htau mice were observed to have spontaneous seizures, the

frequency was lower than observed in C57BL/6J or tau$^{-/-}$ mice. All IHK-treated mice exhibited spiking on EEG regardless of whether convulsive seizures were observed. This spiking was not observed in any sham-treated mice.

The study of spontaneous seizures in the IHK-treated mice reveals two major points. First, convulsive seizure prevalence determined by 24 hour vEEG monitoring differed dramatically from prevalence determined by behavior monitoring. Whereas convulsive seizures were observed in 50-100% of vEEG monitored mice, convulsive seizure prevalence assessed by video monitoring of behavior in tau$^{-/-}$, htau, and C57BL/6J mice was 0%, 50%, and 22%, respectively. Notably, 75% of seizures observed on vEEG occurred at night while the animals were most active, whereas all behavioral monitoring occurred during the day. Therefore, studies which only monitor for seizures during the day likely underrepresent actual seizure prevalence.

Additionally, although htau mice experienced a high mortality rate after IHK, they were observed to develop fewer spontaneous seizures compared to C57BL/6J mice or tau$^{-/-}$ mice that developed spontaneous seizures. This finding suggests tau affects survival and epileptogenesis by different mechanisms. IHK treatment occurred at 1.5-2 months of age, when htau mice exhibit reduced excitability at the perforant pathway-DGC synapse. Although this reduction in excitability did not improve overall survival, it may have contributed to the apparent reduction in seizure frequency. It is not known whether other hippocampal synapses exhibit similar patterns of excitability at this age. Reduced synaptic excitability throughout the hippocampus would likely inhibit seizure propagation and could underlie the reduced frequency of convulsive seizures in htau mice. However, the frequency of spontaneous seizures in tau$^{-/-}$ complicate this picture. Tau$^{-/-}$ mice exhibited

similar synaptic activity described in htau mice. Spontaneous seizures were not observed in two of four tau$^{-/-}$ mice during 24 hour vEEG after IHK, but the frequency of seizures in the other two tau$^{-/-}$ did not differ from C57BL/6J mice. One possible conclusion is that tau deficiency inhibits epileptogenesis, but has little effect on seizures in animals that do become epileptic. This conclusion is not fully supported from the current data, however, because EEG spikes were present in all IHK treated mice. Although not conclusive, this observation raises the possibility that the mice without observed seizures had become epileptic. Since seizures often develop in clusters (Lim et al., 2018; Williams et al., 2009) and EEG monitoring was not continuous throughout the observation period, it is possible that these two mice had seizures between vEEG recordings.

5.3.4 Future directions

While the current study provides new insight on tau's role in epileptogenesis, more work is needed to clearly define this role. The data presented here suggest a reduction in epileptogenesis in tau$^{-/-}$ mice, but limitations in monitoring could be responsible for this finding. Longer term, continuous vEEG monitoring would provide a better assessment of epileptogenesis after IHK. Furthermore, IHK in the current study was performed at 1.5-2 months of age, corresponding with reduced excitability at the perforant pathway-mossy fiber synapse as previously discussed. It is not clear from the current data whether this reduced excitability influenced development of SE or epileptogenesis. However, since this synapse exhibited normal excitability later in life, performing IHK at later ages could clarify this question. A small cohort of older tau$^{-/-}$ and htau mice displayed no obvious differences after IHK compared to the current study, but full investigation is warranted

before making any conclusions. Given the increased rate of SUDEP in htau mice, NTS function should be assessed after IHK in tau$^{-/-}$ and htau mice.

5.4 Dentate granule cell excitability after IHK in tau$^{-/-}$ and htau

5.4.1 Electrophysiology in IHK treated mice

Epilepsy is associated with extensive changes in DGC function. Mossy fibers form aberrant synapses with DGCs, resulting in recurrent excitatory circuits (Butler et al., 2015; Hunt et al., 2010; Winokur et al., 2004). Animal models of epilepsy also frequently develop inhibitory neuron dysfunction (Butler et al., 2017; Hunt et al., 2011; Kang et al., 2021) and alterations to $GABA_A$ receptor function (Boychuk et al., 2016; Peng et al., 2004). Although C57BL/6J mice exhibit resistance to epileptogenesis after SE induced by systemic KA administration (McKhann et al., 2003; McLin and Steward, 2006; Schauwecker and Steward, 1997), IHK reliably induces epileptogenic changes (Kang et al., 2021; Welzel et al., 2020).

This study describes the first application of IHK in tau$^{-/-}$ and htau mice. DGC excitability was measured 6-8 weeks after IHK. In IHK-treated C57BL/6J mice, the perforant pathway-DGC synapse exhibited increased excitability compared to sham control, demonstrated by increased neurotransmitter release and more frequent sEPSCs. These changes were detected both contralateral and ipsilateral to the injection. Additionally, peak evoked action potential frequency was increased in DGCs contralateral, but not ipsilateral, to the injection. These changes were largely abrogated in tau$^{-/-}$ and htau mice. Synaptic hyperexcitability was reduced, but not entirely prevented, in both transgenic

strains. Evoked action potential frequency was not affected in DGCs from tau$^{-/-}$ and htau mice after IHK.

The difference in functional changes after IHK in tau$^{-/-}$ and htau mice compared to C57BL/6J mice suggest the altered tau expression affected the process of epileptogenesis after IHK. DGCs contralateral to the injection in C57BL/6J mice exhibited increased intrinsic excitability. However, this effect was not observed ipsilateral to the injection. Kainate was injected into the dorsal CA1, whereas the DGCs studied were in the ventral dentate gyrus. The absence of changes in intrinsic excitability in DGCs ipsilateral to the injection suggests that seizures did not readily propagate along the septotemporal axis within the hippocampus. Changes in DGCs contralateral to the injection suggests involvement of the commissural inputs or secondary generalization of seizures in driving changes during epileptogenesis. This notion is supported by the increased synaptic excitability observed in DGCs from IHK-treated C57BL/6J mice. If synaptic hyperexcitability develops generally in IHK-treated mice, then epileptiform discharges could spread more easily, promoting widespread propagation of seizures.

The assessment of excitability in DGCs from tau$^{-/-}$ and htau mice is consistent with and may explain, in part, the potentially reduced seizure prevalence/frequency previously discussed. Synaptic hyperexcitability was reduced, though not entirely abrogated, in DGCs from both transgenic strains, consistent with the reduction in seizure prevalence in tau$^{-/-}$ and seizure frequency in htau mice suggested here. If increased synaptic transmission could promote seizure propagation, then it stands to reason that preventing this hyperexcitability could inhibit seizure development. Furthermore, the incomplete prevention of

hyperexcitable changes observed is consistent with the modification, but not prevention, of epileptogenesis described previously.

5.4.2 Future directions

The results of this study suggest tau contributes to IHK-induced changes in electrophysiological function. However, this study only investigated intrinsic and synaptic function in the DGCs. Although DGCs play a critical role in epileptogenesis, several additional cells, including hilar interneurons, are also involved in epileptogenesis. Further studies are needed to determine whether changes in tau expression modifies epileptogenic changes in other neuron populations. The current study also only investigated broad electrophysiological function in DGCs. More detailed investigation is needed to determine which specific processes involved in epileptogenesis might be influenced by tau. Furthermore, changes in excitability may occur throughout the trisynaptic circuit and should be investigated.

5.5 Final conclusions

This dissertation presented new insights regarding tau's role in DGC function and IHK-induced epileptogenesis. The results presented here largely agreed with those of previous studies, but with a few important exceptions. Most notably, these results indicate that persistent expression of murine tau in most models of tauopathy may confound results by exacerbating pathology. The htau mice studied here did not display the same dysfunction described in other models. Although it is not entirely clear why these differences occurred, the potential for murine tau to interact with the various forms of human expressed in other animal tauopathy models should be considered and avoided

where feasible. The existing body of literature studying tau's role in neuronal and synaptic excitability using specific electrophysiological techniques is sparse. More studies employing whole-cell recordings are needed to better understand the specific effects of tau deletion or hyperphosphorylation on neuronal function. While the current study was intended to address this paucity of information, it only measured broad electrophysiological function and should be considered a starting point for further research. Still, the insights gained here have identified several avenues for future research. Furthermore, studies of pathological tau have largely focused on hyperphosphorylation. Recent studies have identified key roles for other pathogenic tau modifications which should be further investigated.

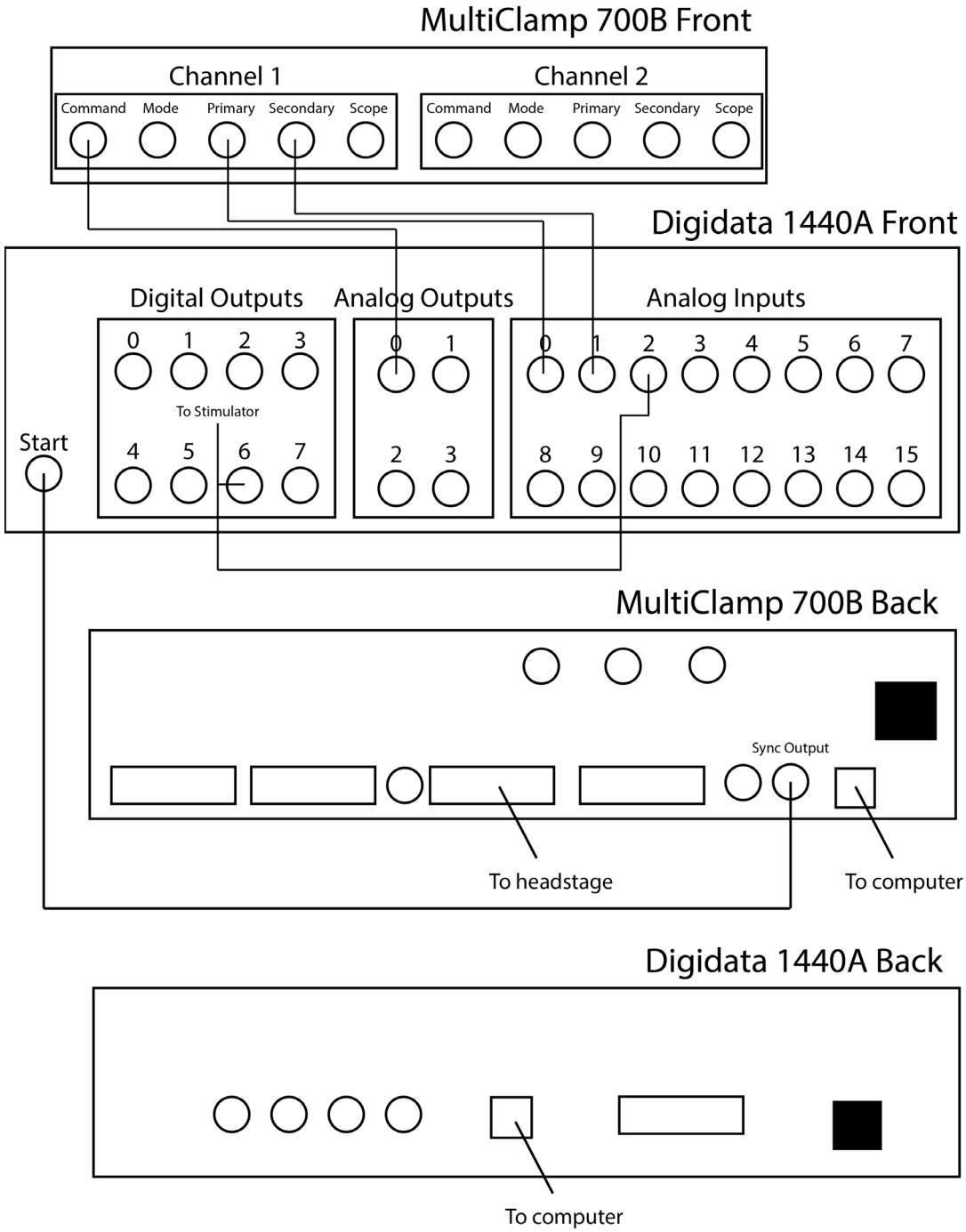
Numerous studies of tau's role in several pathologic processes suggest tau's involvement in disease may be even more significant than currently recognized. In addition to tauopathies, tau plays an increasingly recognized role in epilepsy. While the majority of the studies showing involvement of tau in epilepsy present correlative data, a growing body of evidence, including this dissertation, suggest the role may be more active. Tau deficient animal models exhibit improved survival in different models of epilepsy and induced seizures. However, improved survival in tau deficient animals is not unique to epilepsy. Tau deletion is also associated with reduced mortality associated with models of AD (Ittner et al., 2010; Roberson et al., 2011; Roberson et al., 2007), Huntington's disease (Fernandez-Nogales et al., 2014), and amyotrophic lateral sclerosis (Roberson 2011). Improved survival associated with tau reduction is often dose dependent. Viewed broadly, this body of literature suggests tau may act as a mediator of neurological dysfunction not limited to primary tauopathies. Furthermore, tau reduction does not appear to be associated

with impaired survival (Li et al., 2014), although other adverse consequences have been identified. Although reduction of tau at the genome level is unlikely to be a viable option for novel therapeutics, ASO-mediated reduction of tau expression shows promise. Selectively modifying tau in the adult nervous system should avoid the negative effects on neuronal structure and development observed in $tau^{-/-}$ mice while still having disease-modifying potential. Since tau's toxic potential seems to be related to soluble oligomers, this approach may even be capable of reversing some neurological diseases. While development of such approaches is still at an early stage, research is underway. At the time of writing this, one phase 1 trial is recruiting patients to begin testing an ASO therapy directed at progressive supranacular palsy. This is unlikely to be last such trial and may open the door to development of disease-modifying therapies for other neurological diseases.

With relevance to this dissertation, ASOs may represent an option to modify tau after the initiation of epileptogenesis. This approach is more realistic in terms of novel therapeutics than modifications at the genome level. While the limited data available suggest tau ASOs are effective at reducing tau expression *in vivo*, it remains to be determined whether this approach could interrupt or reverse epileptogenesis. Although the current study suggests such an approach may not be entirely preventative or curative, it does suggest that it may provide meaningful disease modification that goes beyond symptomatic control.

APPENDICES

APPENDIX 1 ELECTROPHYSIOLOGY RECORDING SETUP



APPENDIX 2 SOLUTIONS USED FOR ELECTROPHYSIOLOGY

A2.1 Cutting/holding solution

Compound	Molecular weight (g)	Concentration (mM)	Grams for 1L (5X)
NaCl	58.4	85	24.82
Sucrose	342.3	75	128.36
KCl	74.6	2.5	0.9325
Glucose	180.2	25	22.525
$NaH_2PO_4 \cdot H_2O$	137.99	1.25	0.862
$MgCl_2 \cdot 6H_2O$	203.3	4	4/066
$CaCl_2 \cdot 2H_2O$	147	0.5	0.3675
$NaHCO_3$	84.01	24	10.081

A2.2 Recording solution

Compound	Molecular weight (g)	Concentration (mM)	Grams for 1L
NaCl	58.4	124	24.82
$NaHCO_3$	84.01	26	10.081
NaH_2PO_4	119.98	1.4	22.525
Glucose	180.2	11	22.525

Solution	Concentration (mM)	Milliliters for 1L
1M KCl	3	3
2M $MgCl_2$	1.3	2.6
2M $CaCl_2$	1.3-2	2.6-4

A2.3 Electrode internal solution

Compound	Molecular weight (g)	Concentration (mM)	Grams for 25mL
K-gluconate	234.2	126	737.7
KCl	74.6	4	7.5
HEPES	238.3	10	59.6
MgATP	507.2	4	50.7
NaATP	523.2	0.3	3.9
PO-Creatine	255.1	10	63.8

- Adjust to pH7.2 by adding 1N KOH
- Adjust osmolality to 290-295 by adding small amounts of MilliQ water
- Aliquot to 50-100µL and store at -20°C. Sonicate at least 5 minutes before use

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Advisor: Dr. Bret N. Smith (2018-present), Dr. Jose F. Abisambra (2017-2018)

Medical School Student Tutor

2017-2019

University of Kentucky College of Medicine, Lexington, KY

Supervisor: Dr. Michelle Lineberry

Medical Student Researcher

2015-2017

Department of Physiology, University of Kentucky College of Medicine, Lexington, KY

Advisor: Dr. Jose F. Abisambra

Operations Technician

2014-2015

BioStorage Technologies, Indianapolis, IN

Supervisor: Rob Dininger

Research Intern

2014

Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Belval, Luxembourg

Supervisor: Dr. Alexander Crawford

Undergraduate Researcher

2012-2014

Chemistry Department, Wabash College, Crawfordsville, IN

Advisor: Dr. Laura M. Wysocki

Scholastic and Professional Honors

2017- Promoted to third year of medical school with distinction

2016- Promoted to second year of medical school with high distinction

2014- Wabash College John Maurice Butler Prize for Scholarship and Character

2014- Earned distinction of senior comprehensive examinations

2014- Winner of American Chemical Society "Speak Simply" poster contest

2013- Elected to Phi Beta Kappa as a junior

2011- Wabash College Paul T. Hurt for All-Around Freshman Achievement

2010- Wabash College Honor Scholarship

2010- Eli Lily Community Scholarship

Funding

2018-2020 "Training Program for Predoctoral Students in Clinical and Translational Sciences" TL1TR001997

2017-2018 "Graduate Training in Integrative Physiology" 1T32GM118292-01A1

2016-2017 "Professional Student Mentored Research Fellowship" IL1TR000117/ UL1TR001998

Professional Publications and Presentations

Publications

Cloyd RA, Koren J 3rd, Abisambra JF, Smith BN. Effects of altered tau expression on dentate granule cell excitability in mice. Exp Neurol. 2021. doi: 10.1016/j.expneurol.2021.113766

Sompol P, Gollihue JL, Kraner SD, Artiushin IA, **Cloyd RA,** Chisti EA, Koren SA, Nation GK, Abisambra JF, Huzian O, Nagy LI, Hackler L Jr, Puskas, LG, Norris, CM. Q134R: Small chemical compound with NFAT inhibitory properties improves behavioral performance and synapse function in mouse models of amyloid pathology. Aging Cell. 10.1111/acel.13416

Koren SA, Hamm MJ, **Cloyd R**, Fontaine SN, Chisti E, Lanzilotta C, Rodriguez-Rivera J, Ingram A, Bell M, Galvis-Escobar SM, Zulia N, Di Domenico F, Duong D, Seyfried NT, Powell D, Vandsberger M, Frolinger T, Hartz AMS, Koren J 3rd, Axten JM, Laping NJ, Abisambra JF. Broad kinase inhibition mitigates early neuronal dysfunction in tauopathy. Int J Mol Sci. 2021. doi: 10.3390/ijms22031186

Cloyd RA, Koren SA, Abisambra JF. Manganese-enhanced imaging: Overview and central nervous system applications with a focus on neurodegeneration. Front in Aging Neurosci. 2018; 10. doi: 10.3389/fnagi.2018.00403; PMCID: PMC6300587

Gusareva ES, Twizere JC, Sleegers K, Abisambra JF, Meier SE, **Cloyd RA**, Weiss B, Dermaut B, Dourlen P, Dupont C, Bessonov K, van der Lee S, Carrasquillo MM, Katsumata Y, Cherkaoui M, Asselbergh B, Bellenguez C, Hofman AA, Ikram AMA, the GERAD1 consortium Harold D, Williams J, Amouyel P, van Dujin CM, Ertekin-Taner N, van Broeckhoven C, Fardo DW, Lambert JC, van Steen K. Male-specific epistasis between *WWC1* and *TLN2* genes is associated with Alzheimer's Disease. Neurobiol Aging. 2018. doi: 10.1016/j.neurobiolaging.2018.08.001

Cloyd RA, Vandsburger M, Abisambra JF. A new opportunity for MEMRI. Aging-US. 2017; 9: 1855-6. doi: 10.18632/aging.101283

Fontaine SN, Ingram A, **Cloyd RA,** Meier SE, Miller E, Lyons D, Nation GK, Mechas E, Weiss B, Lanzillotta C, Di Domenico F, Schmitt F, Powell DK, Vandsburger M, Abisambra JF. Identification of changes in neuronal function as a consequence of aging and tauopathic neurodegeneration using a novel and sensitive magnetic resonance imaging approach. Neurobiol Aging. 2017; 56: 78-86. doi: 10.1016/j.neurobiolaging.2017.04.007

Kitley WR, Santa Maria PJ, **Cloyd RA,** Wysocki, LM. Synthesis of high contrast fluorescein-diethers for rapid bench-top sensing of palladium. Chem Commun. 2015; 51 (40): 8520-3. doi: 10.1039/c5cc02192h

Presentations

2019- American Epilepsy Society Annual Meeting (Poster)
2019- Association for Clinical and Translational Science Annual Meeting (Poster)

2019- University of Kentucky Center for Clinical and Translational Science Spring Conference (Talk)
2018- University of Kentucky Neurology Department Resident Research Day (Poster and talk)
2018- Wabash College Chemistry Department Seminar Series (Talk)
2018- Society for Neuroscience Annual Meeting (Poster)
2018- University of Kentucky Center for Clinical and Translational Science Spring Conference (Poster)
2017- Sanders-Brown Center on Aging Markesbery Symposium on Aging and Dementia (Poster)
2017- Alzheimer's Association International Conference (Poster)
2017-University of Kentucky Center for Clinical and Translational Sciences Spring Conference (Poster and talk)
2016- Sanders-Brown Center on Aging Markesbery Symposium on Aging and Dementia (Poster)
2016- AOA Groves Memorial Research Day (Poster)
2014- American Chemical Society National Conference, Dallas (Poster)
2013-American Chemical Society National Conference, New Orleans (Poster)
2012- Eli Lilly Research Symposium (Poster)