

DISSERTATION

THE ROLE OF MICROTUBULE ASSOCIATED TAU PROTEIN IN THE
EPILEPTOGENESIS AND ASSOCIATED INHIBITORY NEUROPLASTICITY OF TLE

Submitted by

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ABSTRACT

THE ROLE OF MICROTUBULE ASSOCIATED TAU PROTEIN IN THE EPILEPTOGENESIS AND ASSOCIATED INHIBITORY NEUROPLASTICITY OF TLE

Studies of the microtubule-associated protein, tau suggest its promise as a potential target for epilepsy disease modification, but mechanisms underlying tau's effects on seizures are not well-defined. Acquired temporal lobe epilepsy (TLE) is the most prevalent form of focal epilepsy, yet the impact of tau expression on the process of TLE development is unexplored. These studies investigated tau's role in the epileptogenesis of acquired TLE using the intrahippocampal kainate (IHK) model in mice lacking tau expression (i.e., tau^{-/-} mice).

Background information on microtubule associated tau protein and its role in cellular and synaptic physiology, organization and circuitry of the dentate gyrus of the hippocampus, TLE, rodent models of TLE, and literature review of tau protein and seizures is provided in Chapter 1. The study in Chapter 3 examined epileptiform activity during status epilepticus (SE) after IHK injection and assessed the subsequent development of spontaneous recurrent seizures (SRS) using video and video-electroencephalography (v-EEG). Results demonstrate that the lack of tau expression did not prevent evoked seizures or the development of TLE but reduced the number of convulsive seizures during SE and the severity of spontaneous seizures after TLE developed which has not been demonstrated in the context of an acquired TLE model. To investigate synaptic changes associated with TLE development in the dorsal dentate gyrus, whole-cell patch clamp electrophysiology was used to assay excitatory and inhibitory synaptic input to dentate granule cells (DGCs). Results show that DGCs in tau^{-/-} mice normally receive less inhibitory

input compared to than in wildtype controls, and after tau^{-/-} mice develop TLE, DGCs receive increased contralateral inhibitory input. This suggests that deletion of tau protein modifies seizure expression, potentially via mechanisms involving inhibitory synaptic circuits in the dentate gyrus but does not prevent epileptogenesis. To further examine how development of TLE modifies inhibitory synaptic reorganization at sites distal to the kainate lesion, in Chapter 4, assays of excitability and synaptic transmission in DGCs in the ventral hippocampus were performed using whole-cell patch clamp electrophysiology. Results demonstrate development of TLE in mice lacking tau expression is associated with widespread inhibitory synaptic reorganization in the dentate gyrus that may contribute to altered seizure expression and epileptogenesis, expanding on the results in Chapter 3. In Chapter 5, I explored the hypothesis that development of TLE in mice lacking tau expression is associated with modified inhibitory synaptic connections and plasticity between inhibitory interneurons and vDGCs. Using whole-cell patch clamp electrophysiology with direct optogenetic stimulation of interneurons in the ventral dentate gyrus. Results demonstrated that TLE development in mice lacking tau expression results in net strengthening of inhibition that is associated with increased synaptic contacts between interneurons and vDGCs. This indicated a regulatory role of tau expression in inhibitory interneuron synaptogenesis that may be responsible for modified epileptogenesis and seizure expression in the IHK model. Taken together, Chapters 3, 4, and 5 suggest a regulatory role of microtubule associated tau protein in inhibitory synaptic transmission in the local dentate gyrus circuit which may underlie its distinct influence on seizures and epileptogenesis.

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DEDICATION

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CHAPTER 1: INTRODUCTION

According to the World Health Organization, there are roughly 50 million people worldwide living with epilepsy. Temporal lobe epilepsy (TLE) is the most common focal epilepsy diagnosis in that approximately 3 million Americans are currently living with this disease. TLE is a type of epilepsy in which seizures begin in the temporal lobe. Mesial TLE is the most common form of TLE accounting for nearly 80% of all cases where the focal site of seizure generation is in the hippocampus and is characterized by hippocampal sclerosis.

Seizures in individuals with TLE are frequently resistant to antiseizure medications, making long-term management challenging and often leading to the need for surgical removal of affected temporal lobe tissue. Although the underlying causes of TLE are relatively well characterized, the cellular and synaptic processes that drive epileptogenesis—the progression toward spontaneous, recurrent seizures—remain poorly defined. Microtubule-associated tau protein has recently been identified as a potential target for treatment of seizures in rodent models of evoked seizures and genetic epilepsies. However, our understanding of tau's role in the epileptogenesis of acquired TLE is limited.

Temporal Lobe Epilepsy

As TLE is an acquired epilepsy, seizures often develop weeks, years, or even decades as a result of an initial precipitating injury like status epilepticus, head trauma, or febrile seizures. The latent period between the initial injury and development of TLE is a distinct etiology of acquired epilepsies. Seizures in patients with TLE are often refractory to drug treatment, making it

difficult to manage seizures long-term, often requiring surgical resection of the temporal lobe in patients whose seizures are untreatable by current anti-epileptic medications. While the etiology of TLE is understood, cellular and synaptic mechanisms involved in the epileptogenesis (e.g., development) of spontaneous seizure expression is not well defined. Therefore, it is imperative to identify new targets for treatment that either interfere with the epileptogenic process to prevent and/or modify epileptogenesis or are more effective at treating refractory seizures in resistant patients.

Patients with Temporal Lobe Epilepsy

Epilepsy is an umbrella term used to characterize disorders in which patients exhibit recurrent seizures. The diagnosis of epilepsy can be difficult as patients can present isolated seizures which are not sufficient for an epilepsy diagnosis. The presentation and diagnosis of epilepsy requires predisposing factors like genetic mutations or injury/insult that alter the threshold for a seizure to generate leading to increases susceptibility to recurrent seizures (J. Engel, 1995; Engel, 1996). Seizures in patients with TLE are often extremely difficult to treat, with TLE being the most common refractory epilepsy. To date, it is unclear how to definitively treat or prevent seizures in patients with epilepsy, making it necessary to identify targets involved in the cellular and synaptic mechanisms underlying epileptogenesis, in order to prevent the process from occurring.

Pathological hallmarks seen in patients with TLE are often limited to the lesion area or seizure foci and include hippocampal sclerosis, cell loss, and neurodegeneration (Acharya *et al.*, 2008). Additionally, there are cellular and synaptic mechanisms observed in patients that are thought to contribute or be a consequence of epileptogenesis and/or seizures. A common histopathological hallmark is abnormal mossy fiber sprouting of DGCs in the dentate gyrus,

which is evident in both human and rodent models (Okazaki *et al.*, 1995; Buckmaster *et al.*, 2002; Scharfman *et al.*, 2003). Sprouting of mossy fiber axons increases the recurrent excitation within the hippocampus, lowering the seizure threshold. This excitatory network increase is also compounded by diminished inhibition through selective cell loss of inhibitory interneurons (Prince, 1978; Dingledine & Gjerstad, 1980; Franck *et al.*, 1995). With the understanding of the overall network changes that more likely contribute to seizure development, there has been a significant increase in the number of anti-epileptic drugs available for patients with TLE. Even so, the epileptogenic synaptic process is not necessarily confined to the latent period, rather epileptogenesis likely continues after the presentation of chronic epilepsy (Pitkanen *et al.*, 2015), making the treatment of seizures difficult throughout a patient's life.

There are currently thirty antiseizure medications available for prescription in the United States. The proposed mechanism of action of the drugs vary from those that enhance the inactivation of sodium channels, inhibit non-inactivating persistent sodium current, block L-type calcium channel, agonists for GABA_A receptors, inhibit NMDA receptors, increase serotonin levels, binds presynaptic calcium channel, antagonist for AMPA receptors, and modulating phasic and tonic GABA_A receptors (Vossler & Gidal, 2024). Even so, seizures in patients with TLE are often resistant to drug treatment with only 29% of patients achieving long-term seizure free periods with prescriptions (Kurita *et al.*, 2016). It is common for a particular prescription to be effective for a period of time but may eventually become less effective, thereby lowering the threshold for a seizure event, leading to reevaluation of dosage and/or new prescription. Sometimes if a patient is not responding to medicine, another common treatment option for patients is surgical resection of the temporal lobe, which is highly common in patients with refractory TLE (Henning, 2022). The surgery involves either a temporal lobectomy or selective

amygdalohippocampectomy, which has been shown to lead to a remission rate of approximately 80% in TLE patients (Engel *et al.*, 2012; Kurita *et al.*, 2016).

Rodent Models of Temporal Lobe Epilepsy

There are many rodent models used to induce epileptiform activity and seizures to allow for investigation of cellular and synaptic mechanisms involved in epileptogenesis. As such, most models can be classified by the way in which seizures are generated and their purpose for study. For example, some models are generally only used to evoke acute convulsive seizures (i.e., electrical kindling or chemoconvulsant administration), while others are used to induce an initial injury (i.e., status epilepticus or traumatic brain injury) that leads to development of spontaneous seizures after a delay (i.e., development of TLE) (Wang *et al.*, 2022). Rodent models of TLE are used to recapitulate key features of TLE including: a seizure foci usually in the hippocampus, entorhinal cortex, and/or amygdala, initiation of an initial brain injury or insult, a latent period following the injury or insult, and neuropathological hallmarks observed in human patients (i.e., hippocampal sclerosis and neuronal loss) (Mathern *et al.*, 1997; Maglócsky *et al.*, 2000; Mathern *et al.*, 2002; Bartolomei *et al.*, 2005). Common models used to study TLE are those in which the initial brain injury is status epilepticus which is defined as a prolonged period of convulsive seizures that trigger epileptogenesis and the development of spontaneous seizures. Studies using rodent models of TLE have elucidated many presumed cellular and synaptic mechanisms involved in epileptogenesis including but not limited to: enhanced activation of excitatory circuits through glutamate receptor modifications, synaptic potentiation, changes in glutamate transporters, modifications of voltage-gated channels, formation of new excitatory circuits, as well as neurogenesis, protein modifications, failure of inhibitory systems, and neuron loss

(Morimoto *et al.*, 2004). In this section, I will introduce and describe key characteristics of rodent models of seizures and TLE.

The first seizure model in rats is the electrical kindling model. Electrical kindling is not widely used in current research studies due to inability to induce neuropathology associated with epilepsy in humans but has been used frequently to test anti-epileptic drugs, making it a more functional model. In the kindling model, an electrode is implanted into the brain, creating a seizure focus when electrical stimulation is repeated. The repeated electrical stimulations in limbic regions result in epileptiform discharges and evoked seizures. Unlike some chemoconvulsant models, the electrical kindling model is a gradual development over time and over stimulations. There are cases in which electrical stimulation can lead to status epilepticus and eventual development of spontaneous seizures (i.e., acquired TLE). Even so this model is not ideal for investigating TLE since neuropathology and neuroplastic changes associated with epilepsy is mild and sometimes rarely develops making it more suitable model of evoked seizures rather than epilepsy.

Chemoconvulsant models of epilepsy are more widely used in basic epilepsy research compared to electrical kindling. Of the chemical kindling models primarily used to induce evoked seizures for a period (i.e., status epilepticus, SE), the general administration is intraperitoneal injections. Chemoconvulsants commonly used are kainic acid, pilocarpine, pentylenetetrazole (PTZ), or picrotoxin (PTX) (Shibley & Smith, 2002b; D'Amour *et al.*, 2015; Kumar *et al.*, 2016; Fan *et al.*, 2019; Tsai *et al.*, 2020). The morphological changes associated with these chemoconvulsant-SE models recapitulate pathological changes commonly seen in human TLE patients.

In the pilocarpine-SE model of SE, pilocarpine is commonly administered intraperitoneally. Pilocarpine is an M1 muscarinic acetylcholine receptor agonist. Injection of pilocarpine induces status epilepticus which is characterized by the expression of convulsive seizures for several hours until seizures self-terminate or administration of a GABA agonist like diazepam or pentobarbital. The pilocarpine-SE model was developed in rats by Turski's group in 1983 (Turski *et al.*, 1983 ; Turski *et al.*, 1984). They documented a relatively quick time to the presentation of sub convulsive behaviors that lasted for over 2 hours until the development of convulsive seizures. The period of convulsive seizures self-terminated roughly 6 hours after pilocarpine injection, with approximately 60% of rats successfully developing status epilepticus. A caveat of the pilocarpine-SE model is that there is a significantly high mortality rate correlating with the duration of status epilepticus, even with modified doses of pilocarpine (Glien *et al.*, 2001). It should be noted that termination of status epilepticus prematurely with diazepam or pentobarbital, often prohibits development of spontaneous seizures after a latent period (Glien *et al.*, 2001). The pathophysiological mechanisms associated with TLE in patients are also demonstrated in the pilocarpine-SE model including hippocampal sclerosis and DGC dispersion, mossy fiber sprouting, interneuron cell loss. Even so, a caveat to this model as a model of TLE is that there is no clear epileptic foci or lesion.

Another commonly used chemoconvulsant model of TLE is the kainic acid model. The kainic acid model was developed in 1978 by Ben-Ari and Lagowska. In these studies, kainic acid was administered directly into the amygdala (i.e., intra-amygdala) (Ben-Ari & Lagowska, 1978; Levesque & Avoli, 2013). Kainic acid is an analog of L-glutamate and acts as an agonist of ionotropic AMPA/kainate receptors. Discovery and characterization of kainic acid was in 1950 when it was extracted from red algae (Murakami *et al.*, 1953). Later it was discovered that

application of kainic acid causes excitatory responses in cortical neurons in rats, identifying kainate as a potent analog of glutamate resulting in excitotoxicity, which is a key feature of epilepsy (Shinozaki & Konishi, 1970). Soon after, it was developed into a chemoconvulsant model of TLE via intracerebral injections in the hippocampus or amygdala, recapitulating initial status epilepticus and development of spontaneous seizures after a latent period (Nadler *et al.*, 1978; Cepeda *et al.*, 1982). Intracerebral injection of kainic acid induces similar seizure responses during status epilepticus as the pilocarpine-SE model. Unilateral focal injections of the intrahippocampal and intra-amygdala kainic acid model have been instrumental in studies examining seizure generation and propagation throughout the brain. After intrahippocampal kainate injection, epileptiform discharges are detected in the ipsilateral hemisphere, presumably where seizures are generated (Bragin *et al.*, 1999; Medvedev *et al.*, 2000; Akaike *et al.*, 2001; Bragin *et al.*, 2005; Jinde *et al.*, 2009; Carriero *et al.*, 2012). Using electroencephalography, it has been shown that the seizures spread to the amygdala ipsilateral to kainate injection and then to the contralateral temporal lobe regions (Tanaka *et al.*, 1982; Leite *et al.*, 1996; Riban *et al.*, 2002). Development of spontaneous seizures after intrahippocampal kainate injection results in neuropathological alternations to the hippocampal formation that are also observed in human patients. Unlike the pilocarpine-SE model, the intrahippocampal kainate model of TLE results in neuropathological changes generally localized in areas ipsilateral to the injection, with selective loss of inhibitory interneurons, hippocampal sclerosis, and mossy fiber sprouting, making it a suitable model for studying cellular and synaptic changes associated with epileptogenesis involved in acquired TLE.

Other chemoconvulsant-SE models are picrotoxin (PTX) and pentylenetetrazole (PTZ). Unlike pilocarpine or kainic acid, the PTX model induces seizures by blocking GABA_A receptor

mediated chloride conductance, reducing inhibition (Kumar *et al.*, 2016). PTZ is a commonly used experimental model to study excitability in rats and mice (Corda *et al.*, 1991). Repeated intraperitoneal injections can induce status epilepticus and development of spontaneous seizures after 4-6 weeks (Morimoto *et al.*, 2004). Cellular pathology associated with the PTZ model includes neuron loss and permanent alterations to inhibition (Corda *et al.*, 1991).

As mentioned previously, acquired epilepsy can develop in humans after a traumatic brain injury (TBI). Different from the other models seizures and acquired epilepsy, animal models of post-traumatic epilepsy use physical trauma to the brain as the precipitating injury rather than SE. Currently used models include fluid percussion injury (FPI), controlled cortical impact injury (CCI), and the impact-acceleration model (Keith & Huang, 2020). While these models do not induce the same precipitating injury, the pathological characteristics in the hippocampus have been shown to be similar to those exhibited in focal SE models like the IHK model (Hunt *et al.*, 2009; 2010a; 2011; Hunt *et al.*, 2012; Butler *et al.*, 2015; 2016; 2017; Kang *et al.*, 2022a).

The Hippocampal Circuit

The hippocampus is comprised of four major subregions: the dentate gyrus, CA3, CA2, and the CA1. Communication and information are passed through these four regions via the glutamatergic circuit known as the trisynaptic circuit. The main source of input to the trisynaptic circuit is from the entorhinal cortex (EC) through the perforant pathway that projects to all subregions of the hippocampal formation. Stellate cells residing in layer II of the EC project to dentate gyrus and CA3, while layer III projects to CA1 (Wittner, 2007). Following the traditional

version of the tri-synaptic circuit, output of DGCs via mossy fiber axons is primarily directed onto mossy cells and inhibitory interneurons within the hilus, as well as directly onto pyramidal cells in the CA3, giving rise to the mossy fiber pathway (Claiborne *et al.*, 1986; Witter, 1989; Seress, 2007). It should be noted that the inhibitory interneurons within the dentate gyrus provide significant local feedback inhibition to DGCs, controlling their output to the CA3 (Johnston & Amaral, 2004). Between the CA3 and CA1 is the less understood CA2 that receive input from both the entorhinal cortex and CA3 pyramidal cells via Schaffer collaterals. From the CA2, Schaffer collaterals then synapse onto CA1 pyramidal cells. Output from the trisynaptic circuits is largely done by projections of CA1 pyramidal neurons. These neurons are able to send projections to many brain regions like the subiculum, perirhinal cortex, prefrontal cortex, and the amygdala but the major output connecting the circuit together is back to the entorhinal cortex (Witter, 1989).

The Dentate Gyrus of the Hippocampus

The dentate gyrus is located within the hippocampus of the temporal lobe of the brain. It is a highly organized brain region consisting of ordered layers where a variety of cell types reside. The dentate gyrus is a subregion within the trisynaptic circuit of the hippocampal formation that controls learning, memory, and other functions. The dentate gyrus has been studied extensively due to its involvement in many different diseases and disorders such as epilepsy, Alzheimer's disease, and neurodevelopmental disorders like autism spectrum disorder. The dentate gyrus is known to be involved in seizure activity in TLE, due to its location in the temporal lobe and involvement within the trisynaptic circuit.

The organization and cell types of the dentate gyrus

Organization

The dentate gyrus is comprised of three main layers: molecular layer, granule cell layer (GCL), and polymorphic cell layer (i.e., hilus). The molecular layer is largely made up of the dendrites of dentate granule cells (DGCs), fibers of the perforant path originating from the entorhinal cortex, and a small number of inhibitory interneurons. The GCL is predominately made up of densely packed DGCs creating a layer. While DGCs make up the majority of the GCL, other cell types like basket cells are in the boundary between the hilus and the GCL. Lastly, the hilus or polymorphic layer is enclosed within the GCL (Amaral *et al.*, 2007). The hilus is made up of a diverse population of cell types such as mossy cells and inhibitory interneurons.

Dentate granule cells

The principal cell type of the dentate gyrus is the dentate granule cell (DGC). The cell body or soma of DGCs is roughly 10 μm in diameter. DGCs are tightly packed together forming what is known as the granule cell layer (GCL) (Seress, 2007). The GCL is organized into three main organizational areas. The dorsal layer is known as the suprapyramidal blade, and the ventral layer is known as the infrapyramidal blade with the crest or apex connecting the two (Witter, 1989). DGCs exhibit a characteristic tree of apical dendrites that extend into the molecular layer where distal dendrites terminate at the hippocampal fissure separating the GCL from the CA1. Axons of DGCs are denoted as “mossy fibers”. The mossy fiber axons of DGCs synapse onto the proximal dendrites of excitatory mossy cells and pyramidal cells within the CA3, with a majority synapses onto inhibitory interneurons in the hilus (Ribak *et al.*, 1985;

Acsady *et al.*, 1998; Amaral *et al.*, 2007). Mossy fiber axons of DGCs rarely innervate near the molecular layer or granule cell layer, otherwise never synapsing onto other DGCs, unless in pathological conditions like temporal lobe epilepsy, where sprouting of mossy fibers into the molecular layer is a pathological hallmark of the disease.

Inhibitory interneurons

As with most regions in the brain there is a mixed population of excitatory and inhibitory cell types. While DGCs are a main excitatory cell type in the dentate gyrus, the other cell type are inhibitory interneurons. Within the dentate gyrus, there is a large, diverse population of GABAergic expressing inhibitory interneurons. An early study identified nearly twenty-one different inhibitory interneuron cell types in the hilar region alone. Even so, interneuron cell types can be differentiated by their morphology, targets (i.e., perisomatic or dendritic), neuropeptide or calcium-binding expression, and physiology (Amaral, 1978; Parra *et al.*, 1998; Maccaferri & Lacaille, 2003; Somogyi & Klausberger, 2004; Jinno & Kosaka, 2006). Further classification of interneuron sub-type is also by their specific location within the three regions (i.e., molecular layer, subgranular layer, and hilus) (Houser, 2007).

One of the most intensively studied sub-type of inhibitory interneuron within the dentate gyrus are parvalbumin (PV) expressing interneurons. Unlike other interneuron subtypes that reside deep in the hilus, basket cells are primarily located along the border between the GCL and the hilus (i.e., sub granular zone (SGZ)), although they have also been identified in smaller numbers in the molecular layer but its uncommon (Ribak *et al.*, 1990). PV-expressing interneurons are often characterized as basket or axo-axonic cells, depending on where they synapse onto target cells. PV-positive basket cells can be morphologically distinguished from DGCs by their substantially larger soma and apical dendrites. First described by Ramón y Cajal,

with aspiny apical dendrite located in the molecular layer that divides into many aspiny branches with additional basal dendrites that further divide and extend into the hilus. As their name describes, the “basket” portion of their name refers to the axon of these cells that forms encompassing synapses with the somas of DGCs. Interestingly, it has been shown that axon plexuses from a single basket cell can extend for distances greater than 900 μm along the transverse axis and roughly 1.5 mm along the septotemporal axis. This would suggest that the axons of a single basket cell could synapse onto as many as 10,000 DGCs. The second type of PV-positive interneurons are those classified as axo-axonic and form synapses exclusively on the axon initial segments of DGCs. Each of their axon branches can make multiple synapses along the axon initial segment of a single DGC, resembling a chandelier. The proximal location of PV-expressing interneurons to the granule cell layer allows them to provide strong inhibition onto DGCs and control the downstream output of DGCs. Even so, the number of PV interneurons in the dentate gyrus is surprisingly low, representing only 20-25% of the population. Identification of PV interneurons can also be done by measuring their electrical properties being that they are typically fast-spiking.

Somatostatin (SST)- expressing interneurons are predominately located in the hilus, making up 55% of all interneurons in this region (Amaral *et al.*, 2007; Tallent, 2007). Due to the location of their somas and dendrites in the hilus, the main excitatory input comes from DGC mossy fibers and mossy cells in the hilus. Unlike, PV-expressing interneurons, axon terminals of SST interneurons exclusively innervate the dendrites of DGCs in the molecular layer, allowing them to modulate the excitatory perforant path input (i.e., hilar-perforant path (HIP)) (Houser, 2007; Tallent, 2007; Savanthrapadian *et al.*, 2014). Being that they are located in the hilus receiving substantial innervation from DGCs, mossy cells, and the perforant path, SST

interneurons are highly susceptible to excitotoxicity, with a highly documented vulnerability in rodent models of TLE (Johansen *et al.*, 1987; Sloviter, 1987; Buckmaster & Dudek, 1997a)

Mossy cells

The other most prominent excitatory/glutamatergic cell type in the dentate gyrus are mossy cells which are located only within the hilus (Amaral, 1978; Soriano & Frotscher, 1994; Amaral *et al.*, 2007; Scharfman & Myers, 2013). The cell bodies of mossy cells are quite large being roughly 25-35 μm and are multipolar in shape. Their dendrites extend long distances throughout the hilus but rarely within the granule cell layer (Ribak *et al.*, 1985). A particular distinctive feature of mossy cells is that they are covered by large and complex dendritic spines resembling thorns where they receive synaptic input from mossy fiber axons of DGCs (Amaral *et al.*, 2007; Scharfman, 2016). Axons of mossy cells synapse on both DGCs and hilar inhibitory interneurons, making mossy cells integral to the local dentate gyrus circuit, regulating feedback or feedforward inhibition to DGCs. Studies have identified projections of mossy cells to the distal contralateral dentate gyrus, and traverse projections across the septotemporal axis (Ribak *et al.*, 1985; Buckmaster & Schwartzkroin, 1994; Milstein & Soltesz, 2017; Scharman, 2018)

Mossy cells have been shown to be particularly vulnerable to insults and injury. Specifically, significant mossy cell loss is observed in TLE (Margerison & Corsellis, 1966). Rodent models of TLE have demonstrated that mossy cell loss can occur after status epilepticus which is the brain insult that triggers spontaneous seizures and TLE development (Buckmaster & Jongen-Relo, 1999; Jiao & Nadler, 2007; Volz *et al.*, 2011; Scharfman & Myers, 2013).

Dorsal and Ventral Hippocampal Function

Even though the hippocampus is a unitary brain structure, evidence suggests that there are different roles for the dorsal (septal axis) and the ventral (temporal axis) regions (Moser & Moser, 1998; Fanselow & Dong, 2009). Studies have demonstrated that intrinsic circuitry of the tri-synaptic circuit is conserved between the two regions but each have distinct input and output connections with cortical areas (Swanson & Cowan, 1977; Strange *et al.*, 2014). Further, the dorsal hippocampus is responsible for cognitive functions (i.e., spatial memory) while the ventral hippocampus regulated stress and emotional behavior (Henke, 1990; Moser *et al.*, 1995). In the context of TLE, seizures in acquired TLE models and patients have been described to develop in the ventral hippocampus, as opposed to the dorsal hippocampus (J. Engel *et al.*, 1990; Buckmaster *et al.*, 2022). Further, it is thought that the ventral hippocampus is more epileptogenic compared to the dorsal hippocampus (Gilbert *et al.*, 1985; Imamura *et al.*, 2001), likely due to differences in the glutamatergic inputs via the entorhinal cortex. Few studies have specifically examined widespread synaptic mechanisms in acquired TLE model with a focal injury site (Hunt *et al.*, 2010a; Kang *et al.*, 2021b; Lee *et al.*, 2024a). Even so, one study found that focal injuries in one model of epilepsy in either the dorsal or ventral hippocampus, result in similar seizure expression and severity (Zeidler *et al.*, 2018).

Microtubule-associated tau protein

Microtubule associated tau protein is highly expressed in the brain and is most known for its pathological state being a hallmark neuropathology associated with neurodegenerative diseases like Alzheimer's disease or frontotemporal dementia. Like its name, tau's main function

is in its binding and stabilization of microtubules, but roles in synaptic plasticity and transmission have also been identified.

Structure

Microtubule-associated tau protein as its name implies is a member of the microtubule associated protein family. All microtubule associated proteins exhibit structures made up of four major domains which consist of a N-terminal domain (NTD), proline-rich region (PRR), microtubule-binding region (MTBR), and C-terminal domain (CTD) (Wang & Mandelkow, 2015).

The NTD is at the beginning of the tau protein covering the first six exons. Exon 2 translated the N1 segment while exon 3 translates the N2 segment. The function of the NTD is that it interacts with various proteins and is thought to regulate the distribution of tau and cellular signaling (Andreadis *et al.*, 1992; Kolarova *et al.*, 2012; Gauthier-Kemper *et al.*, 2018; Brandt *et al.*, 2020; Fischer & Baas, 2020). Once tau is bound to microtubules, the NTD regulates axonal transport by altering the spacing between the microtubules (Kolarova *et al.*, 2012; Fischer & Baas, 2020). Tau protein is asymmetrically charged and intrinsically disordered with the NTD being primarily negatively charged (Corsi *et al.*, 2022). Differences in the protein construct between human and mouse tau is primarily in the NTD with the human tau sequence containing 11 amino acids, making it longer than mouse tau (Hernandez *et al.*, 2020). It is thought the additional length of the NTD in human tau likely modifies the “paperclip” confirmation that tau adopts with the NTD and CTD folding onto the MTBR (Carmel *et al.*, 1996). Further, the extended length of NTD could alter the interaction with CTD, making it more likely for human tau to adopt the pathological configuration compared to murine tau (Heraud *et al.*, 2014).

The PPR is encoded by exon 7 and part of exon 9. PPR is the main region of the tau protein that interacts with protein kinases and protein phosphatases, making it involved in regulating the phosphorylation of tau microtubule assembly and the stability of the MTBR (Sibille *et al.*, 2012; Brandt *et al.*, 2020). Additionally, the PPR interacts with proteins controlling conformational folding and those regulating cellular signaling (Goode *et al.*, 1997; He *et al.*, 2009; Brandt *et al.*, 2020). Unlike NTD, the PPR is positively charged with the highest concentration of serine and threonine residues, making phosphorylation sites higher than other regions or domains of tau. The state of phosphorylation is extremely important in determining conformational changes of the protein controlled by interactions between the PPR and the MTBD (Xia *et al.*, 2020; Zhang *et al.*, 2022).

The 3rd region, the MTBR, is encoded by exons 9-12. Alternative splicing of exon 10 determines the length of the region and the final protein, whether the second repeat (i.e., R2 segment) is included or not. If R2 is included, the MTBR is at its longest and has been shown to be the isoform with the highest affinity for microtubule binding (Kolarova *et al.*, 2012; Goedert, 2018; Wang *et al.*, 2022). The positively charged nature of the MTBR interacts with high affinity to the negatively charged CTD, allowed it to dynamically bind to microtubules (Janning *et al.*, 2014; Brandt *et al.*, 2020; Costa *et al.*, 2022). Additionally, the MTBR region is involved in the self-aggregation of tau into paired-helical filaments and tau tangles, which is the pathological state of the tau protein (Kolarova *et al.*, 2012; Drummond & Wisniewski, 2016; Wegmann *et al.*, 2021).

Lastly, CTD is encoded by exon 13. The functional role of CTD is its contribution to microtubule binding. As mentioned, prior, this region interacts with the NTD forming the “paperclip” conformation, where all the tau domains fold in on each other (Jeganathan *et al.*,

2006). This confirmation has been shown to prohibit self-aggregation and tangle formation and is crucial for the physiological functions of tau. Post-translational modifications like phosphorylation can disrupt the “paperclip” confirmation, thereby increasing the propensity for tau aggregation and formation of pathological species (Wang & Mandelkow, 2015; Ruiz-Gabarre *et al.*, 2022).

Alternative splicing and isoform diversity

In addition to the unique structure of tau protein, alternative splicing of the microtubule associated tau protein gene introduces a diverse set of isoforms that play important roles in tau’s structure and functional behavior. Human tau protein is encoded by the microtubule-associated tau protein gene on chromosome 171 and spans 134 kb while murine tau is on chromosome 11 and is 100 kb in length (Pookaj *et al.*, 2001; Avila *et al.*, 2004; McMillan *et al.*, 2008; Wang *et al.*, 2022). Even so, there are no obvious structural differences in the tau gene between humans and mice. Alternative splicing of the tau gene gives rise to six different isoforms in the central nervous system (Goedert *et al.*, 1989; Andreadis, 2006) Some of the exons are constitutively expressed in all tau isoforms (i.e., exons 1, 4, 5, 7, 9, 11, 12 and 13), while some exons are minimally expressed in the central nervous system (i.e., exons 4a, 6, and 8). Of the constitutive exons, alternative splicing between exons 1-4 determines the inclusion of the N1 or N2 regions and if the N-terminal domain is 0N (1-4), 1N (1-2-4), 2N (1-2-3-4). Alternative splicing of exon 10 and inclusion of the R2 region determines the expression of the 3R or 4R isoforms of the microtubule-binding region. The resulting alternative splicing of the human tau gene results in the different isoforms: 2N4R, 1N4R, 0N4R, 2N3R, 1N3R, and 0N3R. The isoforms of tau are differentially expressed across cellular compartments and cell types. Specifically, the 3R isoform of tau is more highly expressed during early stages of development compared to the 4R isoform

which is preferentially expressed in the adult brain (Avila *et al.*, 2004; Wang & Mandelkow, 2015). After development, both the 3R and 4R isoforms are similarly expressed in humans but the ratio of expression between the two are suggested to contribute to tau aggregation, misfolding, and resulting in diseases known as tauopathies (Goedert, 2018; Hefti *et al.*, 2018). In the adult mouse, the expression of the tau isoforms are different from that of the adult human brain. Specifically, the 3R isoform is not present in adult mouse brains, where there is a definitive shift to 4R isoform expression (Goedert *et al.*, 1989; Spillantini & Goedert, 1998). Specifically, in mice, during adult neurogenesis within neurogenic niches (i.e., dentate gyrus, subventricular zone) neural progenitor cells expressing doublecortin exclusively express the 3R isoform with the tau protein is highly phosphorylated (Bullmann *et al.*, 2006; Hernandez *et al.*, 2020). Through the process of neurogenesis, neural progenitor cells develop into mature dentate granule cells (DGCs) that exclusively express the 4R isoform, with tau being less phosphorylated (Hernandez *et al.*, 2020).

Physiological Function

Microtubule associated tau protein was first identified by its main functional role as a microtubule associated protein. Tau protein is differentially expressed in the brain depending on cell type, regulating neuronal morphogenesis, differentiation, axon transport, and signaling between neurons (Arendt *et al.*, 2016; Liang *et al.*, 2022). Additional identified functions of tau include neurogenesis, synaptic plasticity, genome stability, and protein synthesis (Colnaghi *et al.*, 2020; Didonna, 2020; Koren *et al.*, 2020; Liang *et al.*, 2022).

The normal function(s) of tau are tightly regulated through post-translational modifications of its distinct isoforms. These post-translation modifications include phosphorylation, acetylation, methylation, ubiquitination, and others. Of the post-translation

modifications listed, there is large body of work on phosphorylation and acetylation of tau, especially in their implications in disease. Phosphorylation is the most studied post-translational modification of tau protein. Phosphorylation of tau can occur at more than 80 residues but primarily in the proline-rich region and the microtubule binding region. Specific regions of phosphorylation along the tau protein like Thr231 and Ser262 primarily alter tau's affinity for microtubule binding. The most-well known kinases responsible for the phosphorylation of tau are glycogen synthase kinase-3 beta (GSK3 β), cyclin-dependent kinase 5 (CDK5), protein kinase A (PKA), mitogen-activated protein kinase (MAPK), calcium/calmodulin-dependent protein kinase II, and casein kinase 1 (CK1) (Martin *et al.*, 2013). GSK3 β kinase is a predominant serine/threonine protein kinase that has been shown to be directly involved in tauopathies like Alzheimer's and Pick's diseases (Ferrer *et al.*, 2002). The regulation of tau phosphorylation in its physiological state is controlled through phosphatases like protein phosphatase 2A (PP2A), which is the best described tau phosphatase (Wang & Mandelkow, 2015). The phosphorylation state of tau is highly regulated during development. For example, during development tau is more phosphorylated as compared to postnatally. It is thought that phosphorylation of tau is significantly important for its functions, where phosphorylation particularly reduces its affinity for microtubule binding (Avila *et al.*, 2004). When tau is abnormally phosphorylated with excessive phosphorylation sites, tau is then in a state of hyperphosphorylation. Abnormal tau hyperphosphorylation is specific to tauopathies as it increases the propensity of tau to self-aggregate into structures like paired-helical filaments and neurofibrillary tangles (Avila, 2009). Additionally, hyperphosphorylated can drive the mis-localization of tau to dendrites where synaptic function of neurons is impaired.

A large body of work on the physiological function of tau in the central nervous system have demonstrated that tau proteins primary function is to regulate axonal microtubule assembly and stabilization in the presynaptic compartment (Andreadis, 2006; Wang & Mandelkow, 2015). While it is widely accepted that tau's main function is to stabilize microtubules, other studies have identified tau's role in microtubule dynamics (Drubin & Kirschner, 1986; Panda *et al.*, 1995; Trinszek *et al.*, 2017; Qiang *et al.*, 2018).

Within neurons, tau is primarily localized to axons in adult neurons where it binds to microtubules via the MBTR and regulates their dynamics which is important in maintaining the structure, function, and viability (Baas & Black, 1990; Baas *et al.*, 1991; Feinstein & Wilson, 2005). While bound to microtubules, tau regulates their growth and stabilization which are essential for axonal projections. In addition to directly stabilizing microtubules, tau has also been shown to indirectly regulate microtubule stabilization through crosslinking microtubules to other cytoskeletal components like actin and tubulin (Guo *et al.*, 2017). Tau protein can also inhibit HDAC6, that serves to deacetylate tubulin, thereby increasing the stability of tubulin and microtubule interactions (Perez *et al.*, 2009).

The interaction between tau and microtubules is primarily determined by tau's phosphorylation state. In normal, healthy physiological conditions, tau is primarily located in the axons of neurons with minimal amounts found in the dendrites. Tau is prevented from moving into the distal axon, soma, and dendrites by the axon initial segment that serves as a barrier (Li *et al.*, 2011). When tau becomes pathological (i.e., hyperphosphorylated) it can destabilize the axon initial segment and impairs cargo compartmentalization, which suggests that tau protein normally regulates the integrity of the axon initial segment (Guo *et al.*, 2017). Another regulatory role of tau in axonal compartments is that tau has been shown to regulate axonal

transport of specific cargoes via competitive binding of cargoes to motor proteins like kinesin, thereby modifying axonal transport. Despite the plethora of literature describing microtubule-associated tau protein's main role in microtubule dynamics, a growing body of evidence suggests many different roles of tau protein in the central nervous system and synaptic mechanisms.

In the context of post-synaptic signaling mechanisms, tau has been shown to be a mediator of synaptic plasticity, even though tau protein has only been shown in small amounts in dendrites and dendritic spines in the absence of tauopathy. Synaptic plasticity (i.e., long-term depression and long-term potentiation), is activity-dependent alterations in excitatory synaptic transmission that is regulated by the presence and trafficking of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA) at the post-synapse. The trafficking of AMPARs is induced by activity of N-methyl-D-aspartate receptors (NMDARs). Evidence suggests that tau is crucial for long-term depression but plays a minimal role in long-term potentiation (Kimura *et al.*, 2014; Regan *et al.*, 2015). One study demonstrated that genetic knockdown of tau expression ablates long-term depression (Kimura *et al.*, 2014). Alternatively, when tau protein is present, evoking long-term depression through low frequency stimulation, increases activity of GSK3 β , thereby increasing phosphorylation of tau (Kimura *et al.*, 2014). In the post synapse, tau has been shown to serve as a scaffolding protein in dendritic spines interacting with Fyn kinase, a Src tyrosine kinase (Lee *et al.*, 1998; Bhaskar *et al.*, 2005; Ittner *et al.*, 2010). Evidence suggests that tyrosine phosphorylation of tau by Fyn, which in turn facilitates the localization of Fyn in the post synapse. The Fyn-tau complex then regulates the activity of NMDAR signaling by forming a complex with the post-synaptic density-95 (PSD-95) (Ittner *et al.*, 2010). By doing so, tau indirectly regulates excitability and the stability of NMDARs within the post-synapse (Putra *et al.*, 2020). Interestingly, this interaction has been implicated in Alzheimer's disease and

frontotemporal dementia where phosphorylated tyrosine sites of tau have been identified but is likely facilitated by hyperphosphorylation of tau (Bhaskar *et al.*, 2005; Ittner & Gotz, 2011). Dynamic regulation of long-term depression by tau has also been shown to facilitate the interaction of protein kinase C binding protein 1 (PICK1) and GluA2, suggesting a role of tau in AMPAR internalization (Regan *et al.*, 2015; Suzuki & Kimura, 2017).

Additional roles of tau protein in network activity have been shown using non-transgenic mice with genetic deletion of tau. Specifically, ablation of murine tau protein reduces spontaneous action potential firing of pyramidal cells and increases their inhibitory input by increasing the intrinsic excitability of inhibitory interneurons (Chang *et al.*, 2021a).

Together these findings highlight a potential relationship between microtubule associated tau protein and synaptic activity. Even so, the likely plethora of physiological functions of tau protein remain to be determined.

Tauopathies

Tauopathies are a morphologically and biochemically heterogeneous population of diseases that have distinct neuropathological phenotypes which are distinguished by brain region, cell types, and differential presences of tau isoforms that determine the pathological protein deposits. Primary tauopathies like Frontotemporal Dementia, Parkinsonism linked to chromosome 17, and Pick's Disease are those in which tau is the primary pathological species (Chung *et al.*, 2021). Differently, secondary tauopathies like Alzheimer's disease, Huntington's disease, Parkinson's disease, and chronic traumatic encephalopathy (CTE) are classified with additional pathological species or etiologies in addition to tau pathology (Katsumoto *et al.*, 2019; Lopez *et al.*, 2019). Tauopathies can be further classified by the differential expression of the 3R

and/or 4R isoforms where diseases can exhibit predominately the 3R-, or 4R- isoforms or both the 3R and 4R isoforms with overexpression of both (Caamano-Moreno & Gargini, 2023).

Pathological mouse models of Alzheimer's disease—whether driven by tau pathology, amyloid- β accumulation, or both—show marked deficits in synaptic plasticity and in the neural processes that support memory formation (Chung *et al.*, 2020). Further, overexpression of phosphorylated tau dampens GABAergic signaling and disrupts the intrinsic electrophysiological properties of GABAergic neurons in both the dentate gyrus and CA1 regions of the mouse hippocampus (Zheng *et al.*, 2020).

Studies investigating secondary tauopathies, like Alzheimer's disease, have explored the effects of tau as a target for treatment. One study demonstrated that reducing endogenous tau expression in a mouse model of Alzheimer's disease, prevented behavioral deficits and network excitability (Roberson *et al.*, 2007). In this study, transgenic mice expressing human amyloid precursor protein (hAPP) with mutations causing an increase in amyloid beta protein were crossed with mice with either a homozygous or heterozygous genetic deletion of tau protein. Tests of learning and memory demonstrated that hAPP mice with a genetic deletion of tau, performed similarly to control mice. Further challenging with chemoconvulsant administration, highlighted how reduction of tau expression is protective against seizure induction (Roberson *et al.*, 2007). The effect of tau reduction was further demonstrated in a following study, examining endogenous tau reduction in hAPP mice overexpressing Fyn kinase, where synaptic transmission and plasticity deficits associated with hAPP mice were prevented. Similarly spontaneous epileptiform activity not observed along with a reduced severity of seizures was observed in transgenic mice with reduced endogenous tau expression. The behavioral findings

were supported by the normalized inhibitory/excitatory current imbalance in hippocampal slices when tau was not expressed (Roberson *et al.*, 2011b).

Literature review of microtubule associated tau protein and seizures

There is growing evidence suggesting an association between epilepsy and Alzheimer's disease from clinical studies. Even so, there are no definitive identified mechanisms of seizures inducing or progressing cognitive decline associated with Alzheimer's disease or that pathologies like amyloid-beta or pathological tau directly induce seizures.

Since the early 2000's microtubule associated tau protein has been discussed as a potential target for the treatment of seizures with the publication of many studies suggesting that genetic deletion or suppression of tau protein expression protects against seizures and associated comorbidities in models of genetic and evoked seizures. Conversely, pathological hyperphosphorylated tau has been suggested to be associated with seizures and TLE development in rodent models and in patients.

Hyperphosphorylated tau

As previously discussed, Alzheimer's disease consists of two main pathological hallmark lesions: amyloid-beta plaques and neurofibrillary tangles from hyperphosphorylated tau (Liang *et al.*, 2009). Additional etiological factors are also thought to contribute to excitotoxicity that is thought to be an underlying mechanism of neurodegenerative diseases like Alzheimer's disease (Mattson, 2003). Even so, the relationship between excitotoxicity and phosphorylated tau is not well understood. If excitotoxicity is thought to be one of the main cellular mechanisms contributing to neurodegeneration does excitation influence pathological species formation and

expression or is there a more synergistic relationship between the two? Additionally, what is the relationship between amyloid-beta and pathological tau outside of Alzheimer's disease development?

Cognitive deficits associated with epilepsy diagnosis and neurodegenerative diseases appear to go hand in hand. The relationship between these two disorders was initially identified because seizures and neurodegenerative disorders are commonly observed in elderly patients. It is thought that cellular mechanisms and pathology that cause Alzheimer's disease are risk factors for epilepsy and seizures in patients and that the likelihood of seizures increases as the neurodegeneration progresses (Pandis & Scarmeas, 2012). Additionally, epileptiform activity has been observed in patients diagnosed with Alzheimer's disease (Vossel *et al.*, 2016; Lam *et al.*, 2020). Presence of pathological species like phosphorylated tau in cerebrospinal fluid has been used as a biomarker for Alzheimer's disease and has been identified in other neurological disorders like brain injury and stroke (Blennow *et al.*, 1995; Hesse *et al.*, 2001; Sussmuth *et al.*, 2001; Ost *et al.*, 2006). It was demonstrated that after an episode of status epilepticus or prolonged period of seizures resulted in increased deposits of tau and phosphorylated tau present in the cerebrospinal fluid from patients that exhibited systemic seizures but otherwise undiagnosed etiology (Palmio *et al.*, 2009; Monti *et al.*, 2015). Studies examining phosphorylated tau expression in resected tissue from the temporal lobe and post-mortem tissue from patients diagnosed with TLE demonstrated a correlation cognitive deficits and epilepsy (Puvenna *et al.*, 2016; Tai *et al.*, 2016; Smith *et al.*, 2019; Gourmaud *et al.*, 2020; Silva *et al.*, 2021). Together, these findings substantiate the association between pathological tau and epilepsy in the context of human epilepsy but do not provide direct evidence.

The associative negative effects of hyperphosphorylated tau and seizures have been identified through dephosphorylation of tau using sodium selenate in models of evoked seizures. Sodium selenate is a modified drug that is an oxidized, less toxic form of selenium which has been shown to decrease phosphorylated tau species by increasing the activity of PP2A (i.e., phosphatase) (Corcoran *et al.*, 2010; Eersel *et al.*, 2010). It was shown that in kindled and chemoconvulsant models of evoked seizures, that chronic administration of sodium selenate via drinking water significantly reduced the expression of evoked seizures in rats (Jones *et al.*, 2012). This finding led to a future study on the effect of sodium selenate on epileptogenesis using models of acquired epilepsy (i.e., kindling, kainic acid induced SE, and post-traumatic epilepsy) (Liu *et al.*, 2016). In this study continual administration of sodium selenate via osmotic mini-pumps to rats for four weeks while kindling took place for three weeks beginning one-week post-surgery. Differently, for chemoconvulsant induced status epilepticus they used intraperitoneal administration of kainic acid and a model of post-traumatic epilepsy prior to sodium selenate treatment that began six weeks post-SE. Continuous monitoring of electrographic seizures occurred for a two-week period and then the sodium selenate was “washed-out” for two-weeks following by additional monitoring. Like the 2012 study, treatment with sodium selenate suppressed seizure expression after status epilepticus and/or epileptogenesis but did not prevent spontaneous seizure development. An additional study identified the positive effect of sodium selenate treatment on cognitive deficits associated with epilepsy development (Casillas-Espinosa *et al.*, 2023). Together these studies not only support a causal effect of seizures on hyperphosphorylated tau expression but also demonstrate a potential role of hyperphosphorylated tau on epileptogenesis.

It has been shown in mice that intraperitoneal injection of kainic acid as a model for excitotoxicity (not for the purpose of seizure development) results in a transient phosphorylation and dephosphorylation of tau, in which there are two phases of phosphorylated tau after induced excitability. Tau undergoes an early phase of dephosphorylation that is correlated with increased activity of PP2A (i.e., phosphatase) that occurs roughly six hours post-injection. The second phase occurred approximately ten hours post-injection in which tau demonstrated significant and sustained phosphorylation at many residues (Concepcion *et al.*, 2023) (Liang *et al.*, 2009). Of the protein kinases examined, CDK5, was identified as having the highest activity during this time point. Other protein kinases demonstrated reduced activity during the initial phase of dephosphorylation, likely playing a role in the amount of phosphorylated sites (Liang *et al.*, 2009). Together, these findings identify the relationship between excitability and tau phosphorylation.

Tau Reduction

The idea of targeting tau protein expression in the treatment of Alzheimer's disease came from the relationship between pathological amyloid-beta and phosphorylated tau with risk for Alzheimer's disease (Lewis *et al.*, 2001; Rapoport *et al.*, 2002; Tanzi & Bertram, 2005). In a mouse model of Alzheimer's disease in which mice express human amyloid precursor protein (hAPP) with reduced endogenous tau expression prevented behavioral deficits and prevented excitotoxicity. Interestingly, in non-transgenic mice with genetic deletion of tau, excitotoxicity was also prevented, suggesting that genetic deletion of tau may be protective against neuronal dysfunction (Roberson *et al.*, 2007). Later when challenged with PTZ to induce evoked seizures, non-transgenic and hAPP mice lacking tau expression demonstrated resistance to evoked seizures with reduced severity as well as prevented hippocampal remodeling associated with

evoked seizures (Roberson *et al.*, 2011b). It appears these studies triggered a slew of future studies examining how reduction of tau may be protective against models of genetic epilepsies and evoked seizures in the absence of additional pathology.

The first study of tau reduction on seizures was using a model of genetic epilepsy that came in 2013 with the investigation of genetic deletion of tau expression in a channelopathy mouse model of genetic epilepsy (Holth *et al.*, 2013). For mice, they used a $Kcna1^{-/-}$ mouse that has a null allele for the alpha subunit of the Kv1.1 voltage-gated potassium channel. Mice with this mutation exhibit spontaneous seizures around 3 weeks postnatally along with early mortality. The results of this study demonstrated that $Kcna1^{-/-}$ Tau double-mutant mice were significantly less likely to exhibit spontaneous seizures compared to $Kcna1^{-/-}$ mice expressing tau protein and decreases cortical epileptiform activity. A common comorbidity of genetic epilepsies is increased mortality, often also studied as models of sudden unexpected death in epilepsy. It has been shown that $Kcna1^{-/-}$ mice prematurely die with only about 10% surviving until 10 weeks of age. In this study, Holth *et al.*, showed that in $Kcna1^{-/-}$ mice with heterozygous deletion of tau had a twofold increase in survival with roughly 59% surviving to 10 weeks of age. Interestingly, homozygous deletion of tau in these mice resulted in 74% survival. Together, decreasing tau expression by 50% has a significantly positive effect on mortality in the $Kcna1^{-/-}$ channelopathy model of epilepsy. Another more common channelopathy mouse model of genetic epilepsy is the Dravet syndrome mouse or “Dravet mice”. Dravet syndrome is one of the most severe channelopathies that is associated with many comorbidities and sudden death or SUDEP occurring in almost 20% of patients. It is caused by mutations in the SCN1A gene that encodes Nav1.1 voltage-gated sodium channel that represents the most prevalent genetic epilepsy in humans. Additional comorbidities include developmental delays, hyperactivity, and autism-like behaviors. Like

Holth et al., this group crossed mice with genetic deletion of tau with a mouse model of Dravet syndrome. The Dravet mice have a knock-in truncation mutation in the *Scn1a* gene that is identical to the mutation seen in human patients. Similar to Holth et al., heterozygous expression of tau and homozygous deletion of tau markedly improved survival of *Scn1a* mutant mice compared to mice with a tau-wildtype background. This study was then followed by a study that examined the effects of conditional tau ablation in neuronal subtypes (Shao *et al.*, 2022b). They created two mouse lines in which tau was knocked out either in excitatory neurons or inhibitory neurons specifically in forebrain regions like the hippocampus and neocortex. They further crossed their respective mouse lines in which tau was either knocked out in excitatory or inhibitory interneurons with their Dravet syndrome mice. Interestingly, they found that ablation of tau expression in excitatory but not inhibitory neurons increased early mortality or SUDEP and reduced epileptiform activity in Dravet syndrome mice. As with many mouse models, genetic modifications often occur embryonically, often making it difficult to rationalize translational relevance for patient treatment. To address this, Mucke and his group took advantage of tau targeting anti-sense oligonucleotides to conditionally knockdown tau expression postnatally in Dravet mice to determine if reduction of tau after development elicits similar effects on seizures and survival. Treatment with the anti-sense oligonucleotides successfully knocked down tau expression in both excitatory and inhibitory neurons in the prefrontal cortex, hippocampus, and cerebellum. Results demonstrated that tau-anti-sense oligonucleotide treatment postnatally significantly increased survival in Dravet mice while also reducing epileptiform activity, similar to embryonic genetic ablation of tau.

Majority of studies examining tau reduction in mouse models of evoked seizures use mouse lines where genetic deletion of tau occurs embryonically, which does not exclude the

possibility of developmental compensation being responsible for the protective effects against evoked seizures. Studies have investigated postnatal knockdown of tau expression on seizures using tau targeting antisense oligonucleotides on evoked seizures. One study demonstrated that postnatal knockdown of tau expression reduced the frequency and severity of PTZ-induced evoked seizures (DeVos *et al.*, 2013). It should be noted that transgenic mice with a genetic deletion of tau embryonically exhibited similar protective effects against evoked seizures. This would suggest that genetic deletion of tau either embryonically or postnatally protects against seizures with no obvious difference in seizure expression between the two. Tau targeting antisense oligonucleotides have also been used to selectively knockdown tau expression postnatally in excitatory and inhibitory neurons (Shao *et al.*, 2022b). It was found that selective ablation of tau in excitatory but not inhibitory neurons reduced epileptiform activity and seizure severity, suggesting that excitatory neurons are the key cell type in which tau promotes or prevents network hyperexcitability and dysfunction associated with seizure expression.

Together, these studies highlight a potential role of microtubule associated tau protein in cellular and synaptic mechanisms involved in seizure generation of different etiologies. Nevertheless, the nature of tau's function in specific cellular and synaptic mechanisms involved in epileptogenesis or seizures remain unexplored.

Rationale for Current Studies

While there is evidence supporting the protect effects of reduced tau expression in models of genetic epilepsies and chemoconvulsant models of evoked seizures, the effect of lack of tau expression on epileptogenesis in a model of acquired TLE, remains unclear. While genetic

deletion of tau appears to suppress seizure generation, the etiology of acquired TLE is distinct, with nuanced cellular and synaptic mechanisms contributing to the development of unprovoked spontaneous seizures. Being that tau protein is suggested to play a role in synaptic plasticity and transmission, and that when endogenous tau is not expression, network activity is lowered, it is hypothesized that lack of tau expression would suppress the epileptogenic process by prohibiting synaptic mechanisms that contribute to epileptogenesis associated neuroplasticity in the hippocampus.

CHAPTER TWO: GENERAL METHODS

Mice

Transgenic B6.Cg-Mapt^{tm1(EGFP)KltTg(MAPT)8cPdav/J} mice were produced in house from breeders obtained from The Jackson Laboratory (JAX; Bar Harbor, ME; stock #005491). 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 the 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). Genotypes and protein expression phenotypes were confirmed in our recent report (Cloyd et al., 2021). Age matched male C57BL/6J control mice were bred in house from breeders obtained from JAX (#000664). Here, we used $\tau^{-/-}$ mice and isogenic C57BL/6J wildtype control mice that express murine tau protein. All mice were housed under a 12-hour light / 12-hour dark cycle in an Association for Assessment and Accreditation of Laboratory Animal Care (AALAC) approved facility. Food and water were available ad libitum. Colorado State University Laboratory Animal and Use Committee approved all procedures. Principles outlined in the Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines and the Basel declaration, including the 3R (Replacement, Reduction, Refinement) concept, were considered when planning the experiments.

Genotyping

DNA was extracted from tail snips or ear punches and genotype was confirmed via PCR according to the protocols supplied by JAX. Tissue was digested in 100 μ l of PNBD buffer and 3 μ l of protease K overnight at 55°C prior to stopping the reaction for 10 minutes at 95°C. Samples were then prepared for polymerase chain reaction (PCR) for 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^{-/-} mice (EGFP) and confirmed lack of presence of MAPT gene at 79 bp. For each reaction, samples contained DreamTaq PCR master mix, 2x (Fisher, FERK1071PM), genomic DNA, 20 μ M of EGFP or MAPT forward and reverse primers (Jax #25730, 25731), and water. The PCR cycle setting was as follows: No touchdown, 55°C annealing temperature, 30 cycles. After PCR reaction, samples were run in a 2% Agarose gel (Agarose RA, VWR) in 0.5x TBE that was pre-stained with 10,000x Gel-Red. To visualize bp, GeneRule 100 bp DNA ladder was used (Thermo Scientific, Cat #FERSM0243). The gel was run at 100 volts for 1 hour (Biorad gel electrophoresis system).

Intrahippocampal kainate (IHK) model

All surgical procedures were performed under isoflurane general anesthesia with 0.05% bupivacaine local anesthesia. Kainic acid (120 nL, 20 mM in 0.9% saline, Tocris Bioscience; Minneapolis, MN) or saline (sham; 120 nL, 0.9% saline) was injected using a syringe pump (Harvard Apparatus Pump 11 Elite; Holliston, MA) into the left dorsal hippocampus (2.0 mm posterior, 1.25 mm left, and 1.6 mm ventral to bregma) in male and female tau^{-/-} and wildtype mice between 6 and 8 weeks of age (Krook-Magnuson *et al.*, 2013b). The injection rate was 100

nL/min, and the needle (Hamilton syringe) was left in place for 5 min before and after injection. Buprenorphine SR (0.05 mg/kg) was administered subcutaneously after surgery. Mice were transferred to a heated cage for recovery and monitored for convulsive behavioral seizures for 4 h to assess development of SE. SE was defined as the occurrence of at least 3 convulsive seizures of Racine scale 3 or higher during the observation period, since convulsive seizures during SE predict the successful conversion to TLE within 2 months in C57BL/5J mice (Racine, 1972; Shibley & Smith, 2002b; Kang *et al.*, 2021c). After 4 h post-IHK or vehicle, diazepam (7.5 mg/kg) was administered intraperitoneally to terminate SE.

Seizure identification

Convulsive behavioral seizures were identified using video recordings. Seizures were classified using a modified Racine rating system for mice (Racine, 1972). The Racine scale for classifying seizures is as follows: Racine 1: mouth and facial movements, Racine 2: head nodding, Racine 3: unilateral forelimb myoclonus; Racine 4: bilateral forelimb myoclonus; Racine 5: postural instability and/or repetitive jumping. Racine 1-2 seizures are difficult to identify and are considered “subclinical” and are therefore not considered convulsive. Racine 3-5 seizures lasting over 30 seconds in duration are considered convulsive with the generalization of seizures and are more easily identified via video recording.

Hippocampal Slice Preparation

Coronal dorsal and horizontal ventral hippocampal slices (300 μ M) were prepared from vehicle and IHK-treated tau^{-/-} and wildtype mice 6–8 weeks after IHK or saline injection. After

cutting in cold, oxygenated sucrose artificial cerebrospinal fluid (ACSF, in mM: 85 NaCl, 75 Sucrose, 2.5 KCl, 25 D-Glucose, 1.25 NaH₂PO₄, 4 MgCl₂, 0.5 CaCl₂, 24 NaHCO₃), slices were incubated for 1 h at 34 °C then held at room temperature until recordings. Slices were transferred to a recording chamber containing ACSF (in mM): 124 NaCl, 26 NaHCO₃, 11 glucose, 3 KCl, 2 CaCl₂, 1.3 MgCl₂, and 1.4 NaH₂PO₄; 32-34 °C. Slices were visualized with an upright microscope (BX51W1, Olympus) with infrared-differential interference contrast optics.

CHAPTER 3: DIFFERENTIAL EFFECTS OF TAU EXPRESSION ON SEIZURES AND EPILEPTOGENESIS¹

Introduction

Temporal lobe epilepsy (TLE) is the most common focal epilepsy and accounts for a majority of drug-resistant epilepsy cases (Semah *et al.*, 1998; Reynolds, 2000; Wiebe, 2000; Pascual, 2007; Asadi-Pooya *et al.*, 2017). Although drug resistant TLE with an identifiable focus often responds well to surgical resection (Engel, 1996; Engel *et al.*, 2003; Engel *et al.*, 2012), development of additional treatment options remains essential to reduce the burden of care and improve quality of life for patients with TLE. Further, there are no approved antiepileptogenic therapies for TLE and improved understanding of the mechanisms that drive TLE development will be crucial to developing therapies to prevent epileptogenesis. One potential mechanistic target that has received attention in recent years is the microtubule-associated protein, tau. Tau protein is highly expressed in the hippocampus where it plays important roles in microtubule stabilization and development of hippocampal neurons (Dawson *et al.*, 2001; McMillan *et al.*, 2008; Cantero *et al.*, 2011). Mice lacking tau expression function normally with no physiological deficits, but exhibit modified neuronal maturation and altered synaptic activity leading to suppression of network hypersynchrony (Dawson *et al.*, 2001; Tucker *et al.*, 2001; Roberson *et*

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al., 2007; Chang *et al.*, 2021a). Additionally, we previously identified age dependent, transient effects of tau deletion on excitability of dentate granule cells (DGCs) (Cloyd *et al.*, 2021).

Genetic deletion or suppression of tau expression with antisense oligonucleotides improves seizure outcomes in animal models of genetic epilepsies (i.e., channelopathies) and chemoconvulsant models of evoked seizures (Roberson *et al.*, 2007; DeVos *et al.*, 2013; Holth *et al.*, 2013; Gheyara *et al.*, 2014; Li *et al.*, 2014a; Putra *et al.*, 2020; Shao *et al.*, 2022b). However, tau's role in acquired TLE remains unclear. This is important because the etiologies of genetic and acquired epilepsies are vastly different. Spontaneous seizures in acquired epilepsies often develop after a delay following an injury (e.g., status epilepticus (SE), stroke, traumatic brain injury) after which cortical circuits become rewired through synaptic reorganization, resulting in an increased probability of unprovoked seizures. Unlike many genetic epilepsies, synaptic reorganization associated with TLE development is characterized by axon sprouting of dentate granule cells (DGCs) and other neurons and selective neuron loss, including subsets of inhibitory interneurons, which ultimately decreases synaptic inhibition and increases recurrent excitation within the dentate gyrus (Cronin *et al.*, 1992a; Buckmaster *et al.*, 2002). Although resistance to evoked seizures has been demonstrated in animals lacking tau (Putra *et al.*, 2020), whether this translates to altered epileptogenesis is not clear and the mechanisms by which tau impacts seizures and epilepsy are not known (Chang *et al.*, 2021a). Furthermore, the degree to which tau promotes seizures and epileptogenesis in the absence of additional, underlying pathology (e.g., channelopathy) has not been adequately studied. Studies have examined the link between tau hyperphosphorylation and epileptogenesis using animal models of TLE, where insults also lead to tau hyperphosphorylation, at least transiently (Canet *et al.*, 2021; Concepcion *et al.*, 2023) and dephosphorylating tau may modify evoked seizures and disease progression in animal models of

TLE (Jones *et al.*, 2012; Liu *et al.*, 2016; Casillas-Espinosa *et al.*, 2023). Even so, few studies have examined how tau deletion alters excitability and network activity of DGCs (Cloyd *et al.*, 2021) and none have examined how this relates to epileptogenesis in a mouse model of acquired TLE.

Here, we used the intrahippocampal kainate (IHK) model of acquired TLE to assess epileptiform activity and convulsive seizure expression during SE and the subsequent development of spontaneous recurrent seizures (SRS) using video and video-electroencephalography (v-EEG) in mice lacking tau expression (i.e., tau^{-/-} mice) and in those expressing native murine tau (i.e., wildtype). We further examined synaptic transmission in DGCs because of their involvement in epileptogenesis and tauopathy-associated pathology (Hunt *et al.*, 2010b; Butler *et al.*, 2015; Cloyd *et al.*, 2021). We tested the hypothesis that tau deletion confers evoked seizure resistance and suppresses - but does not prevent - the process of epileptogenesis in acquired TLE.

Materials and Methods

Animals

Transgenic B6.Cg-Mapt^{tm1(EGFP)KltTg(MAPT)8cPdav/J} mice were produced in house from breeders obtained from The Jackson Laboratory (JAX; Bar Harbor, ME; stock #005491). 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 the 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). Here, we used $\tau^{-/-}$ mice and isogenic C57BL/6J wildtype control mice that express murine tau protein.

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 $\tau^{-/-}$ mice. Age matched male C57BL/6J control mice were bred in house from breeders obtained from JAX (#000664). All mice were housed under a 12 hr light / 12 hr dark cycle in an Association for Assessment and Accreditation of Laboratory Animal Care (AALAC) facility. Food and water were available ad libitum. Colorado State University and University of Kentucky Laboratory Animal and Use Committees approved all procedures. Principles outlined in the Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines and the Basel declaration, including the 3R (Replacement, Reduction, Refinement) concept, were considered when planning the experiments.

IHK mouse model of TLE

All surgical procedures were performed under isoflurane general anesthesia with 0.05% bupivacaine local anesthesia. Kainic acid (120 nL, 20 mM in 0.9% saline, Tocris Bioscience; Minneapolis, MN) or saline (sham; 120 nL, 0.9% saline) was injected using a syringe pump (Harvard Apparatus Pump 11 Elite; Holliston, MA) into the left dorsal hippocampus (2.0 mm posterior, 1.25 mm left, and 1.6 mm ventral to bregma) in male and female $\tau^{-/-}$ and wildtype mice between 6 and 8 weeks of age (Krook-Magnuson *et al.*, 2013a). The injection rate was 100

nL/minute, and the needle (Hamilton syringe) was left in place for 5 minutes before and after injection. Buprenorphine SR (0.05 mg/kg) was administered subcutaneously after surgery. Mice were transferred to a heated cage for recovery and monitored for convulsive behavioral seizures for 4 hours to assess development of SE. SE was defined as the occurrence of at least 3 convulsive seizures of Racine scale 3 or higher during the observation period, since convulsive seizures during SE predict the successful conversion to TLE within two months in C57BL/5J mice (Racine, 1972; Shibley & Smith, 2002a; Kang et al., 2021a). After 4 hours post-IHK or vehicle, diazepam (7.5 mg/kg) was administered intraperitoneally to terminate SE.

Video and video-EEG monitoring during SE induction and development of spontaneous seizures

Randomly selected cohorts of mice were fitted with surface EEG head mounts to allow for v-EEG recording of seizures during the SE induction period and for up to three weeks after IHK or vehicle injection. Kainic acid or saline was injected as described above. One channel screw with wire lead was placed 2.0 mm left of midline and 1.0 mm posterior to bregma. Ground and reference screws with wire leads were placed 2.0 mm left and right of midline and 5.0 mm posterior to bregma. Once screws were in place, a tethered 6-Pin Surface Mount Mouse Head mount EEG (Pinnacle Technology model #8235-SM; 2721 Oregon St., Lawrence, KS 66046) was soldered to wire leads and secured with dental cement. Mice were left to recover from anesthesia as described above. v-EEG recording began ~1 hr after surgery and continued until the administration of diazepam as described above and again for 72 hr beginning 3 wks after SE induction. Recordings were sampled at 2 kHz and high pass filtered at 1 kHz and low pass filtered at 0.1 kHz.

The same cohorts fitted with surface EEG head mounts and additional cohorts without EEG head mounts were monitored for spontaneous seizures by video-EEG or video three weeks after the IHK or vehicle injection. Each animal underwent a minimum of one recording session of 72 continuous hours. The videos were reviewed at 4x speed by an investigator(s) blind to genotype and treatment; all seizures were verified by a second investigator. Mice that exhibited at least one spontaneous behavioral and electrographic seizure during the recording period were defined as developing TLE.

Electrographic seizures were analyzed with Neuroscore (Data Sciences International; DSI; St. Paul, MN). Electrographic seizures were confirmed by behavioral assessment of convulsive seizures (≥ 30 s duration) using a modified Racine rating system analysis for mice (i.e., Racine 3-5; Racine 3: unilateral forelimb myoclonus; Racine 4: bilateral forelimb myoclonus; Racine 5: postural instability and/or repetitive jumping). Quantification of epileptiform spikes (i.e., spike frequency) during convulsive seizures were defined by a threshold ratio of 2 from the baseline signal. Seizure onset was defined as behavioral signs (i.e., Racine scale) with concurrent electrographic epileptiform activity exceeding twice the baseline signal amplitude. Termination was marked by cessation of behavioral seizure hallmarks and the onset of postictal electrographic activity (≤ 0.5 μ V of baseline signal). Duration of convulsive seizures, either during SE or spontaneously, was defined by seizure onset and termination as described above. Mice that did not exhibit convulsive seizures during SE induction demonstrated high amplitude, low frequency electrographic spiking therefore spike frequencies (i.e., spikes/sec) in mice that did not exhibit convulsive SE was calculated over one hour at similar times post IHK injection. For spectral analysis, electrographic seizures were defined like above, segmented into 10 second epochs and subjected to fast Fourier transformation (FFT) for the estimation of power

spectral density from 0.5 – 100 Hz with standard frequency bands being defined as: delta: 0.5-4 Hz, theta: 4-8 Hz, alpha: 8-12 Hz, sigma: 12-16 Hz, beta: 16-30 Hz, and gamma: 30-100 Hz. Periodogram analysis allowed for power estimates at each frequency. Slower background spikes (e.g. movement artifacts) were disregarded during analysis.

Whole-cell patch clamp electrophysiological recordings

Coronal dorsal hippocampal slices (300 μ M) were prepared from vehicle and IHK-treated tau^{-/-} and wildtype mice 6-8 weeks after IHK or saline injection. After cutting in cold, oxygenated sucrose artificial cerebrospinal fluid (ACSF), slices were incubated for one hour at 34°C then held at room temperature until recordings. Slices were transferred to a recording chamber containing ACSF (in mM): 124 NaCl, 26 NaHCO₃, 11 glucose, 3 KCl, 2 CaCl₂, 1.3 MgCl₂, and 1.4 NaH₂PO₄; 32-34°C. Slices were visualized with an upright microscope (BX51W1, Olympus) with infrared-differential interference contrast optics.

Whole-cell patch-clamp recordings were obtained from hippocampal DGCs identified by location and morphological characteristics. To examine synaptic properties of DGCs, spontaneous excitatory post-synaptic currents (sEPSCs) and spontaneous inhibitory post-synaptic currents (sIPSCs) were recorded at a holding potential of -70 mV and 0 mV respectively in voltage-clamp configuration. Recording pipettes were pulled from borosilicate glass (open tip resistance 3-5 M Ω ; King Precision Glass Co. Claremont, CA). The pipette recording solution contained (in mM) 140 Cs-gluconate, 4 CsCl, (or 126 K-gluconate with 4 KCl) 10 HEPES, 4 MgATP, 0.3 NaGTP, and 10 Phosphocreatine; pH was adjusted to 7.2 with CsOH or KOH with an osmolarity of 290-295 mOsm. Electrophysiological recordings were performed using a Multiclamp 700B amplifier (Molecular Devices), low pass filtered at 3 kHz using a Bessel filter

and digitized at 20 kHz with Digidata 1440A analog-digital interface (Molecular Devices). Series resistance was $<25\text{ M}\Omega$ and was monitored periodically during the recordings. Recordings were discontinued if series resistance changed by more than 20% during the recording or reached $25\text{ M}\Omega$. Electrophysiological traces were analyzed using Mini Analysis (version 6.0.7, Synaptosoft Inc.). Frequency and amplitude of sEPSCs and sIPSCs were detected and included if the amplitude of the synaptic current was greater than three times root mean square noise level using Mini Analysis.

Hippocampal DGC Dispersion

After electrophysiology recordings, coronal hippocampal slices were placed in 4% paraformaldehyde in phosphate-buffered saline (PBS). Slices were rinsed in PBS then equilibrated in 30% sucrose solution in PBS prior to cryo-sectioning. Slices were re-sectioned with a thickness of $30\text{ }\mu\text{M}$ and mounted on Superfrost microscope slides with Vectashield mounting medium containing DAPI. Images of the dorsal dentate gyrus were acquired using a SPOT RT-Slider camera (Diagnostic Instruments, Sterling Heights, MI) on an upright microscope (BX43, Olympus) using SPOT Advanced Software (Diagnostic Instruments). Hippocampal DGC dispersion was quantified using ImageJ software. Dispersion of granule cells was defined as being at least two times the average linear micron distance of the vehicle DGC suprapyramidal blade measured from the hilar border to the outer margin of the DGC layer (Moura et al 2019). Dispersion was only detected in mice that developed spontaneous recurrent seizures.

Statistical Analysis

Statistical measures were performed with Prism (GraphPad, San Diego, CA). Data were disaggregated by sex; no sex-dependent differences were detected for any measure, so sexes were combined for all analyses. Statistics of sex-dependent differences are shown in Supplemental Table 1 using chi-square test for seizure distribution and unpaired-tests for electrophysiology measurements comparing males and females within each genotype and treatment. Data were tested for normality by a Shapiro-Wilk test, and non-parametric tests were used where appropriate. Seizure analysis (i.e., seizure number, duration, latency, and spiking frequency) was compared using an unpaired t-test between genotypes. Distribution analysis of spontaneous seizure development was compared using chi-square analysis. Electrophysiological data were analyzed using two-way ANOVA with Tukey test for multiple comparisons. Data are presented as mean $\pm$ SEM and statistical significance was set to $p < 0.05$ for all tests. See Supplemental Table 2 for description of statistical analyses for all figures.

Results

A lower percentage of mice lacking tau expression developed SE with fewer seizures during the induction period

Intrahippocampal kainate-induced SE leads to the development of spontaneous seizures after a latent period, where convulsive seizures during SE reliably result in TLE (Bouilleret *et al.*, 1999a; Kang *et al.*, 2021a; Rusina *et al.*, 2021). We previously determined that at least three convulsive seizures (Racine seizure scale 3-5; Racine, 1972) after intraperitoneal pilocarpine injection predicted epileptogenesis in C57BL/6J and other mouse strains (Shibley & Smith, 2002a; Winokur *et al.*, 2004b; Bhaskaran & Smith, 2010), so emergence of multiple convulsive

seizures after IHK was defined as status epilepticus (SE) that was sufficient to trigger TLE. Unilateral IHK injection in the dorsal hippocampus results in SE in mice starting ~1-2 hours after the injection; wildtype C57BL/6J mice reliably develop convulsive SE after IHK (Welzel *et al.*, 2020; Kang *et al.*, 2021a). Injection of kainate resulted in 85.71% of wildtype controls (42/49 mice) and 34.69% of $\tau^{-/-}$ (17/46 mice) exhibiting convulsive SE during the induction period ($p < 0.05$; Fig. 3.1A). Mice that were not characterized as exhibiting convulsive SE did not develop convulsive seizures at any point during the induction period but demonstrated epileptiform spiking in EEG recordings, which has been reported by others (Haussler *et al.*, 2012). $\tau^{-/-}$ mice that exhibited convulsive SE during the induction period had fewer convulsive (i.e., Racine 3-5) and electrographic seizures compared to wildtype controls ($p < 0.05$; Fig. 3.1B). Further there was no difference in the latency to the first convulsive seizure between $\tau^{-/-}$ (84 ± 14.37 min) and wildtype controls (82 ± 7.98 min) ($p = 0.36$, unpaired-test; Fig. 3.1C), with majority of animals experiencing the first convulsive seizure 1-2 hours after recovery from anesthesia. Overall, the results suggest that susceptibility to SE is lower in $\tau^{-/-}$ mice, consistent with results of previous studies showing reduced seizure activity in mice lacking tau expression in models of genetic epilepsies and lower seizure susceptibility to chemo-convulsant evoked seizures (Roberson *et al.*, 2007; DeVos *et al.*, 2013; Gheyara *et al.*, 2014; Li *et al.*, 2014a; Putra *et al.*, 2020).

To determine potential differences in the severity of convulsive seizures during SE, we assessed epileptiform spiking frequency and power spectral density during the ictal phase of seizures and the duration of the epileptiform activity (Fig. 3.1D-G). After injection of kainate or saline, a subset of mice (IHK: wildtype N = 18, $\tau^{-/-}$ N = 15; vehicle: wildtype N = 7, $\tau^{-/-}$ N = 9) were fitted with a 4-channel surface EEG connector with one channel screw implanted near

the injection burr hole and recorded as described in the methods section. Electrographic seizures were assessed during behavioral seizures described in the methods. There were no significant differences detected in spiking frequency ($p > 0.05$) or electrographic seizure duration ($p > 0.05$) between $\tau^{-/-}$ or wildtype mice (Fig. 3.1D- G). We further analyzed spectral analysis of convulsive seizures during SE from wildtype and $\tau^{-/-}$ animals. The periodogram demonstrated no significant difference in the total power or any difference in the peak at specific frequencies, with convulsive seizures from both wildtype and $\tau^{-/-}$ mice exhibiting the dominant signal frequency within the delta band frequency range, suggesting similar signal strength and oscillatory pattern during SE ($p > 0.05$, unpaired t-test; Fig. 3.1H). Interestingly the summed power across specific frequency bands of seizures demonstrated a significant increase in power in the delta and theta power bands in $\tau^{-/-}$ animals compared to wildtype animals, suggesting that while the signal strength does not differ, the power has shifted to a more hypersynchronous signal activity ($p < 0.05$, 2-way ANOVA; Fig. 3.1H inset). Thus, whereas fewer $\tau^{-/-}$ mice expressed SE after IHK, with a lower convulsive seizure frequency, tau deletion did not overtly alter spiking frequency, electrographic activity, or mean seizure duration but may alter cortical network activity of individual seizures during SE.

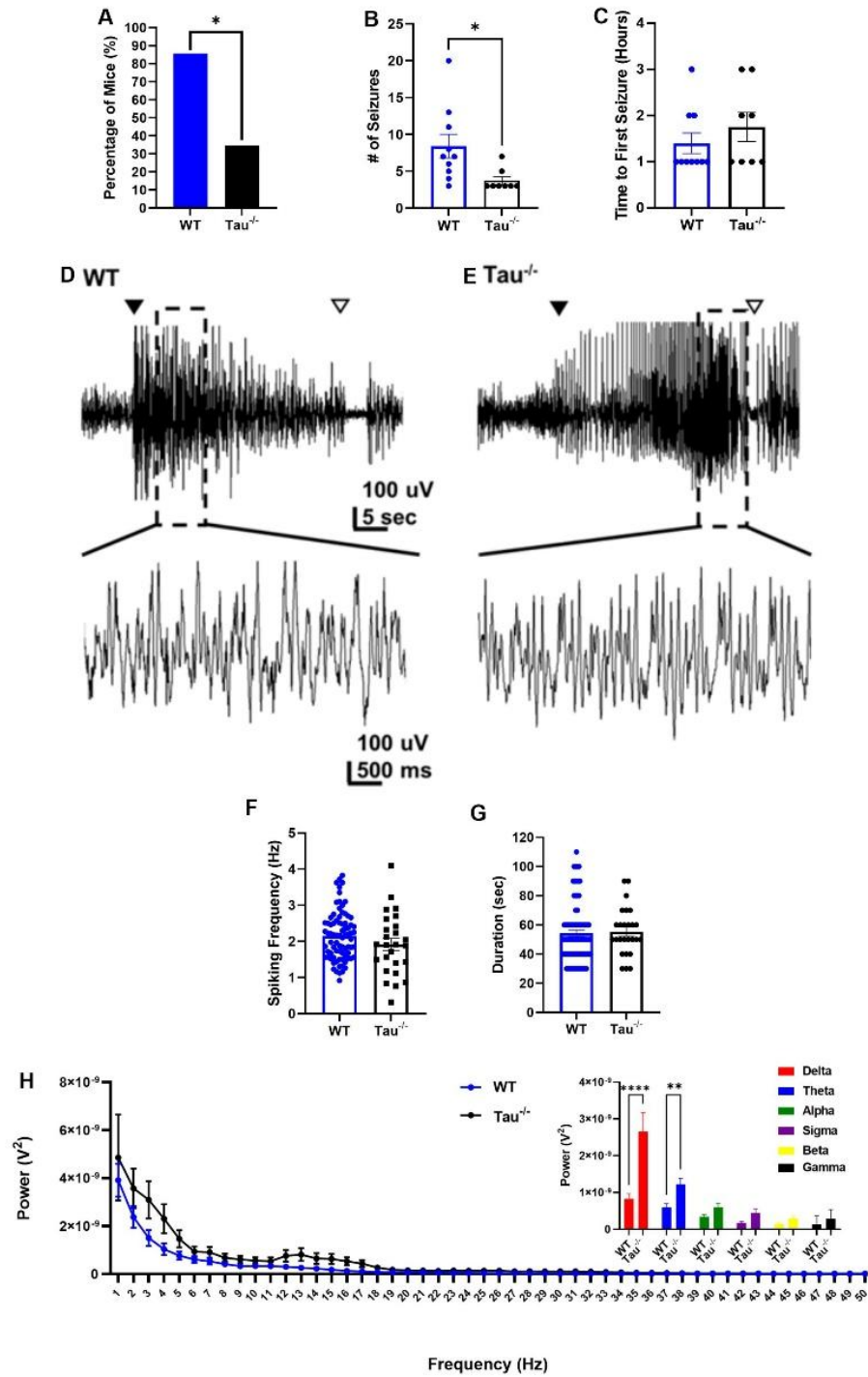


Figure 3.1. Tau^{-/-} mice are less likely to exhibit SE after intrahippocampal kainate (IHK) injection and if SE develops, mice exhibit fewer convulsive seizures compared to wildtype controls. (A) Percentage of wildtype (WT) control (42/49) and tau^{-/-} mice (17/44) that developed SE after kainate injection; *p<0.01, chi-square test. (B) Number of seizures from mice that exhibited SE defined by electroencephalography (EEG) and behavior (WT N = 10, tau^{-/-} N = 8; *p<0.05, unpaired-t test) between the time of recovery from anesthesia and 4 hours post-recovery. Each point represents the number of seizures from a mouse from either genotype. (C) Time to first convulsive seizure after recovery from mice fitted with EEG (WT N = 10, tau^{-/-} N = 8; p>0.05; unpaired-t test) (D) Representative EEG traces of convulsive seizures from WT control and (E) tau^{-/-} mice during SE. Closed arrow indicates the start of the behavioral/electrographic seizure and open arrow indicates the end. Temporally expanded sections of each trace are shown below. (F) Epileptiform activity quantified as spiking frequency (i.e., spikes per second) in convulsive seizures during SE. Each point represents a seizure from either genotype (p>0.05, unpaired t-test). (G) Duration of epileptiform activity of each convulsive seizure during the induction period after IHK injection from either genotype (WT N =10, tau^{-/-} N = 8, p>0.05, unpaired t-test). (H) Periodogram of electroencephalography signal during convulsive seizures from both genotypes ranging from 0.5 Hz to 50 Hz. Both genotypes exhibited a frequency peak at 1 Hz indicating dominant frequency range within the delta frequency band. No significant difference detected in total power calculated as area under the curve (AUC; p = 0.0749; unpaired t-test). Inset is summed power within each frequency band. Tau^{-/-} mice exhibit greater power during SE seizures within the delta and theta frequency bands compared to wildtype mice (****p<0.0001; **p<0.01). Frequency bands were defined as: delta: 0.5 – 4 Hz; theta: 4 – 8 Hz; alpha: 8 – 12 Hz; sigma: 12 – 16 Hz; beta: 16 – 30 Hz; gamma: 30 – 100 Hz.

Development of TLE in Tau^{-/-} mice

Emergence of multiple convulsive seizures after IHK (i.e., SE) is considered to be sufficient to trigger TLE development in C57BL/6J mice (Kang *et al.*, 2021a; Rusina *et al.*, 2021), similar to systemic chemo-convulsant treatments to induce TLE development (Shibley & Smith, 2002a). It is possible that the lower severity of SE in tau^{-/-} mice predicts a lower proportion of those mice developing spontaneous seizures. However, studies examining tau reduction or deletion have not described whether resistance to chemoconvulsant-induced SE

alters spontaneous seizure development independent of other pathologies in a model of acquired TLE (Roberson *et al.*, 2007; Roberson *et al.*, 2011a; DeVos *et al.*, 2013).

Using video and video-EEG, we investigated whether lack of tau expression alters chronic seizure expression in TLE by examining the percentage of mice that exhibited SRS three weeks after IHK. Of the animals that exhibited SE during the induction period and developed spontaneous seizures (i.e., SE:SRS), a significantly smaller proportion of only 10/17 (58.82%) tau^{-/-} animals exhibited both while 38/42 (90.47%) wildtype mice exhibited both convulsive SE and developed SRS (Fig. 3.2A; $p < 0.05$). Chi-square analysis of the distribution of animals that developed SRS after exhibiting SE, suggests that there is an associative effect of tau expression on TLE epileptogenesis after SE.

Interestingly, spontaneous seizures developed in subsets of wildtype and tau^{-/-} mice that did not exhibit convulsive SE (i.e., No SE:SRS) (Fig. 3.2B). In the subset of mice that did not exhibit convulsive SE, there was a similar proportion of wildtype and tau^{-/-} mice that developed spontaneous seizures (wildtype: 2/7, 28.57%; tau^{-/-}: 8/29, 27.59%; $p > 0.05$). Chi-square analysis revealed that there was no effect of tau expression on SRS development when convulsive SE was not expressed after the IHK injection. Further, the number of spontaneous seizures did not significantly differ between the groups that did or did not exhibit convulsive SE but developed SRS ($p > 0.05$; Fig 3.2C). Overall, these results demonstrate that mice lacking tau expression develop acquired TLE in the IHK model, which has not been shown previously.

In mice of both genotypes (i.e., wildtype and tau^{-/-}) that did not experience SE after IHK injection, EEG traces during the induction period displayed epileptiform spiking after kainate administration (Fig. 3.2D), suggesting that nonconvulsive epileptiform activity caused by the IHK injection may be sufficient to trigger or positively influence the epileptogenic process in

mice. Due to the low number of electroencephalographically recorded wildtype mice (N=1) that failed to develop SE but went on to develop SRS compared to tau^{-/-} mice (N=2), quantitative comparison of epileptiform spiking between the two genotypes was not possible. Even so, the epileptiform spike frequency during the induction period of tau^{-/-} mice in the No SE/SRS group (1.331 ± 0.552) was not obviously different from the wildtype mouse (1.119). It is possible differences may not have been observed due to a small cohort of animals.

Hippocampal sclerosis and dispersion of DGCs have been described in the IHK model of TLE (Suzuki *et al.*, 1995; Bouilleret *et al.*, 1999a; Riban *et al.*, 2002; Marx *et al.*, 2013b) and may result from kainate toxicity or SE, or it may be an emergent property of epileptogenesis. Mice of both genotypes that exhibited convulsive SE and developed SRS (i.e., SE:SRS) demonstrated DGC dispersion ipsilateral, but not contralateral, to IHK injection, consistent with previous reports of TLE development in IHK treated mice (Fig. 3.3A, B). We measured the distance of the observed dispersion, as described in the Methods. Intriguingly, the subset of mice that did not exhibit convulsive seizures during SE but nonetheless developed SRS (i.e., No SE:SRS) also exhibited granule cell dispersion ipsilateral to IHK injection that was similar to that observed in mice that experienced convulsive SE and SRS (Fig. 3.3B,C). Mice that failed to develop SRS (i.e., No SE:No SRS and SE:No SRS) did not develop DGC dispersion (Fig. 3.3D). Ipsilateral DGC dispersion in tau^{-/-} mice that developed SRS after SE (SE:SRS) (198.21 ± 23.01 , N = 3) was not numerically different from wildtype mice (WT: 195.89 ± 23.69 , N = 5; $p > 0.05$, unpaired-test). On the contralateral hemisphere where dispersion was not observed in any genotype that developed SRS after SE, the width of the granule cell layer was also not numerically different between wildtype (71.34 ± 8.05 , N = 5) and tau^{-/-} mice (66.06 ± 10.61 , N = 3) ($p > 0.05$, unpaired t-test, nor was it different from vehicle treated animals (77.69 ± 4.23 , N = 4,

unpaired t-test). Due to the low percentage of wildtype mice that failed to develop convulsive SE or SRS, quantitative analysis with comparison to tau^{-/-} animals was not possible. Even so, we did not observe differences in hippocampal dispersion between tau^{-/-} mice that did not exhibit SE but developed SRS (No SE:SRS; 219.38 μM; N=1) and those that did exhibit SE and develop SRS (SE:SRS). Together, these findings suggest that dispersion of the granule cell layer is an outcome of TLE development rather than kainate toxicity or SE alone.

Overall, lack of tau expression reduces the likelihood of TLE development, but it does not prevent epileptogenesis in mice after IHK injection. Additionally, tau expression did not affect TLE development in mice that did not experience SE, suggesting that epileptogenesis itself is not dependent on tau expression and neither is it entirely dependent on convulsive SE.

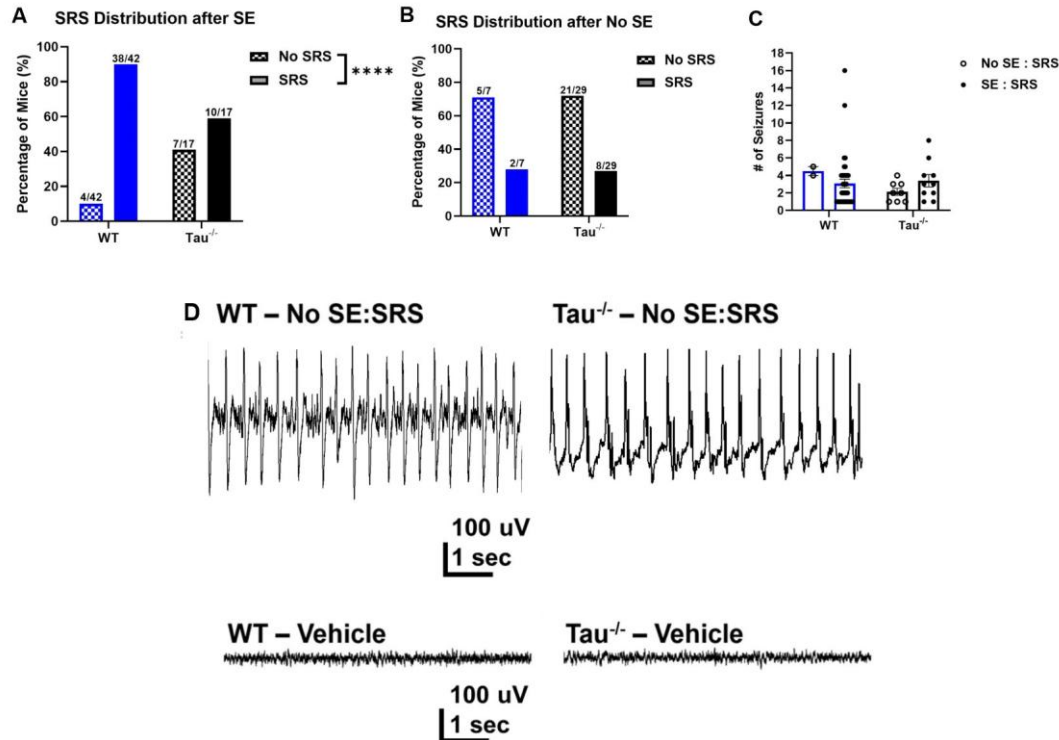


Figure 3.2. Tau^{-/-} mice develop spontaneous seizures after SE, but at a lower rate (A) Percentage of WT control and tau^{-/-} mice that developed spontaneous seizures after exhibiting SE (WT N = 42; tau^{-/-} N = 17; **** p < 0.0001, chi-square test). After experiencing convulsive SE, fewer tau^{-/-} mice developed SRS compared to wildtype controls. (B) Percentage of wildtype controls and tau^{-/-} mice that developed spontaneous seizures without exhibiting convulsive SE (WT N = 7; tau^{-/-} N = 29; p > 0.05, chi-square test). There was no difference in the percentage of mice of either genotype that developed SRS without convulsive SE. (C) Number of spontaneous recurrent seizures per 72 hours from mice defined by electroencephalography and behavior. Each point represents the number of seizures from a mouse of either genotype. No difference was detected across genotypes or SE expression. (D) Representative EEG traces of epileptiform spiking (upper traces) shortly after IHK treatment from mice that did not develop convulsive seizures after IHK injection but developed spontaneous seizures after a latent period (No SE: SRS). Representative EEG traces from vehicle-treated mice are also shown (lower traces).

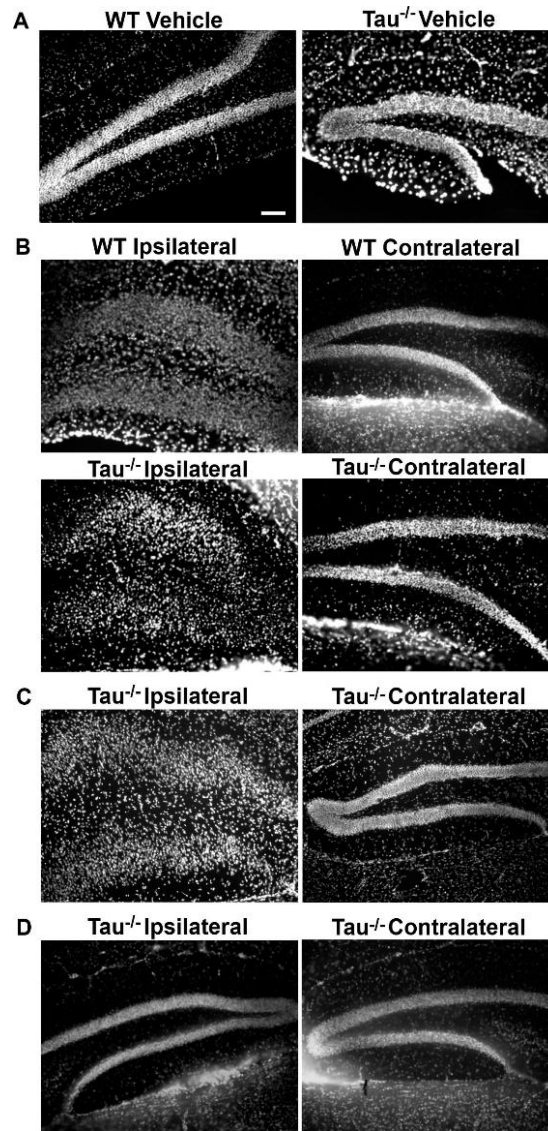


Figure 3.3. Hippocampal dispersion in $\tau^{-/-}$ mice is dependent on SRS development regardless of convulsive SE Representative images of DAPI staining in the dentate gyrus. (A) Vehicle treated wildtype and $\tau^{-/-}$ mice. (B) Wildtype and $\tau^{-/-}$ mice that exhibited SE and later developed SRS demonstrating dispersion of granule cell layer ipsilateral to IHK (SE:SRS). (C) $\tau^{-/-}$ mouse that did not exhibit SE but developed SRS (No SE:SRS) demonstrating dispersion of the granule cell layer ipsilateral to IHK. (D) IHK treated $\tau^{-/-}$ mouse that did not exhibit SE or develop SRS (No SE:No SRS). No dispersion observed ipsilateral to IHK injection. Scale bar = 50 μ M.

Spontaneous seizures in tau^{-/-} mice that develop TLE are less severe

Since we found that tau^{-/-} mice develop SRS at a lower rate than wildtype controls, we investigated how lack of tau expression altered electrographic seizures once TLE developed (Fig. 3.4). Spontaneous electrographic seizures in tau^{-/-} mice that developed TLE exhibited lower epileptiform spiking frequency during the ictal phase ($p < 0.05$) and the duration of the seizures was significantly shorter compared to wildtype mice ($p < 0.05$; Fig. 3.4C-D). We found no difference in spike frequency or duration of spontaneous seizures between tau^{-/-} mice that did not exhibit SE but developed SRS (i.e., No SE:SRS) (spike frequency: 4.19 ± 0.35 ; duration: 30 ± 0.0 , $N = 2$) and those that exhibited both (i.e., SE:SRS) (spike frequency: 4.88 ± 0.73 ; duration: 35 ± 2.89 , $N = 2$) ($p > 0.05$). Therefore, we included spike frequency from both groups of tau^{-/-} mice in Fig. 3.4. Spectral analysis of spontaneous seizures from both genotypes revealed an overall greater total power contribution in tau^{-/-} mice compared to wildtype ($p < 0.05$; Fig. 3.4E). Interestingly, there was a shift in the dominant frequency in tau^{-/-} mice to the alpha power frequency band (i.e., 8-12 Hz) compared to wildtype mice whose seizures peaked in the delta range (i.e., 0.5 – 4 Hz). It should be noted that the large variation in the dominant frequency shift to alpha power can be attributed to seizures from one tau^{-/-} animal. Even so, there was no significant difference detected in the alpha power band ($p > 0.05$; Fig 3.4E inset). Overall, spectral analysis indicates that underlying seizure dynamics are modified in tau^{-/-} mice after development of TLE. Quantitative analysis comparing spike frequency of spontaneous seizures in mice that did not exhibit SE (No SE:SRS) between genotypes was not possible due to only one wildtype animal exhibiting this seizure phenotype out of the 16 recorded using EEG. Additionally, of the 49 IHK-treated wildtype mice, only 2 developed spontaneous seizures without exhibiting convulsive SE after IHK injection.

Thus, after IHK, mice lacking tau expression were less likely to develop TLE and if they did, their spontaneous seizures were less severe, suggesting tau protein may facilitate epileptogenesis-associated neuroplasticity, increasing the severity of individual spontaneous unprovoked seizures. Therefore, lack of tau expression is somewhat protective against spontaneous seizure development if convulsive SE is reached after IHK and if mice lacking tau expression develop TLE their seizures are less severe, but they exhibit altered ictal oscillatory patterns. Further, SE expression has little effect on spontaneous seizure burden once TLE develops. Together, this highlights a significant role of tau protein expression on evoked seizure generation after IHK, which impacts acquired TLE epileptogenesis, and that tau expression also plays a role in unprovoked spontaneous seizures once TLE has developed.

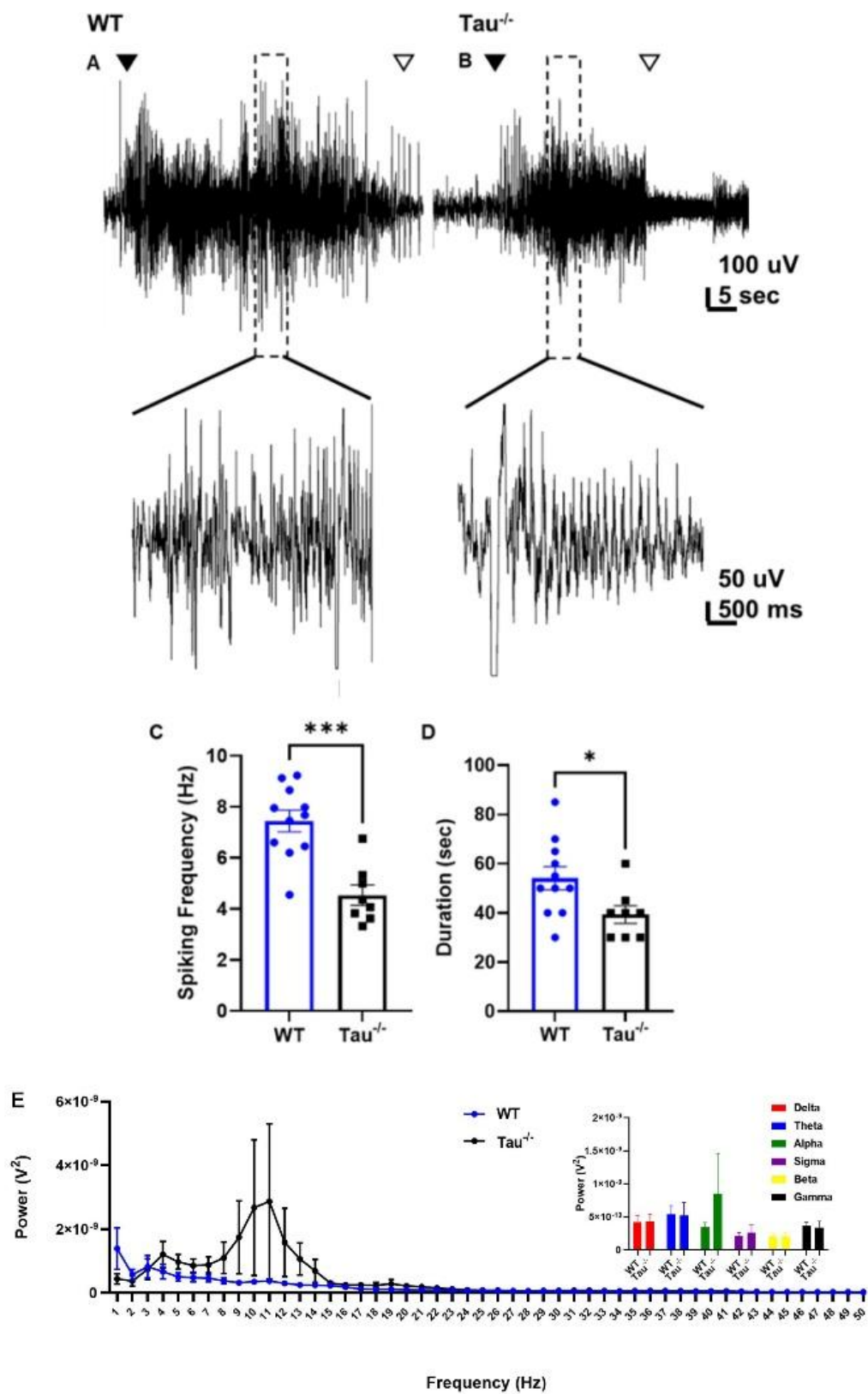


Figure 3.4. Spontaneous seizures in $\tau^{-/-}$ exhibit lower spike frequency and are shorter in duration (A) Representative EEG traces of spontaneous seizures from WT control and (B) $\tau^{-/-}$ mice three weeks after IHK injection. The closed arrow indicates the start of the seizure, and the open arrow indicates the end. Expanded sections of traces are shown at a higher magnitude below. (C) Epileptiform activity is quantified as spiking frequency (i.e., spikes per second) during the ictal portion of spontaneous seizures. Each point represents a convulsive seizure from either genotype. Spike frequency during spontaneous seizures from $\tau^{-/-}$ mice was lower than in WT mice ($***p<0.001$, unpaired t-test). (D) Duration of epileptiform activity during each spontaneous seizure from either genotype. Spontaneous seizures in $\tau^{-/-}$ mice were shorter compared to wildtype controls ($*p<0.05$, unpaired t-test). (E) Periodogram of EEG signal during spontaneous convulsive seizures after development of TLE from both genotypes ranging from 0.5 Hz to 50 Hz. $\tau^{-/-}$ genotypes exhibited a frequency peak in at 1 Hz suggesting a dominant frequency range within the delta frequency band. Total power calculated as area under the curve (AUC) suggests greater total power during convulsive seizures from $\tau^{-/-}$ mice ($*p<0.05$; unpaired t-test). Inset is summed power within each frequency band. No significant difference was detected across the frequency bands between wildtype and $\tau^{-/-}$ mice ($p>0.05$). Frequency bands were defined as: delta: 0.5 – 4 Hz; theta: 4 – 8 Hz; alpha: 8 – 12 Hz; sigma: 12 – 16 Hz; beta: 16 – 30 Hz; gamma: 30 – 100 Hz.

After development of TLE, excitatory synaptic input to DGCs increases in all mice regardless of tau expression

We hypothesized that tau expression was involved in the synaptic reorganization associated with the development of spontaneous seizures, where lack of expression suppresses the synaptic reorganization in DGCs that occurs during the development of TLE. Formation of new recurrent excitatory synaptic connections among DGCs has been shown to be associated with acquired TLE development in rodents (Cronin *et al.*, 1992a; Buckmaster & Dudek, 1997b; Bouilleret *et al.*, 1999a; Winokur *et al.*, 2004b; Hunt *et al.*, 2009). As an assay of overall excitatory synaptic input, we examined the frequency of sEPSCs in DGCs from $\tau^{-/-}$ and wildtype control mice that developed TLE, confirmed by behavioral and electrographic seizure monitoring (Fig. 3.5). In all mice that developed TLE after IHK, significantly increased sEPSC

frequency was observed in DGCs ipsilateral to the injection site compared to vehicle-treated controls, regardless of tau expression ($p < 0.05$; Fig. 3.5D, Table 3.1). There was no significant difference in sEPSC frequency between DGCs from vehicle treated mice and those contralateral to IHK injection ($p > 0.05$), suggesting development of TLE is not associated with an increase in excitatory synaptic input to DGCs contralateral to IHK. Further we found no detectable difference between sEPSC frequency in DGCs from tau^{-/-} or wildtype mice that developed TLE ipsilateral ($p > 0.05$) or contralateral ($p > 0.05$) to the IHK injection; sEPSC frequency was also not affected by genotype in vehicle treated mice ($p > 0.05$). In addition, there was no difference in sEPSC frequency between vehicle- and IHK-injected mice of either genotype that did not develop SRS ($p > 0.05$), suggesting that the increased excitatory synaptic input to DGCs ipsilateral to IHK injection is associated with TLE development rather than kainate treatment or SE alone. There was also no difference detected in the amplitude of sEPSCs events between vehicle- and IHK-injected mice of either genotype ($p > 0.05$; Fig. 3.5E, Table 3.1).

Together these results suggest that tau protein likely does not affect the excitatory synaptic reorganization in the local dentate gyrus circuit that is associated with TLE development. Therefore, the differential effects of tau deletion on evoked and spontaneous seizures in the IHK model are likely to be a result of other circuit reorganization that may modulate seizure initiation and epileptogenesis.

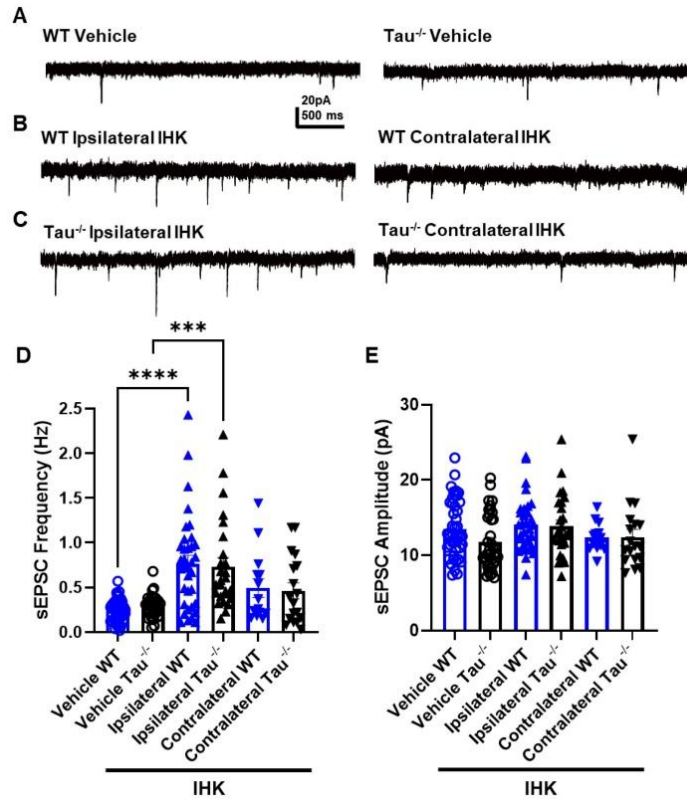


Figure 3.5. Excitatory synaptic input to DGCs is increased ipsilateral to IHK in all mice that developed TLE. (A) Representative sEPSC traces recorded in DGCs from vehicle-treated wildtype (WT) control and tau^{-/-} mice. (B) Representative sEPSC traces from wildtype mice ipsilateral (left) and contralateral (right) to IHK injection. (C) Representative sEPSC traces from IHK-treated tau^{-/-} mice. (D) Frequency of sEPSCs from sham and IHK-treated mice. Each point represents the frequency of events from one cell. After development of TLE, sEPSC frequency is increased similarly in both genotypes ipsilateral to IHK injection. (E) Amplitude of sEPSCs from sham and IHK-treated mice. Each point represents the average event amplitude from one cell; no significant differences were detected in any group. WT: sham N = 11, n = 46; IHK = 12, Ipsilateral n = 32, Contralateral n = 15. Tau^{-/-}: sham N = 7, n = 37; IHK N = 8, Ipsilateral n = 26, Contralateral n = 18). ****p < 0.0001, ***p < 0.001, *p < 0.05, Two-way ANOVA.

Table 3.1. Summary of sEPSCs

* different from vehicle wildtype;

** different from vehicle tau^{-/-};⁺ different from ipsilateral same genotype;[#] different from contralateral same genotype

Treatment and Genotype			Point Estimate ± SEM (n)	
			sEPSC Frequency (Hz)	sEPSC Amplitude (Hz)
Wildtype (Ipsilateral and Contralateral)		Veh	0.2405 ± 0.0188 (44)	13.496 ± 0.5765 (44)
Tau ^{-/-} (Ipsilateral and Contralateral)		Veh	0.2897 ± 0.0199 (37)	11.7616 ± 0.6059 (37)
Wildtype	Ipsilateral	IHK	0.7686 ± 0.0894 (35)*	14.029 ± 0.6327 (35)
	Contralateral	IHK	0.4943 ± 0.1051 (14)	12.34 ± 0.4991 (14)
Tau ^{-/-}	Ipsilateral	IHK	0.7346 ± 0.0997 (26)**	13.8929 ± 0.8209 (26)
	Contralateral	IHK	0.4622 ± 0.0891 (18)	12.412 ± 1.0146 (18)

DGCs receive less inhibitory synaptic input in Tau^{-/-} mice, but development of TLE increases contralateral inhibition in mice lacking tau expression

The development of spontaneous seizures is often accompanied by interneuron loss, especially ipsilateral to IHK injection, which may contribute to hippocampal network excitability (Kobayashi & Buckmaster, 2003a). We assessed the possibility that SRS development in tau^{-/-} mice is associated with preservation of inhibitory synaptic input to DGCs by recording sIPSCs in DGCs from mice that developed spontaneous seizures (Fig. 3.6, Table 3.2). Unsurprisingly, inhibitory synaptic input was reduced ipsilateral to IHK injection in both tau^{-/-} and wildtype mice compared to vehicle treated controls ($p < 0.05$; Fig. 3.6D, Table 3.2), similar to previous reports in TLE models (Morin *et al.*, 1998; Kobayashi & Buckmaster, 2003a). There was no difference in event frequency between IHK-treated tau^{-/-} and wildtype mice in DGCs after development of TLE ($p > 0.05$). Interestingly, tau^{-/-} mice that developed TLE exhibited significantly increased sIPSC frequency in DGCs contralateral to IHK injection compared to vehicle-treated tau^{-/-} mice and ipsilateral to the IHK injection ($p < 0.05$; Fig. 3.6D, Table 3.2), whereas sIPSC frequency in contralateral DGCs from IHK-treated wildtype mice was reduced compared to vehicle-treated wildtype mice ($p < 0.05$). Together this suggests that after tau^{-/-} mice develop TLE, DGCs contralateral to the IHK lesion receive more inhibitory synaptic input, which may contribute to the lower spontaneous seizure burden in these mice. Interestingly, DGCs from vehicle-treated tau^{-/-} mice normally receive less inhibitory synaptic input compared to wildtype controls, since sIPSC frequency is significantly lower in vehicle-treated tau^{-/-} mice ($p < 0.05$; Fig. 3.6D; Table 3.2), which has not been shown before. No difference was detected in the average amplitude of sIPSC events from DGCs across genotype or treatment ($p > 0.05$; Fig 3.6E, Table 3.2). Thus, tau likely plays a role in spontaneous inhibitory synaptic transmission within the dentate gyrus, and

its expression influences the inhibitory synaptic reorganization that occurs during TLE development, which may play a role in the modified seizure expression in tau^{-/-} mice.

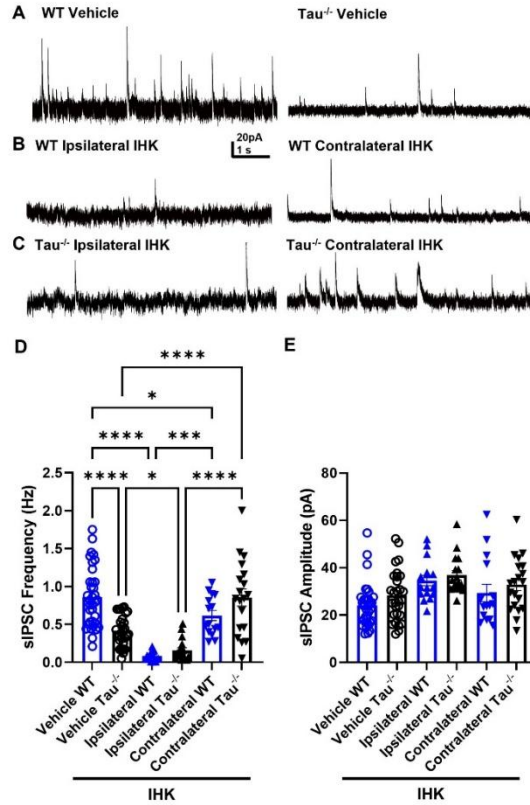


Figure 3.6. DGCs receive less inhibitory synaptic input in naïve tau^{-/-} mice and epileptogenesis decreases sIPSC frequency in both genotypes ipsilateral to IHK but increases contralateral inhibition in tau^{-/-} mice. (A) Representative sIPSC traces recorded in DGCs from vehicle-treated wildtype (WT) control and tau^{-/-} mice. (B) Representative sIPSC traces from IHK-treated wildtype mice that developed TLE. (C) Representative sIPSC traces from IHK-treated tau^{-/-} mice that developed TLE. (D) Frequency of sIPSCs from sham and IHK-treated wildtype mice. Each point represents the frequency of events from one cell. sIPSC frequency is lower in vehicle tau^{-/-} mice compared to vehicle WT ($p < 0.05$). sIPSC frequency is reduced ipsilateral to IHK injection in both tau^{-/-} and wildtype mice compared to their vehicle controls ($p < 0.05$). sIPSC frequency is reduced in IHK-treated wildtype mice contralateral to IHK compared to vehicle-treated wildtype mice ($p < 0.05$). sIPSC frequency is increased contralateral to IHK injection in tau^{-/-} mice compared to vehicle-treated tau^{-/-} mice and to sIPSC frequency in the ipsilateral hemisphere after IHK ($p < 0.05$). (E) Amplitude of sEPSCs from sham and IHK-treated mice. Each point represents the average event amplitude from one cell. No difference detected in amplitude across genotypes or treatments ($p > 0.05$). WT: sham $N = 8$, $n = 35$; IHK = 5, Ipsilateral $n = 18$, Contralateral $n = 15$. Tau^{-/-}: sham $N = 8$, $n = 29$; IHK $N = 6$, Ipsilateral $n = 19$, Contralateral $n = 20$). **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$, Two-way ANOVA.

Table 3.2. Summary of sIPSCs

* different from vehicle wildtype;

** different from vehicle tau^{-/-};⁺ different from ipsilateral same genotype;[#] different from contralateral same genotype

Treatment and Genotype			Point Estimate ± SEM (n)	
			sIPSC Frequency (Hz)	sIPSC Amplitude (Hz)
Wildtype (Ipsilateral and Contralateral)		Veh	0.8611 ± 0.0063 (35)	24.081 ± 1.5300 (35)
Tau ^{-/-} (Ipsilateral and Contralateral)		Veh	0.4179 ± 0.0375 (29)*	28.3152 ± 1.9804 (29)
Wildtype	Ipsilateral	IHK	0.0819 ± 0.0149 (16)* #	34.5543 ± 0.0149 (16)
	Contralateral	IHK	0.6153 ± 0.0672 (15) ⁺ *	29.3133 ± 3.6616 (15)
Tau ^{-/-}	Ipsilateral	IHK	0.1576 ± 0.0354 (17)** #	36.8018 ± 1.9492 (17)
	Contralateral	IHK	0.8536 ± 0.1124 (19) ⁺ **	32.9285 ± 2.5330 (19)

Discussion

The results in this study demonstrate that lack of tau expression does not prevent evoked seizures or the subsequent development of TLE using the IHK model, which has not been previously shown. Rather, deletion of tau elicits differential effects on IHK-evoked SE and

epileptogenesis. Mice lacking tau expression were less likely to exhibit convulsive SE after IHK injection, consistent with other studies on the effects of tau deletion or reduction on evoked seizures (DeVos *et al.*, 2013; Li *et al.*, 2014a; Putra *et al.*, 2020; Shao *et al.*, 2022b). Whereas genetic deletion of tau influenced evoked seizure initiation, it did not quell convulsive seizures once SE developed nor did it prevent the development of TLE. To date there have been limited studies investigating how lack of tau expression influences epileptogenesis in mouse models of acquired TLE. Canonically, SE triggers the process of epileptogenesis in chemoconvulsant models of TLE (Riban *et al.*, 2002). While tau^{-/-} mice developed TLE at a lower rate after experiencing convulsive SE, a subset of mice from both genotypes developed SRS without exhibiting convulsive SE after IHK injection and the rate at which tau^{-/-} mice developed TLE in the absence of IHK induced SE was virtually identical to that in wildtype mice. This suggests the influence of tau expression on TLE development is mainly related to components of the epileptogenic process that are related to SE severity and not the process of epileptogenesis itself. A mouse model of post-traumatic epilepsy (i.e., CCI) often triggers the process of epileptogenesis independent of SE (Hunt *et al.*, 2009; Hunt *et al.*, 2010b; Kang *et al.*, 2022b). Another chemoconvulsant-SE model (i.e., intraperitoneal kainate treatment) results in varying severity of SE (Puttachary *et al.*, 2015), but the mechanisms of how a brain insult like SE contributes to TLE development remain unclear. Our results suggest that nonconvulsive seizures or epileptiform spiking may be sufficient to trigger the development of spontaneous seizures in the IHK model in both genotypes, and tau deletion did not impede the epileptogenic process.

Pathological murine models of Alzheimer's Disease (i.e., tauopathies and/or amyloid beta) exhibit impairments in synaptic plasticity and memory information processing (Chung *et al.*, 2020). Here, we investigated whether mice lacking tau expression exhibited altered

epileptiform activity during convulsive seizures after IHK injection (i.e., during SE) or after TLE developed (i.e., during SRS). Although tau^{-/-} mice exhibited reduced seizure expression during SE, the seizure duration and epileptiform spike frequency of convulsive seizures were not different from wildtype controls. Even so, spectral analysis revealed increased power in the delta and theta frequency bands, suggesting increased neural activity of specific circuit level recruitment. To wit, theta oscillation is associated with the function of both parvalbumin and somatostatin expressing interneurons with activation of these subtypes influencing theta rhythm in the hippocampus (Amilhon *et al.*, 2015; Maheshwari, 2020). Unlike seizures during SE, spontaneous seizures that occurred after TLE developed in tau^{-/-} mice exhibited lower spike frequency and were shorter in duration than in wildtype controls. Interestingly, spectral analysis of spontaneous seizures in tau^{-/-} mice exhibited greater total power and peak frequency shift to the theta/alpha range. Together, this suggests that although the duration and mean spike frequency during the ictal phase is less, the overall signal strength is higher as compared to wildtype controls. It is likely that seizure dynamics are modified once TLE develops in mice lacking tau expression, with a more synchronized neural network with greater oscillatory power between the theta and alpha frequencies. Mechanistically, convulsive seizures after IHK injection in mice lacking tau expression may therefore involve activity of cell types responsible for generating frequencies that may be acting in an anti-epileptogenic manner and further contributing to modified epileptogenesis. Therefore, tau deletion affects seizures in a manner that is consistent with a mechanistic effect on neuronal excitability, but effects of tau deletion on epileptogenesis *per se* were not as apparent.

Tau deficient mice have previously been shown to exhibit slower theta oscillations in the hippocampus and reduced gamma synchronization in the neocortex and hippocampus (Cantero *et*

al., 2011). Distinct populations of interneurons are thought to contribute to hippocampal and neocortical theta and gamma oscillations (Sohal *et al.*, 2009; Lisman & Jensen, 2013; Chung *et al.*, 2020). Dysfunctions in interneuron populations could underlie impaired oscillations in models of AD where reduced power in lower frequency bands corresponds with reduced inhibitory synaptic signaling to excitatory pyramidal cells (Chung *et al.*, 2020), and interneuron circuit function is altered after TLE development in rodents (Buckmaster & Dudek, 1997b; Buckmaster & Wen, 2011). Studies examining brain oscillations commonly analyze local field potentials in specific regions (e.g., hippocampus and/or neocortex) that allows for elucidating the generation of oscillation frequencies from specific cell types (Lisman & Jensen, 2013; Pernia-Andrade & Jonas, 2014; Chung *et al.*, 2020). In this study, we employed the use of single-channel cortical EEG recordings to measure generalized seizure activity. Our use of surface EEG did not allow sufficient spatial resolution to identify regional differences in spiking. This technique did, however, allow for analysis of distinct frequency bands in which we demonstrated altered delta/theta frequency band power and peak frequency shift during the ictal phase of convulsive seizures in tau^{-/-} mice that may correlate with modified inhibitory synaptic transmission.

Synaptic reorganization among DGCs occurs in models of acquired TLE, possibly contributing to regional excitability that supports seizures (Buckmaster & Dudek, 1997b; Buckmaster *et al.*, 2002; Winokur *et al.*, 2004b) and we hypothesized that expression of tau was involved in the synaptic reorganization associated with the development of spontaneous seizures. Even so, we have only a limited understanding of how tau expression alters synaptic transmission in the local dentate gyrus circuit in the absence of additional pathologies (Roberson *et al.*, 2007; Roberson *et al.*, 2011a). We found that sEPSC event frequency was not significantly

different between mice lacking tau expression and wildtype controls normally, similar to our previous study (Cloyd *et al.*, 2021). After TLE developed, sEPSC frequency was increased similarly in both wildtype and tau^{-/-} mice, suggesting that tau expression does not alter the excitatory synaptic reorganization among DGCs that develops during epileptogenesis in this model. Congruently, we examined hippocampal dispersion associated with TLE development in both genotypes of mice and did not observe any obvious differences between tau^{-/-} mice or wildtype controls. Additionally, hippocampal dispersion was exclusively observed in mice of both genotypes that developed SRS, regardless of whether IHK injection evoked SE, suggesting hippocampal dispersion is associated with TLE development in the IHK model and not kainate toxicity or convulsive SE alone.

Being that IHK induced epileptogenesis may affect additional circuits outside of the dentate gyrus (Sheybani *et al.*, 2018; Fernandez *et al.*, 2022; Franz *et al.*, 2023), further studies are required to elucidate potential differences in excitatory synaptic transmission in other regions that may contribute to modified epileptogenesis after IHK in mice lacking tau expression. For example, global tau ablation in excitatory neurons has been shown to reduce evoked seizure expression after pentylenetetrazole (PTZ) injection in mice (Shao *et al.*, 2022b) and deletion of tau reduces spontaneous action potential firing in pyramidal cells in the somatosensory cortex (Chang *et al.*, 2021a). Even so, our results do not implicate a role for tau protein in the excitatory synaptic reorganization in the local dentate gyrus circuit that is associated with epileptogenesis.

We found that sIPSC frequency in DGCs ipsilateral to the IHK lesion was reduced similarly in tau^{-/-} and wildtype controls, consistent with either a loss of inhibitory neurons or a reduction in their ability to release GABA in the dentate gyrus (Kobayashi & Buckmaster, 2003a). Differently, sIPSC frequency was increased contralateral to the IHK lesion in tau^{-/-} mice

that developed TLE compared to vehicle-treated tau^{-/-} mice. It seems possible that this increase in synaptic inhibition could inhibit and/or modify SRS generation, especially seizures that secondarily generalize to involve the hemisphere contralateral to the presumptive seizure initiation zone and may be responsible for reduced epileptiform activity and duration of spontaneous seizures after development of TLE. Axon sprouting and synaptic reorganization of surviving interneurons is associated with development of TLE in rodent models of acquired TLE (Buckmaster & Wen, 2011; Peng *et al.*, 2013; Kang *et al.*, 2022b; Lee *et al.*, 2024b), and similar or more robust synaptic reorganization may occur in contralateral inhibitory circuits in tau^{-/-} mice. DGCs, including adult born DGCs, form new connections with interneurons after TLE development in multiple models (Buckmaster *et al.*, 2002; Halabisky *et al.*, 2010; Butler *et al.*, 2017; Kang *et al.*, 2022b). The restorative increase in synaptic inhibition that occurs contralateral to IHK lesion in the absence of tau expression implies that tau protein may normally suppress this process, but further studies to test this hypothesis directly are needed.

We found that sIPSC event frequency in DGCs was constitutively lower in naïve tau^{-/-} mice. This suggests that tau protein normally plays a role in regulating local inhibitory synaptic transmission in the dentate gyrus. Overexpression of phosphorylated tau suppresses GABAergic neurotransmission and alters intrinsic properties of GABAergic neurons within the dentate gyrus and area CA1 in mice (Zheng *et al.*, 2020). Lack of tau expression in the somatosensory cortex demonstrates a similar dysfunction in inhibitory synaptic transmission (Chang *et al.*, 2021a), but whether interneurons in the dentate gyrus are similarly affected by deletion of tau is unclear. Our results identify a novel potential function of tau protein in local inhibitory circuitry in the dentate gyrus, which has not been shown previously.

Conclusions

This study highlights the impact of tau expression on the development of acquired TLE and uncovers potential inhibitory synaptic mechanisms in the dentate gyrus that may underlie tau's effects on seizures. Here we show that deletion of tau suppresses individual seizures but does not protect against the process of epileptogenesis, highlighting that mice lacking tau expression develop acquired TLE, which has not been demonstrated previously. This study also identifies effects of tau expression on inhibitory synaptic reorganization in the dentate gyrus after epileptogenesis occurs, which may contribute to differential seizure expression in tau^{-/-} mice.

CHAPTER 4: LACK OF TAU EXPRESSION MODIFIES EPILEPTOGENESIS ASSOCIATED NEUROPLASTICITY IN THE VENTRAL DENTATE GYRUS

Introduction

Studies in which the microtubule associated protein tau is genetically deleted or suppressed in models of genetic epilepsies (i.e., channelopathies) and chemoconvulsant-evoked seizures demonstrate that lack of tau expression is protective against seizure expression (DeVos *et al.*, 2013; Holth *et al.*, 2013; Gheyara *et al.*, 2014; Li *et al.*, 2014b; Putra *et al.*, 2020; Shao *et al.*, 2022a), but studies examining how deletion of tau modifies epileptogenesis in acquired TLE models are limited. Using the intrahippocampal kainate (IHK) model of acquired TLE, we showed that genetic deletion of tau protein reduces that rate of TLE development and modifies individual spontaneous seizures expression (Moseley *et al.*, 2025). This highlights one potential role of tau protein in cellular and synaptic mechanisms that drive epileptogenesis of acquired TLE, that has not been adequately investigated.

In the IHK model of acquired TLE, focal injection of kainic acid into the dorsal hippocampus typically results in development of status epilepticus (SE) and after a delay of 2-3 weeks, mice develop unprovoked spontaneous seizures. The development of spontaneous recurrent seizures in the IHK and other rodent models of TLE is often associated with pathological hallmarks that recapitulate characteristics seen in human patients with TLE such as loss of subsets of inhibitory interneurons, dentate granule cell DGC axon sprouting and synaptic reorganization of DGC connections, and (DGC) dispersion (Lanerolle *et al.*, 1989; Sutula *et al.*, 1989; Leite *et al.*, 1996; Bouilleret *et al.*, 1999b; Sundstrom *et al.*, 2001; Lanerolle *et al.*, 2003; Sutula & Dudek, 2007; Marx *et al.*, 2013a; Sieu *et al.*, 2017; Zeidler *et al.*, 2018; Kang *et al.*,

2021b; Lee *et al.*, 2024a). Unlike other rodent models in which TLE is induced after intraperitoneal chemoconvulsant administration, TLE associated neuropathology in the unilateral IHK model is largely limited to the ipsilateral hippocampus with distant sites remaining relatively intact. This allows for the investigation of cellular and synaptic changes associated with epileptogenesis in brain regions remote to the injury, like the ventral hippocampus (Bouilleret *et al.*, 1999b; Riban *et al.*, 2002; Gouder *et al.*, 2003; Groticke *et al.*, 2008; Zeidler *et al.*, 2018; Kang *et al.*, 2021b). Synaptic reorganization that is limited to the dorsal hippocampus however, may be ineffective in regulating or inducing seizures, and seizures in other acquired TLE models and patients with TLE often develop in the ventral hippocampus (J. Engel *et al.*, 1990; Buckmaster *et al.*, 2022). While mice lacking tau expression (i.e., tau^{-/-} mice) developed TLE at a lower rate than wildtype controls, with reduced severity of spontaneous seizures, development of TLE in tau^{-/-} mice was additionally associated with increased inhibitory synaptic drive to DGCs contralateral to the kainate injection in the dorsal hippocampus (Moseley *et al.*, 2025). This suggests discrete inhibitory synaptic reorganization may be a potential mechanism by which tau deletion suppresses seizure expression once epilepsy develops. Therefore, we sought to examine how a focal insult (kainate injection) in the dorsal hippocampus alters epileptogenesis-associated neuroplasticity in the ventral hippocampus, an area remote from the insult, and if lack of tau expression modifies neuroplastic changes associated with the development of TLE.

Here, we used the IHK model of acquired TLE to access neuronal excitability and synaptic transmission in DGCs of the ventral dentate gyrus, not only because of their involvement in epileptogenesis and tauopathy-associated pathology, but also to investigate if epileptogenesis associated neuroplasticity is evident at sites distal from the focal lesion in the

dorsal hippocampus in mice lacking tau expression (i.e., tau^{-/-} mice) and wildtype C57Bl/6J controls. We tested the hypothesis that tau deletion contributes to the synaptic reorganization of inhibitory circuits during epileptogenesis in acquired TLE.

Material and Methods

Animals

Transgenic B6.Cg-Maptm1(EGFP)KltTg(MAPT)8cPdav/J mice were produced in house from breeders obtained from The Jackson Laboratory (JAX; Bar Harbor, ME; stock #005491). All breeding mice were homozygous for the 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 reports (Cloyd et al., 2021, Moseley et al., 2025). Here, we used tau^{-/-} mice and isogenic C57BL/6J wildtype control mice that express murine tau protein.

Age matched male C57BL/6J control mice were bred in house from breeders obtained from JAX (#000664). All mice were housed under a 12 hr light / 12 hr dark cycle in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved facility. Food and water were available ad libitum. The Colorado State University Laboratory Animal and Use Committee approved all procedures. Principles outlined in the Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines and the Basel declaration, including the 3R (Replacement, Reduction, Refinement) concept, were considered when planning the experiments.

Intrahippocampal kainate model of TLE

All surgical procedures were performed under isoflurane general anesthesia with 0.05% bupivacaine local anesthesia. Kainic acid (120 nL, 20 mM in 0.9% saline, Tocris Bioscience; Minneapolis, MN) or saline (sham; 120 nL, 0.9% saline) was injected into the left dorsal hippocampus in male and female tau^{-/-} and wildtype mice between 8 and 10 weeks of age (Krook-Magnuson *et al.*, 2013a), as described previously (Moseley *et al.*, 2025). After injection, mice were monitored for status epilepticus (SE) and diazepam (7.5 mg/kg) was administered intraperitoneally to terminate SE.

Development of TLE with the expression of spontaneous seizures in mice was determined by a continuous 72 hr video recording session at 5-6 weeks after the IHK or vehicle injection. Mice lacking tau expression develop TLE but at a lower rate than non-transgenic controls in this model (Moseley *et al.*, 2025). All IHK-treated mice used in this study exhibited at least one spontaneous seizure during the observed video-recording period and were considered to have developed TLE. We included both males and females from both genotypes and treatments (Vehicle: WT: Males N=7, Females N=11; tau^{-/-}: Males N=9, Females N=8. IHK: WT: Males N=6, Females N=8; tau^{-/-}: Males N=10, Females N=6).

Whole-cell patch clamp electrophysiological recordings

Horizontal slices containing the ventral hippocampus (300 μM) from vehicle and IHK-treated tau^{-/-} and wildtype mice 6-8 weeks after IHK or saline injection were prepared in cold, oxygenated sucrose artificial cerebrospinal fluid (ACSF; in mM: 85 NaCl, 75 Sucrose, 2.5 KCl, 25 D-Glucose, 1.25 NaH₂PO₄, 4 MgCl₂, 0.5 CaCl₂, 24 NaHCO₃). Slices were incubated for one

hour at 34°C then transferred to a recording chamber containing ACSF (in mM): 124 NaCl, 26 NaHCO₃, 11 glucose, 3 KCl, 2 CaCl₂, 1.3 MgCl₂, and 1.4 NaH₂PO₄; 32-34°C for recordings.

Whole-cell patch-clamp recordings were obtained from ventral DGCs (vDGCs) identified by location within the granule cell layer and morphological characteristics. Electrophysiological recordings were performed as previously described (Moseley *et al.*, 2025) using a Multiclamp 700B amplifier (Molecular Devices, San Jose, CA) and acquired using the pClamp software suite (Molecular Devices). Recordings were discarded if series resistance changed by more than 20% during the recording or reached 25 MΩ. Recording pipettes were pulled from borosilicate glass (open tip resistance 3-5 MΩ; King Precision Glass Co., Claremont, CA). Current clamp recordings were obtained using a pipette recording solution which contained (in mM) 130 K-gluconate, 10 HEPES, 1 NaCl, 2 MgATP, 1 MgCl₂, 1 CaCl₂, and 5 EGTA; pH adjusted to 7.2 with KOH with an osmolarity of 290-295 mOsm. Voltage clamp recordings were obtained using a pipette recording solution which contained (in mM) 140 Cs-gluconate, 10 HEPES, 1 NaCl, 2 MgATP, 1 MgCl₂, 1 CaCl₂, and 5 EGTA; pH adjusted to 7.2 with CsOH with an osmolarity of 290-295 mOsm. Liquid junction potential was calculated using JPCalc (pClamp, Molecular Devices) to be +15.56 mV for K-gluconate and +16.57 mV for Cs-gluconate internals.

Intrinsic properties of vDGCs were recorded in current clamp configuration and membrane properties assessed using 1 s hyperpolarizing and depolarizing current steps of 20 pA increments from -200 pA to 200 pA from a membrane potential of -70 mV. Resting membrane potential was recorded in I=0 configuration and averaged over 60 seconds. Input resistance was measured using currents steps from -20 to 20 pA. Rheobase was defined as the first depolarizing current step that elicited action potentials. Current clamp recordings were analyzed using Clampfit 11.2 (Molecular Devices). Synaptic properties of vDGCs were measured by recording

spontaneous excitatory post-synaptic currents (sEPSCs) at a holding potential of -70 mV and spontaneous inhibitory post-synaptic currents (sIPSCs) at a holding potential of 0 mV in voltage-clamp configuration. Action potential independent, miniature excitatory post-synaptic currents (mEPSCs) and miniature inhibitory post-synaptic currents (mIPSCs) were recorded as spontaneous events in the presence of 1 μ M tetrodotoxin (TTX, Alomone Labs). Voltage clamp recordings were analyzed using Mini Analysis (version 6.0.7, Synaptosoft Inc.). Synaptic current amplitude was required to be greater than three times root mean square noise level to be included in s/mEPSCs and s/mIPSC frequency and amplitude analyses.

Timm's Stain of Mossy Fiber Sprouting

Timm's stain was performed on slices used for electrophysiology recordings. Ventral hippocampal slices used for recordings were incubated in 0.37% sodium sulfide solution for 20 minutes followed by 4% paraformaldehyde in 0.15 M phosphate buffer overnight. Slice were washed 3 times for 10 minutes in 0.01 M phosphate buffered saline (PBS) and cryoprotected overnight in 30% sucrose solution in PBS. Slices were sectioned at 20 μ M, rinsed in 0.15 M phosphate buffer and mounted on charged slides (Superfrost Plus; Fisher Scientific) and dried for 48 hours. Sections were developed as previously described in acid washed glassware (Hunt *et al.*, 2010a; Hunt *et al.*, 2012; Butler *et al.*, 2015; Kang *et al.*, 2022a). The developer consisted of 4 individual solutions combined in order: A: 170 ml of 50% Gum Arabic solution, B: 7.65 g anhydrous citric acid and 7.05 g Na-citrate in 30 ml H₂O, C: 0.398 g Ag-lactate in 45 ml H₂O , D: 2.5 g hydroquinone in 45 ml H₂O. Slides were incubated for 2 hours in the developer then rinsed in water for 15 min. After rinsing slides were dehydrated in increasing ethanol concentrations then placed in xylene prior to mounting with Permount. Timm's scoring was

performed by two-blind investigators to treatment and genotype as described in (Cavazos *et al.*, 1991).

Statistical Analysis

Statistical measures were performed with Prism (GraphPad, San Diego, CA). Electrophysiological data were analyzed using two-way ANOVA with Tukey test for multiple comparisons. Data are presented as mean $\pm$ SEM and statistical significance was set to $p < 0.05$ for all tests. All statistical tests are shown in the Statistics Table.

Results

Neuronal excitability of ventral DGCs increases in all mice that develop TLE

In animal models of TLE and in patients (Stegen *et al.*, 2012), epileptogenesis is associated with an increase in neuronal excitability of DGCs, which can be at least partly attributed to axonal sprouting of DGCs (i.e., mossy fiber sprouting, MFS), increased synaptic connectivity between DGCs, and reduced synaptic inhibition (Scharfman *et al.*, 1999; Kobayashi & Buckmaster, 2003b; Winokur *et al.*, 2004a; Hunt *et al.*, 2010a; Althaus *et al.*, 2015; Lee *et al.*, 2024a). Differently, unilateral injection of kainate results in severe hippocampal damage in the dorsal hippocampus and is associated with a significant reduction in intrinsic excitability of dorsal DGCs (dDGCs) ipsilateral to the IHK injection (Fig. 4.1). After development of TLE, dDGCs in wildtype and tau^{-/-} mice exhibit significantly reduced action potential firing in response to depolarizing current steps, higher rheobase, and lower input resistance compared to dDGCs from vehicle-controls and dDGCs contralateral to the IHK injection (Fig. 4.1B,D,E, Table 4.1; $p < 0.05$, Two-way ANOVA). Interestingly, there was no significant difference detected

in the resting membrane of dDGCs across genotypes or treatments (Fig. 4.1C, Table 4.1; $p>0.05$, Two-way ANOVA). There was no difference detected in any intrinsic property between saline-treated wildtype and $\tau^{-/-}$ mice, ipsilateral dDGCs from IHK-treated wildtype and $\tau^{-/-}$ mice, or contralateral dDGCs from IHK-treated wildtype and $\tau^{-/-}$ mice ($p>0.05$; Two-way ANOVA). These results suggest DGCs in the dorsal dentate gyrus, ipsilateral to the focal kainate injection where hippocampal sclerosis is most severe, exhibit significantly reduced excitability after TLE develops, regardless of tau expression. Since the site near the IHK injection may not reflect more widespread patterns of excitability, we examined DGCs in the ventral hippocampus to elucidate how development of TLE may alter cellular excitability and synaptic transmission of vDGCs.

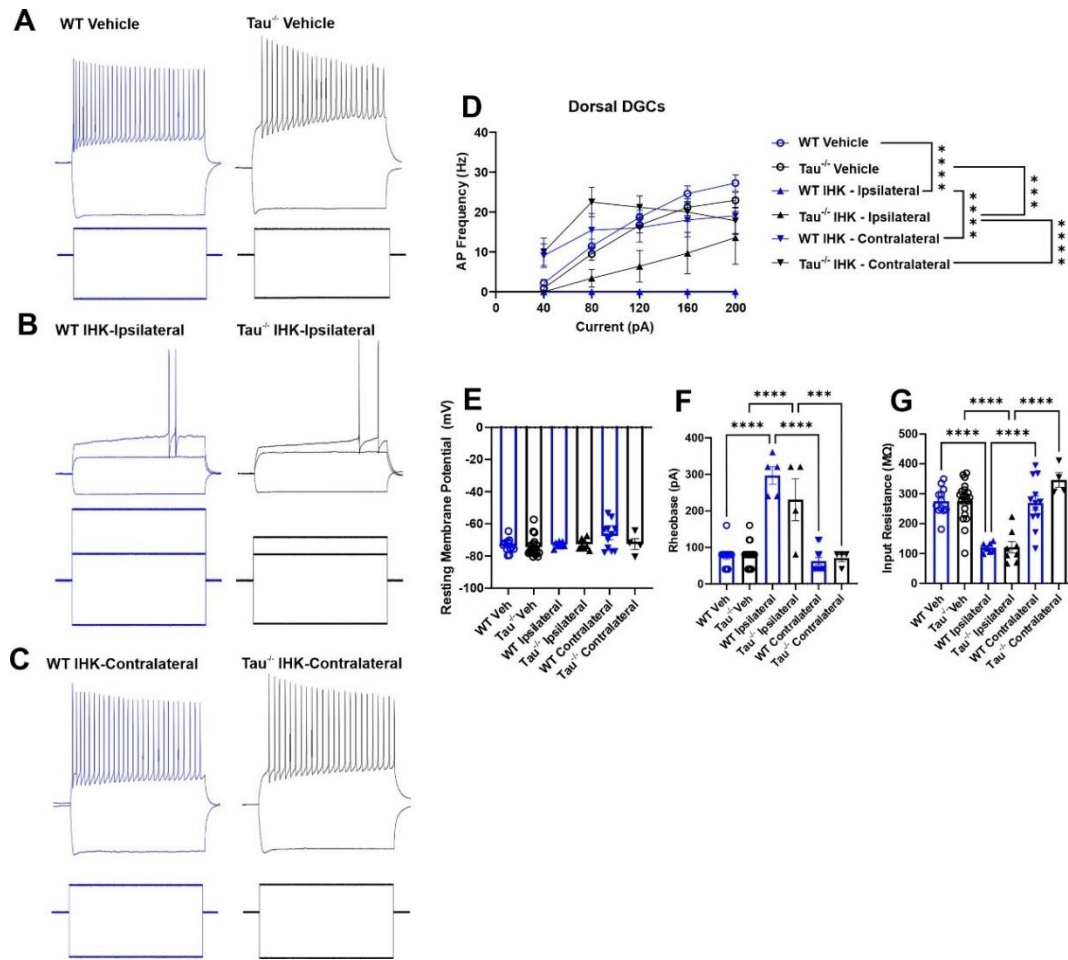


Figure 4.1. Dorsal DGCs near IHK injection exhibit reduced excitability in all mice that develop TLE. Action potential firing of dDGCs in response to current injection steps (A) dDGCs recorded from wildtype (WT) and $\text{tau}^{-/-}$ mice in vehicle controls demonstrate voltage responses at -200 pA and 120 pA. (B) dDGCs ipsilateral to IHK injection in WT and $\text{tau}^{-/-}$ mice. WT IHK-ipsilateral trace demonstrates voltage responses at -200 pA, 120 pA, and 240 pA. Rheobase of this cell was at 240 pA. $\text{Tau}^{-/-}$ IHK-ipsilateral demonstrates voltage responses at -200 pA, 120 pA, 200 pA. Rheobase of this cell was at 200 pA. (C) dDGCs contralateral to IHK injection in WT and $\text{tau}^{-/-}$ mice demonstrate voltage responses at -200 pA and 120 pA. (D) Input-out curve of action potential frequency of dDGCs from vehicle and IHK-treated wildtype and $\text{tau}^{-/-}$ mice (** $p < 0.001$, **** $p < 0.0001$, Two-way ANOVA). (E) Resting membrane potential of dDGCs from vehicle and IHK-treated wildtype and $\text{tau}^{-/-}$ mice ($p > 0.05$, Two-way ANOVA). (F) Rheobase (i.e., minimum current that elicits action potentials) of dDGCs from vehicle and IHK-treated wildtype and $\text{tau}^{-/-}$ mice (** $p < 0.001$, **** $p < 0.0001$, Two-way ANOVA). (G) Input resistance of dDGCs from vehicle and IHK-treated wildtype and $\text{tau}^{-/-}$ mice (** $p < 0.0001$; Two-way ANOVA). WT Vehicle: N=4, n=19; $\text{Tau}^{-/-}$ Vehicle: N=4, n=21; WT IHK: N=2, ipsilateral: n=5, contralateral: n=11; $\text{Tau}^{-/-}$ IHK N=3, ipsilateral: n=5, contralateral: n=4).

Table 4.1. Summary of resting membrane potential, rheobase, and input resistance from Dorsal DGCs

* different from vehicle wildtype;

** different from vehicle tau^{-/-};

⁺ different from ipsilateral same genotype;

Treatment and Genotype			Dorsal DGCs		
			RMV (mV)	Rheobase (pA)	Input Resistance (mOhm)
Wildtype (Ipsilateral and Contralateral)		Veh	-73.59 ± 1.196 (12)	76.67 ± 9.156 (12)	275.2 ± 13.47 (12)
Tau ^{-/-} (Ipsilateral and Contralateral)		Veh	-74.58 ± 1.265 (21)	78.00 ± 7.157 (21)	276.3 ± 14.45 (21)
Wildtype	Ipsilateral	IHK	-72.92 ± 0.7083 (5)	296.0 ± 24.00 (5)*#	119.7 ± 6.097 (5)*#
	Contralateral	IHK	-67.44 ± 2.610 (11)	61.82 ± 9.892 (11) ⁺	269.20 ± 23.53 (11) ⁺
Tau ^{-/-}	Ipsilateral	IHK	-71.13 ± 1.418 (4)	230.0 ± 57.45 (4)***	110.46 ± 17.24 (4)***
	Contralateral	IHK	-72.47 ± 3.317 (4)	70.00 ± 10.00 (4) ⁺	346.3 ± 24.26 (4) ⁺

To test the hypothesis that development of TLE modifies neuronal excitability of vDGCs and to determine if excitability is attenuated once epilepsy develops in mice lacking tau expression, we assayed intrinsic properties in vehicle and IHK-treated wildtype and tau^{-/-} mice that developed spontaneous seizures (Fig. 4.2). Action potential firing frequency of vDGCs both ipsilateral and contralateral to the IHK injection was increased in all IHK-treated mice that developed spontaneous seizures compared to vDGCs in vehicle-treated controls, with no detectable differences between genotypes (Fig. 4.2B-C, Table 4.2; $p < 0.05$). There was no significant difference in firing frequency of vDGCs between vehicle-treated wildtype and tau^{-/-} mice ($p > 0.05$). Interestingly, the resting membrane potential of vDGCs ipsilateral and contralateral to IHK injection in wildtype mice that developed TLE exhibited a significantly depolarized resting membrane potential compared to vehicle-treated wildtype controls and IHK-treated tau^{-/-} mice that developed TLE (Fig 4.2D, Table 4.2; $p < 0.05$). Even so, there was no significant difference detected in rheobase or input resistance across treatments or genotypes (Fig. 4.2E-F, Table 4.2; $p > 0.05$). Together this suggests that in the ventral dentate gyrus, DGCs exhibit increased action potential firing in response to a depolarizing stimulus once epilepsy develops in the IHK model. Additionally, it is likely that tau protein does not play a role in endogenous firing properties of DGCs in mice of this age (14-16 weeks old), similar to a previous report (Cloyd *et al.*, 2021).

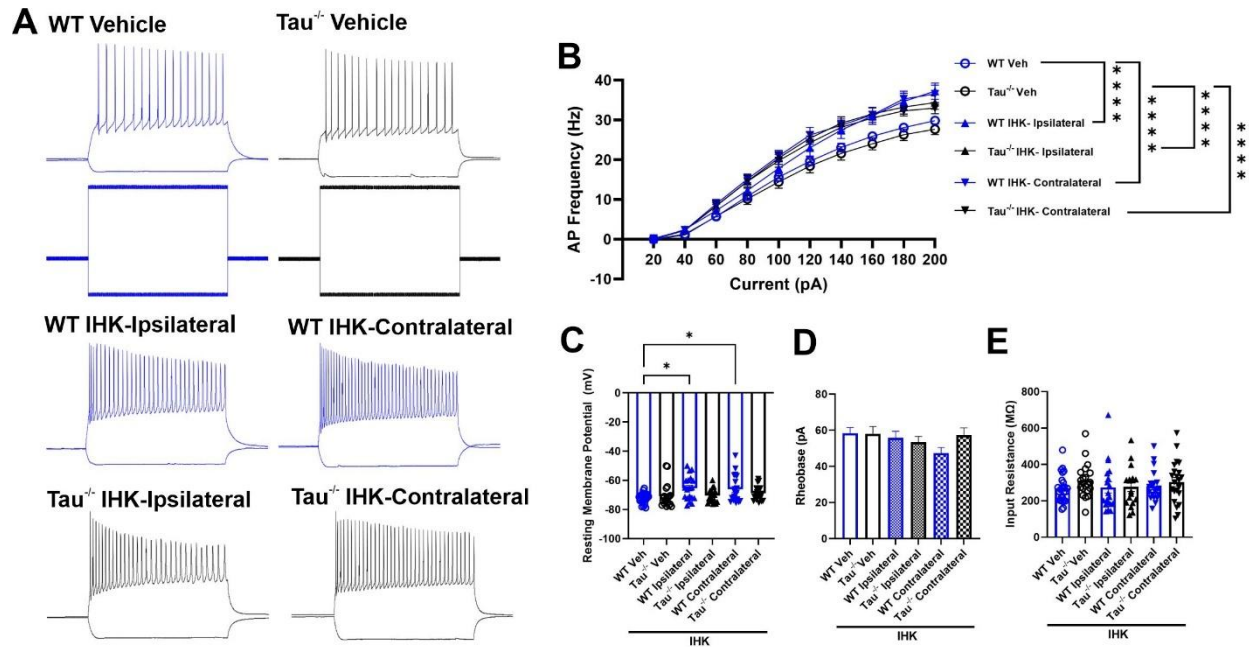


Figure 4.2. Development of TLE increases ventral DGC excitability in all mice. (A) Representative evoked action potential firing of vDGCs in response to current injection steps. (B) Input-output curves of action potential frequencies for vDGCs from vehicle and IHK-treated WT and $\text{tau}^{-/-}$ mice (*, different from vehicle-treated wildtype controls, +, different from vehicle-treated $\text{tau}^{-/-}$ mice; $p < 0.05$, Two-way ANOVA). (C) Resting membrane potential of vDGCs from vehicle and IHK-treated WT and $\text{tau}^{-/-}$ mice ($p > 0.05$, Two-way ANOVA). (D) Rheobase (i.e., minimum current that elicits action potentials) of vDGCs from vehicle and IHK-treated WT and $\text{tau}^{-/-}$ mice ($p > 0.05$, Two-way ANOVA). (E) Input resistance of vDGCs from vehicle and IHK-treated WT and $\text{tau}^{-/-}$ mice. ($p > 0.05$, Two-way ANOVA). WT Vehicle: N=7, n=25; $\text{Tau}^{-/-}$ Vehicle: N=6, n=20; WT IHK: N=10, ipsilateral: n=19, contralateral: n=20 ; $\text{Tau}^{-/-}$ IHK N=11, ipsilateral: n=18, contralateral: n=22).

Table 4.2. Summary of resting membrane potential, rheobase, and input resistance from Ventral DGCs

* different from vehicle wildtype;

** different from vehicle tau^{-/-};

⁺ different from ipsilateral same genotype;

Treatment and Genotype			Ventral DGCs		
			RMV (mV)	Rheobase (pA)	Input Resistance (mOhm)
Wildtype (Ipsilateral and Contralateral)		Veh	-72.16 ± 0.6856 (25)	58.33 ± 3.166 (25)	267.4 ± 15.28 (25)
Tau ^{-/-} (Ipsilateral and Contralateral)		Veh	-70.87 ± 1.784 (20)	58.00 ± 4.078 (20)	311.4 ± 21.02 (20)
Wildtype	Ipsilateral	IHK	-65.34 ± 1.890 (19)*	55.79 ± 3.613 (19)	237.6 ± 30.47 (19)
	Contralateral	IHK	-66.16 ± 2.026 (20)*	47.367 ± 3.138 (20)	280.3 ± 18.78 (20)
Tau ^{-/-}	Ipsilateral	IHK	-70.38 ± 1.087 (18)	53.33 ± 3.234 (18)	276.5 ± 25.40 (18)
	Contralateral	IHK	-68.91 ± 1.007 (22)	57.27 ± 4.012 (22)	302.9 ± 24.79 (22)

Development of TLE in tau^{-/-} mice does not lead to excitatory synaptic reorganization in the ventral dentate gyrus

We recently showed that the development of spontaneous seizures results in significantly increased excitatory synaptic input to dDGCs ipsilateral to the IHK lesion in both wildtype and tau^{-/-} mice (Moseley *et al.*, 2025). We hypothesized that seizure expression or epileptogenesis is modified in mice lacking tau expression due to suppressed excitatory synaptic reorganization distant from the kainate lesion. As an assay of overall excitatory synaptic input (action potential

dependent and independent), we examined the frequency of sEPSCs in vDGCs from tau^{-/-} and wildtype mice that developed TLE. In wildtype mice that developed spontaneous seizures, significantly increased sEPSC frequency was observed in DGCs contralateral to the IHK injection compared to ipsilateral DGCs and DGCs from IHK-treated tau^{-/-} mice (Fig. 4.3D, Table 4.3; p<0.05). Unlike the wildtype mice, development of TLE in mice lacking tau expression did not result in differences in sEPSC frequency compared to vehicle-treated tau^{-/-} mice (Fig. 4.3D, Table 4.3; p>0.05). Interestingly, we found that sEPSC frequency in vDGCs from naive, vehicle-treated tau^{-/-} mice was significantly lower than in wildtype controls (Fig 4.3D, Table 4.3; p<0.05), suggesting tau expression may play a role in excitatory synaptic transmission in the local dentate gyrus network. As in the dorsal dentate gyrus (Moseley *et al.*, 2025), differences in the amplitude of unitary sEPSC events were not detected in the vDGCs across all mice Fig 4.3E, Table 4.3; p>0.05). Thus, increase sEPSC frequency in vDGCs was a feature of epileptogenesis in wildtype mice that was not conserved in tau^{-/-} mice.

To determine if the increase in excitatory synaptic input to ventral DGCs in the wildtype mice that developed TLE was a result of action potential dependent or independent release of glutamate onto ventral DGCs, we assayed mEPSCs in the presence of TTX. In all mice, regardless of treatment or genotype, no significant differences in mEPSC frequency or amplitude were observed in vDGCs from mice with TLE (Fig. 4.3I; Table 4.3; p>0.05). Furthermore, unlike sEPSCs, there was no significant difference in mEPSC frequency between naïve vehicle-treated tau^{-/-} mice and wildtype controls, suggesting that lack of tau expression reduces action-potential dependent glutamate release to vDGCs. Similar to sEPSC event amplitude, there was no

detectable difference in mEPSC amplitude across treatment or genotype (Fig. 4.3J; Table 4.3; $p < 0.05$).

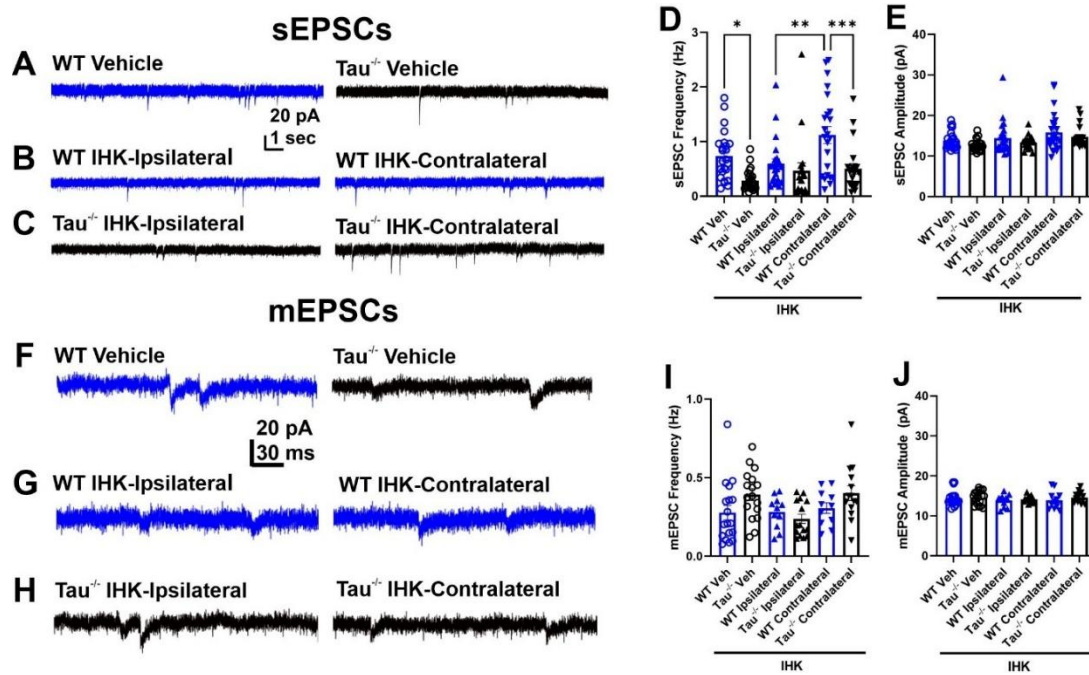


Figure 4.3. Ventral DGCs in mice lacking tau expression do not receive increased synaptic excitability after development of TLE.

(A) Representative traces showing sEPSCs from vehicle-treated WT and $\tau^{-/-}$ mice. (B) Representative sEPSC traces from vDGCs ipsilateral and contralateral to IHK injection in IHK-treated WT mice. (C) Representative sEPSC traces from vDGCs ipsilateral and contralateral to IHK injection in IHK-treated $\tau^{-/-}$ mice. (D) sEPSC frequency from vehicle and IHK-treated wildtype and $\tau^{-/-}$ mice (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Two-way ANOVA). (E) Amplitude of sEPSC events from vehicle and IHK-treated wildtype and $\tau^{-/-}$ mice ($p > 0.05$, Two-way ANOVA). WT Vehicle: $N=7$, $n=25$; $\tau^{-/-}$ Vehicle: $N=8$, $n=25$; WT IHK: $N=10$, ipsilateral: $n=23$, contralateral: $n=23$; $\tau^{-/-}$ IHK $N=10$, ipsilateral: $n=18$, contralateral: $n=21$). (F) Representative mEPSC traces from vehicle-treated wildtype and $\tau^{-/-}$ mice. (G) Representative mEPSCs traces from vDGCs ipsilateral and contralateral to IHK injection in IHK-treated wildtype mice. (H) Representative mEPSC traces from vDGCs ipsilateral and contralateral to IHK injection in IHK-treated $\tau^{-/-}$ mice. (I) mEPSC frequency from vehicle and IHK-treated wildtype and $\tau^{-/-}$ mice ($p > 0.05$, Two-way ANOVA). (J) Amplitude of mEPSC events from vehicle and IHK-treated wildtype and $\tau^{-/-}$ mice ($p > 0.05$, Two-way ANOVA). Traces from WT mice are blue; from $\tau^{-/-}$ are black for display purposes. WT Vehicle: $N=4$, $n=18$; $\tau^{-/-}$ Vehicle: $N=4$, $n=17$; WT IHK: $N=4$, ipsilateral: $n=11$, contralateral: $n=11$; $\tau^{-/-}$ IHK $N=3$, ipsilateral: $n=15$, contralateral: $n=14$).

Table 4.3. Summary of spontaneous and miniature excitatory post-synaptic currents

* different from vehicle wildtype;

** different from vehicle tau^{-/-};

⁺ different from ipsilateral same genotype;

[#] different from contralateral same genotype;

^a different from ipsilateral WT-IHK

^b different from contralateral WT-IHK

Treatment and Genotype			Point Estimate ± SEM (n)			
			sEPSC Frequency (Hz)	sEPSC Amplitude (pA)	mEPSC Frequency (Hz)	mEPSC Amplitude (pA)
Wildtype (Ipsilateral and Contralateral)		Veh	0.7375 ± 0.0905 (25)	13.84 ± 0.4340 (25)	0.2777 ± 0.046 (18)	14.44 ± 0.4665 (18)
Tau ^{-/-} (Ipsilateral and Contralateral)		Veh	0.2833 ± 0.0351 (27)*	12.56 ± 0.2539 (27)	0.3923 ± 0.038 (17)	14.57 ± 0.4185 (17)
Wildtype	Ipsilateral	IHK	0.5930 ± 0.0909 (23) [#]	14.41 ± 0.8492 (23)	0.2813 ± 0.0306 (11)	13.80 ± 0.4747 (11)
	Contralateral	IHK	1.1213 ± 0.1543 (23) ⁺	15.86 ± 1.005 (23)	0.3064 ± 0.0336 (11)	13.89 ± 0.2125 (11)
Tau ^{-/-}	Ipsilateral	IHK	0.4662 ± 0.1451 (18)	13.35 ± 0.4249 (18)	0.2382 ± 0.0293 (15)	14.16 ± 0.2125 (15)
	Contralateral	IHK	0.5038 ± 0.0945 (21) ^b	14.71 ± 0.6170 (21)	0.4010 ± 0.0473 (14)	14.60 ± 0.3630 (14)

Spontaneous seizure development in rodent TLE models and in patients with TLE has been shown to be associated with excitatory synaptic reorganization that is supported by MFS into the inner molecular layer of the dentate gyrus (Franck *et al.*, 1995). It is hypothesized that the sprouting of DGC axons onto other DGCs contributes to recurrent excitation in the circuit leading to a lower spontaneous seizure threshold (Buckmaster & Dudek, 1997a). While it is debated whether the severity of MFS is associated with seizure burden, it is relatively unknown

if modified epileptogenesis contributes to varying degrees of MFS. Mice lacking tau expression develop TLE but at a lower rate with a reduced severity of spontaneous seizures than wildtype controls. In several rodent models of TLE, it has been shown that MFS is detectable in the ventral dentate gyrus once epilepsy develops (Zeidler *et al.*, 2018). Therefore, we investigated whether tau^{-/-} mice exhibit MFS in the ventral dentate gyrus associated with development of TLE after focal injection of kainate in the dorsal hippocampus. To visualize potential MFS, we used Timm's stain that allows for detection of zinc containing axons of DGCs. Timm's scoring was quantified as described in (Cavazos *et al.*, 1991). The presence and distribution of Timm positive granules in the molecular layer was scored on a scale of 0-5 (0: no granules, 1: sparse granule distribution, 2: continuous granule distribution, 3: prominent continuous granules, 4: granules forming a dense laminar band in the molecular layer, 5: confluent dense laminar band extending farther into the molecular layers). After development of TLE, wildtype mice (N=5) and tau^{-/-} mice (N=4) that develop TLE exhibited MFS in the inner molecular layer both ipsilateral and contralateral to IHK injection (Fig. 4.4). Semi-quantitative analysis of Timm's score did not reveal any detectable differences in the severity of MFS in the ventral dentate gyrus between IHK-treated wildtype and tau^{-/-} mice (Fig. 4.4D). Notably, MFS was not observed in vehicle treated mice of either genotype (wildtype: N=2; tau^{-/-} N=2) or in IHK-treated wildtype and tau^{-/-} mice that did not exhibit spontaneous seizures during the video recording period (WT: N=3, Tau^{-/-} N=1). Therefore, development of TLE in the IHK model results in MFS in the ventral dentate gyrus, distant from the focal kainate injection in the dorsal hippocampus. Further, it may be that increased excitatory input to vDGCs does not entirely correlate with the presence of MFS.

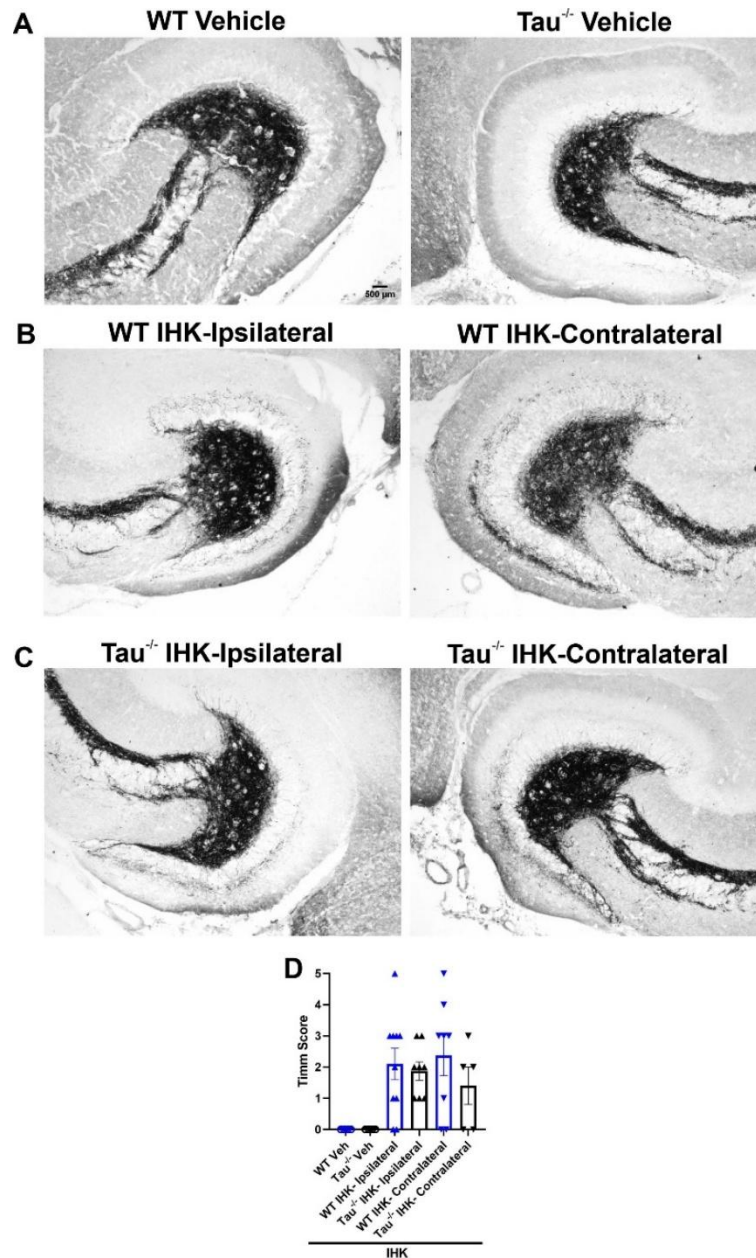


Figure 4.4. After development of TLE mossy fiber sprouting is detected in the ventral dentate gyrus inner molecular layer of IHK treated mice in both genotypes. (A) Representative Timm's stain in the ventral dentate gyrus from vehicle-treated wildtype (WT) and $\tau^{-/-}$ mice (WT: N=2, $\tau^{-/-}$: N=2). (B) Representative Timm's stain in the ventral dentate gyrus from IHK-treated wildtype mice (WT: N=5). (C) Representative Timm's stain in the ventral dentate gyrus from IHK-treated $\tau^{-/-}$ mice ($\tau^{-/-}$: N=5). (D) Semi-quantitative assessment of Timm score of ventral dentate gyrus sections from vehicle and IHK-treated wildtype and $\tau^{-/-}$ mice. 0=no granules, 1=sparse granules in the supragranular layer, 2=more granules in a continuous distribution, 3=prominent granules in the supragranular layer, 4=prominent granules in the supragranular layer forming a dense band, 5=confluent laminar band in the supragranular layer. MFS was detected after IHK in both WT and $\tau^{-/-}$ mice while no MFS was observed in vehicle treated controls.

Ventral DGCs in wildtype mice receive less inhibitory synaptic input but development of TLE increases inhibitory synaptic input in mice lacking tau expression

In our previous work in the dorsal dentate gyrus, we demonstrated that development of TLE in tau^{-/-} mice results in increased inhibitory synaptic input to dorsal DGCs contralateral to the IHK injection compared to vehicle-treated controls; this increase was not observed in wildtype mice (Moseley *et al.*, 2025). We investigated whether similar inhibitory synaptic reorganization was detectable at more distal sites in the ventral dentate gyrus. We recorded sIPSCs in vDGCs as an assay for overall inhibitory synaptic transmission (Fig. 4.5). Unlike sEPSC frequency, and unlike sIPSC frequency in dDGCs (Moseley *et al.*, 2025), there was no difference in sIPSC frequency in wildtype controls that developed TLE compared to vehicle-controls (Fig. 4.5D, Table 4.4; $p > 0.05$), suggesting that inhibitory synaptic input remains intact in the ventral dentate gyrus distant from the focal injury in the dorsal hippocampus. Interestingly, sIPSC frequency was significantly increased both ipsilateral and contralateral to the IHK injection in tau^{-/-} mice that developed TLE compared to vehicle treated controls (Fig. 4.5D, Table 4.4; $p < 0.05$), suggesting inhibitory synaptic reorganization previously seen in the dorsal contralateral hemisphere in tau^{-/-} mice occurs throughout the dentate gyrus in these mice. Additionally, like sEPSC frequency, sIPSC frequency was also significantly lower in naïve, vehicle-treated tau^{-/-} mice compared to wildtype controls (Fig. 4.5D, Table 4.4; $p < 0.05$), like what we observed in the dorsal dentate gyrus (Moseley *et al.*, 2025). There was no detectable difference in sIPSC event amplitude across genotypes or treatments (Fig. 4.5E, Table 4.4; $p > 0.05$).

To assess action potential independent inhibitory synaptic events, we recorded mIPSCs in the presence of TTX (Fig. 4.5). After development of TLE, mIPSC frequency was significantly

lower both ipsilateral and contralateral to the IHK injection in wildtype mice compared to vehicle treated controls (Fig. 4.5I, Table 4.4; $p < 0.05$). Like sIPSC frequency, mIPSC frequency was significantly increased in $\tau^{-/-}$ mice that developed TLE compared to vehicle-treated $\tau^{-/-}$ mice (Fig. 4.5I, Table 4.4; $p < 0.05$). Together this suggests that after the development of TLE, there is a potential loss of functional inhibitory synapses and/or reduced vesicular release of GABA onto vDGCs in mice that express tau, whereas development of TLE in mice lacking tau expression results in increased inhibitory synaptic transmission bilaterally. Interestingly, sIPSC frequency and mIPSC frequency were significantly lower in vehicle-treated $\tau^{-/-}$ mice compared to wildtype controls (Fig. 4.5I, Table 4.4; $p < 0.05$). There was no significant difference detected in mIPSC event amplitude between IHK-treated mice of either genotype that developed TLE compared to their vehicle-treated controls, consistent with preserved postsynaptic GABA receptor function in vDGCs even after epilepsy develops (Fig. 4.5J, Table 4.4; $p > 0.05$). This highlights a potential mechanism of tau protein's endogenous function in inhibitory synaptic transmission in which lack of tau expression may suppress the function of GABAergic synapses normally and contribute to epileptogenesis, whereas TLE development increases functional interneuron connectivity with DGCs in the local dentate gyrus network, possibly in a compensatory manner, once epilepsy develops.

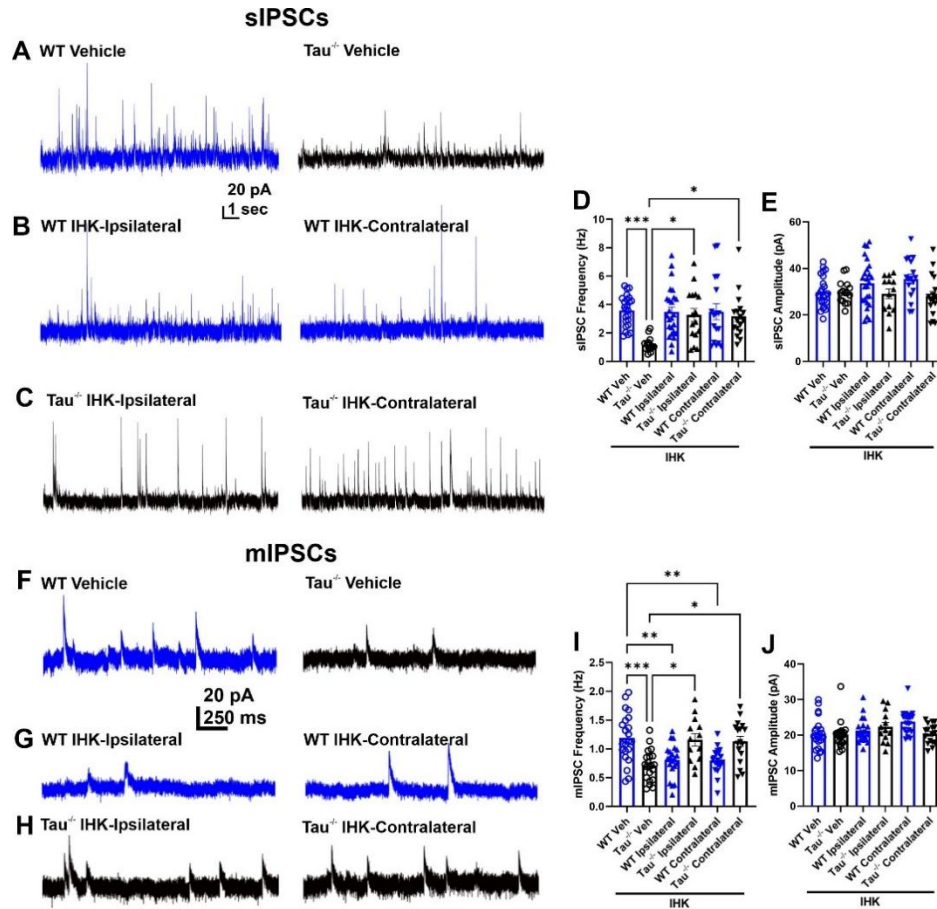


Figure 4.5. Ventral DGCs in mice lacking tau expression receive less inhibitory synaptic input and development of TLE increases inhibitory input in $\tau^{-/-}$ but not WT mice. (A) Representative traces showing sIPSC from vehicle-treated wildtype (WT) and $\tau^{-/-}$ mice. (B) Representative sIPSC traces from vDGCs ipsilateral and contralateral to IHK injection in IHK-treated wildtype mice. (C) Representative sIPSC traces from vDGCs ipsilateral and contralateral to IHK injection in IHK-treated $\tau^{-/-}$ mice. (D) sIPSC frequency from vehicle and IHK-treated wildtype and $\tau^{-/-}$ mice (* $p < 0.05$, *** $p < 0.001$, Two-way ANOVA). (E) Amplitude of sIPSC events from vehicle and IHK-treated wildtype and $\tau^{-/-}$ mice ($p > 0.05$, Two-way ANOVA). WT Vehicle: N=7, n=24; $\tau^{-/-}$ Vehicle: N=8, n=15; WT IHK: N=10, ipsilateral: n=25, contralateral: n=17; $\tau^{-/-}$ IHK N=10, ipsilateral: n=16, contralateral: n=17). (F) Representative mIPSC traces from vehicle-treated wildtype and $\tau^{-/-}$ mice. (G) Representative mIPSCs traces from vDGCs ipsilateral and contralateral to IHK injection in IHK-treated wildtype mice. (H) Representative mIPSC traces from vDGCs ipsilateral and contralateral to IHK injection in IHK-treated $\tau^{-/-}$ mice. (I) mIPSC frequency from vehicle and IHK-treated wildtype and $\tau^{-/-}$ mice (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Two-way ANOVA). (J) Amplitude of mEPSC events from vehicle and IHK-treated wildtype and $\tau^{-/-}$ mice ($p > 0.05$, Two-way ANOVA). WT Vehicle: N=3, n=18; $\tau^{-/-}$ Vehicle: N=4, n=17; WT IHK: N=7, ipsilateral: n=11, contralateral: n=11; $\tau^{-/-}$ IHK N=5, ipsilateral: n=15, contralateral: n=14).

Table 4.4. Summary of spontaneous and miniature inhibitory post-synaptic currents

* different from vehicle wildtype;

** different from vehicle tau^{-/-};⁺ different from ipsilateral same genotype;[#] different from contralateral same genotype;^a different from ipsilateral WT-IHK^b different from contralateral WT-IHK

Treatment and Genotype			Point Estimate ± SEM (n)			
			sIPSC Frequency (Hz)	sIPSC Amplitude (pA)	mIPSC Frequency (Hz)	mIPSC Amplitude (pA)
Wildtype (Ipsilateral and Contralateral)		Veh	3.579 ± 0.2226 (24)	29.83 ± 1.360 (24)	1.923 ± 0.0903 (22)	20.41 ± 0.9307 (22)
Tau ^{-/-} (Ipsilateral and Contralateral)		Veh	1.175 ± 0.1288 (15)*	29.83 ± 1.294 (15)	0.7141 ± 0.0577 (21)*	20.12 ± 0.8074 (21)
Wildtype	Ipsilateral	IHK	3.482 ± 0.3475 (25)	33.63 ± 2.014 (25)	0.8066 ± 0.0626 (21)*	21.27 ± 0.7384 (21)
	Contralateral	IHK	3.498 ± 0.5692 (17)	35.44 ± 2.102 (17)	0.7995 ± 0.0491 (20)*	23.79 ± 0.7460 (20)
Tau ^{-/-}	Ipsilateral	IHK	3.263 ± 0.4572 (16) ⁺	29.10 ± 2.140 (16)	1.158 ± 0.1085 (13) ⁺	22.30 ± 1.240 (13)
	Contralateral	IHK	3.181 ± 0.3824 (17) [#]	28.05 ± 2.141 (17)	1.130 ± 0.0879 (17) [#]	20.38 ± 0.6688 (17)

Discussion

Genetic deletion or suppression of the microtubule associated protein, tau has been shown to reduce seizure burden in models of genetic epilepsies and to suppress evoked seizures in rodents (DeVos *et al.*, 2013; Holth *et al.*, 2013; Gheyara *et al.*, 2014; Shao *et al.*, 2022a). The etiologies between genetic and acquired TLE models are different, in that spontaneous seizures

in acquired epilepsies develop after a delay following an insult compared to seizures in genetic epilepsies that are a consequence of voltage-gated channel mutations. We have shown that genetic deletion of tau reduces the likelihood of evoked seizures and inhibits seizure severity but does not prevent the epileptogenic process involved in acquired epilepsy in the IHK model of TLE (Moseley *et al.*, 2025). Therefore, lack of tau expression does not prevent the epileptogenic process from occurring but is hypothesized to attenuate network activity of individual spontaneous seizures. In this study, we demonstrate a potential mechanism within the dentate gyrus local circuitry in which lack of tau expression may modify the development of epilepsy and reduce spontaneous seizure burden in a manner consistent with synaptic reorganization of inhibitory circuitry. Tau^{-/-} mice that develop TLE not only exhibit inhibitory synaptic reorganization, resulting in increased inhibitory synaptic tone in DGCs at sites distant from the initial focal insult, but also do not exhibit widespread excitatory synaptic neuroplastic changes that are associated with epileptogenesis in this and similar TLE models and in human TLE (Franck *et al.*, 1995; Buckmaster *et al.*, 2009; Buckmaster *et al.*, 2022). Further, we uncovered unappreciated functions of murine tau in normal excitatory and inhibitory synaptic transmission in the dentate gyrus circuit.

In rodent models of acquired TLE, DGCs develop increased action potential output, suggesting that epilepsy development promotes hyperactivity of DGCs that may result from recurrent excitatory synaptic connections between DGCs, which is linked to sprouting of axon collaterals of DGCs themselves (Cronin *et al.*, 1992b; Althaus *et al.*, 2015; Kelly & Beck, 2017). Few studies have examined excitability changes of DGCs associated with TLE using the IHK model. Interestingly, our results demonstrate a significant decrease in excitability of dDGCs near the IHK injection where severe hippocampal damage and DGC dispersion are detected, as

previously described (Moseley *et al.*, 2025). It is possible that development of spontaneous seizures lowers the excitability of DGCs near the focal injury, suggesting that spontaneous seizures that develop in this model may not initiate in the ipsilateral hemisphere near the injury site. Even so, future studies are required to elucidate potential alterations to voltage-gated channel expression and conductance that may be responsible for reduced excitability in dDGCs.

Here, we investigated whether there are changes in intrinsic excitability of DGCs in the ventral dentate gyrus associated with epilepsy development in the IHK model. We demonstrated that evoked action potential firing frequency of vDGCs is increased in all mice that develop epilepsy both ipsilateral and contralateral to the focal injection compared to vehicle controls, regardless of tau expression. Interestingly, vDGCs ipsilateral and contralateral to IHK injection in wildtype mice that developed TLE exhibited a depolarized resting membrane potential compared to vehicle-treated controls and tau^{-/-} mice that developed TLE, but other intrinsic properties were unaffected. Together these results highlight an associative effect between TLE development and increased excitability of DGCs at sites distal to the focal injury, indicating a potential widespread effect of epileptogenesis on neuronal excitability throughout the dentate gyrus, regardless of tau expression. Further studies are required to delineate mechanisms by which epileptogenesis modifies ion channel expression responsible for the depolarization of DGC membrane potential in mice expressing tau.

Development of spontaneous seizures after epileptogenesis is associated with an increase in excitatory synaptic input to DGCs in the dorsal dentate gyrus, as we and others have shown in rodent models of acquired TLE (Cronin *et al.*, 1992b; Buckmaster & Dudek, 1997a; Wuarin & Dudek, 2000; Sutula & Dudek, 2007; Hunt *et al.*, 2010a; Althaus *et al.*, 2016; Moseley *et al.*, 2025). In the context of tau deletion and epileptogenesis, we have shown that while there is an

increase in excitatory input to dDGCs ipsilateral to the kainate injection in both wildtype and tau^{-/-} mice, the increase was similar between the two genotypes. Although epilepsy development increases excitatory input to dDGCs near the IHK injection site similarly across the genotypes, mice lacking tau expression exhibit less severe spontaneous seizure expression. This supports a role for excitatory synaptic reorganization in the evolution of spontaneous seizure development in the IHK model, but it does not intuitively account for the difference in expression of individual seizures across the genotypes once TLE has developed. Here, we found no detectable change in excitatory input to vDGCs in mice lacking tau expression after epileptogenesis, whereas a significant increase in excitatory input was detected in wildtype mice after development of TLE, suggesting an attenuation of the TLE associated excitatory synaptic reorganization in the ventral dentate gyrus in tau^{-/-} mice that could contribute to lower seizure expression in this genotype. To investigate if the modified excitatory input to vDGCs is associated with excitatory synaptic reorganization, we measured excitatory synaptic events independent of action potentials. Interestingly, there was no change in stochastic glutamatergic events regardless of epileptogenesis or tau expression, suggesting the observed excitatory synaptic changes arise from activity of afferent excitatory neurons such as those in the entorhinal cortex or hilar mossy cells in mice expressing endogenous tau protein that is not observed in the absence of tau expression. While excitatory synaptic reorganization of DGCs is associated with MFS after TLE development, we did not observe any obvious differences in MFS between IHK-treated wildtype and tau^{-/-} mice. It has been shown that sprouted axons of DGCs can synapse onto other DGCs, increasing their overall excitatory synaptic input. Even so, our results indicating an increase in excitatory synaptic input to vDGCs in wildtype but not tau^{-/-} mice is inconsistent with the similar MFS in the molecular layer of the dentate gyrus. Differences in

sprouted mossy fiber synapse maturity or in the dynamics of the synapses themselves may account for the mismatch between the anatomical and electrophysiological outcomes. Even so, our results demonstrate that overall excitatory synaptic input is increased to DGCs in the ventral hippocampus in the IHK model once epilepsy develops but tau deletion does not significantly increase excitatory input to vDGCs in mice with TLE. Further, DGCs in the ventral dentate gyrus in naïve tau^{-/-} mice receive less overall excitatory synaptic input compared to wildtype controls, highlighting a role of tau protein in glutamatergic signaling upstream of the dentate gyrus that may be associated with decreased susceptibility to evoked seizure expression and SE that we and other have observed in tau^{-/-} mice (Li *et al.*, 2014b; Putra *et al.*, 2020; Shao *et al.*, 2022a; Moseley *et al.*, 2025).

Development of seizures in rodent models of TLE often results in a significant loss of inhibitory synaptic input to DGCs, usually due to a loss of select groups of inhibitory interneurons (Buckmaster & Dudek, 1997a; Kobayashi & Buckmaster, 2003b; Hunt *et al.*, 2011; Butler, 2017; Moseley *et al.*, 2025). We recently showed that development of TLE is associated with an increase in inhibitory synaptic input in dDGCs contralateral to the IHK injection in mice lacking tau expression, suggesting that inhibitory synaptic reorganization is possible at sites distal to the focal injury (Moseley *et al.*, 2025). It has been proposed that increased synaptic excitation of GABAergic interneurons due to sprouted mossy fibers or other afferents synapsing on interneurons in the dentate gyrus may partially compensate for interneuron loss in rodent models of acquired TLE and post-traumatic epilepsy (Sloviter, 1992; Hunt *et al.*, 2011; Butler *et al.*, 2015; Kang *et al.*, 2022a). In this study, we found that overall inhibitory input was preserved in the ventral dentate gyrus in wildtype mice, suggesting survival of inhibitory interneurons distal to the kainate injection, even in the ipsilateral hemisphere. Vulnerability of cholecystokinin

(CCK)-expressing interneurons has been reported in the ipsilateral ventral CA1 in epileptic mice after IHK injection, but synaptic connectivity of surviving interneurons with CA1 pyramidal cells was preserved (Kang *et al.*, 2021b). While overall inhibitory synaptic input was unchanged in epileptic wildtype mice here, we found a decrease in mIPSC frequency after development of TLE, suggesting a loss of functional inhibitory synapses onto vDGCs in wildtype mice.

Conversely, there is an increase in inhibitory synaptic input to vDGCs after development of TLE in mice lacking tau expression, both ipsilateral and contralateral to the IHK injection. Together with findings from the dorsal dentate gyrus, this suggests that epileptogenesis results in increased inhibitory synaptic signaling at sites distant from the focal injury in tau^{-/-} mice that does not occur in wildtype mice. Mice lacking tau expression are less likely to develop spontaneous seizures, and when the epileptogenic process occurs, spontaneous seizures are less severe. The increase in inhibitory tone in DGCs may contribute to this difference in seizure expression. Increases in inhibitory input have been associated with axon sprouting of surviving inhibitory interneurons (Halabisky *et al.*, 2010) and could also involve increased DGC input to surviving interneurons (Kang *et al.*, 2021b; Kang *et al.*, 2022a). Further experiments are required to determine the nature of the synaptic connections between surviving inhibitory interneurons and vDGCs in mice lacking tau expression.

Conclusions

In this study we have shown that neuroplastic changes associated with TLE development in the IHK model are detectable in the ventral dentate gyrus. Additionally, we identified a potential synaptic mechanism involving inhibitory synaptic reorganization by which lack of tau expression confers protection from seizures but not from the process of epileptogenesis. At the same time, we have identified a potential role of tau protein in synaptic inhibition in the dentate

gyrus Further studies are required to elucidate tau protein's role in the inhibitory circuitry within the ventral dentate gyrus that will allow for the potential identification of targets to either suppress the epileptogenic process after an insult or modify seizure expression once epilepsy has already developed.

CHAPTER 5: INHIBITORY SYNAPTIC REORGANIZATION IN THE VENTRAL DENTATE GYRUS AFTER DEVELOPMENT OF TLE IN MICE LACKING TAU EXPRESSION

Introduction

Previous studies have highlighted a role of tau protein in epileptogenesis and associated neuroplasticity in the dentate gyrus using mice with genetic deletion of tau protein (Moseley *et al.*, 2025). Specifically, we demonstrated a role for tau protein in inhibitory synaptic transmission within the hippocampal circuit that may be responsible for in mice lacking tau expression. This suggests that tau protein is involved in the cellular and synaptic mechanisms that are involved in epileptogenesis and that the absence of tau somehow suppresses these mechanisms.

Tau protein has been shown to be important for cellular processes like axon transport, microtubule stabilization and synaptic plasticity (Liang *et al.*, 2009; Arendt *et al.*, 2016). Most studies examine inhibition in the context of tau primarily focus on tauopathies. For example, impaired network oscillations observed in models of Alzheimer's disease likely occur from dysfunction in inhibitory synaptic signaling and hyperphosphorylated tau expression suppresses GABAergic neurotransmission and alters excitability of interneurons in the hippocampus (Chung *et al.*, 2020; Zheng *et al.*, 2020). Other studies have identified a role of tau protein in excitatory synaptic signaling, which is suggested to underlie the protective effects of lack of tau expression on evoked seizures (Shao *et al.*, 2022a; Voskobiynyk *et al.*, 2024). Even so, few studies have recognized a role of endogenous tau protein in the hippocampal circuit and specifically in inhibitory signaling using a model for TLE epileptogenesis (Moseley *et al.*, 2025).

Loss of inhibition in the dentate gyrus in rodent models of TLE and patients is associated with epileptogenesis and seizures (Sloviter, 1987; Buckmaster & Dudek, 1997a; Maglowsky *et al.*, 2000; Lee *et al.*, 2024a). Few studies have examined inhibition changes after development of TLE in the ventral hippocampus in the IHK model, where kainate is injected into the dorsal hippocampus. We have previously shown that development of TLE in mice lacking tau expression results in increased inhibitory input to vDGCs which may be due to increased excitability of interneurons, increase presynaptic release of GABA at synapses, or recruitment of more inhibitory synapses from interneurons (Sloviter, 1987; Bausch, 2005; Halabisky *et al.*, 2010; Peng *et al.*, 2013; Lee *et al.*, 2024a). Therefore, this study sought to investigate the nature of widespread inhibitory neuroplasticity associated with epileptogenesis in mice with genetic deletion of tau.

Here, we used the IHK model of acquired TLE and expression of an adenoassociated virus with channelrhodopsin (ChR2) to access inhibitory synaptic transmission between individual inhibitory interneurons and vDGCs in tau^{-/-} mice. We tested the hypothesis that increased inhibitory synaptic input to vDGCs observed in tau^{-/-} mice after development of TLE is a result of an increase in inhibitory synapses from sprouted inhibitory interneurons and increase in synaptic strength of connecting interneurons and vDGCs.

Methods

Animals

Transgenic B6.Cg-Maptm1(EGFP)KltTg(MAPT)8cPdav/J mice were produced in house from breeders obtained from The Jackson Laboratory (JAX; Bar Harbor, ME; stock #005491).

All breeding mice were homozygous for the 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. Offspring were either full tau knockout ($\tau^{-/-}$) or express only human tau (htau), and genotypes and protein expression phenotypes were confirmed in our recent reports (Cloyd et al., 2021, Moseley et al., 2025). Here, we used $\tau^{-/-}$ mice and isogenic C57BL/6J wildtype control mice that express murine tau protein. Age matched male C57BL/6J control mice were bred in house from breeders obtained from JAX (#000664). All mice were housed under a 12 hr light / 12 hr dark cycle in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved facility. Food and water were available ad libitum. The Colorado State University Laboratory Animal and Use Committee approved all procedures. Principles outlined in the Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines and the Basel declaration, including the 3R (Replacement, Reduction, Refinement) concept, were considered when planning the experiments.

Intrahippocampal kainate model of TLE

All surgical procedures were performed under isoflurane general anesthesia with 0.05% bupivacaine local anesthesia. Kainic acid (120 nL, 20 mM in 0.9% saline, Tocris Bioscience; Minneapolis, MN) or saline (sham; 120 nL, 0.9% saline) was injected into the left dorsal hippocampus in male and female $\tau^{-/-}$ and wildtype mice between 8 and 10 weeks of age, as described previously (Moseley *et al.*, 2025). After injection, mice were monitored for status epilepticus (SE) and diazepam (7.5 mg/kg) was administered intraperitoneally to terminate SE 4 hours after recovery.

Development of TLE with the expression of spontaneous seizures in mice was determined by a continuous 72 hr video recording session at 5-6 weeks after the IHK or vehicle

injection. Mice lacking tau expression develop TLE but at a lower rate than non-transgenic controls in this model (Moseley *et al.*, 2025). All IHK-treated mice used in this study exhibited at least one spontaneous seizure during the observed video-recording period and were considered to have developed TLE. We included both males and females from both genotypes and treatments (Vehicle: WT: Males N=7, Females N=11; tau^{-/-}: Males N=9, Females N=8. IHK: WT: Males N=6, Females N=8; tau^{-/-}: Males N=10, Females N=6).

Adeno-associated viral injections in the ventral hippocampus

In the same surgery as saline or kainate injections, 500 nL of rAAV-mDlx-ChR2-mCherry-Fishell-3 (Addgene; #83898-AAV9) was injected bilaterally into the ventral hippocampus (-3.5 mm posterior; ±2.8 mm medial/lateral, -3.8 mm ventral). The injection rate was 10 nL/minute, and the needle (Hamilton syringe) was left in place 5 minutes before and after injection.

Whole-cell patch clamp electrophysiology

300 µm horizontal slices containing the ventral hippocampus from vehicle and IHK-treated wildtype and tau^{-/-} mice 6-8 weeks after IHK or saline injection were prepared in cold, oxygenated sucrose artificial cerebrospinal fluid (ACSF). These slices were incubated prior to being transferred to a recording chamber containing oxygenated ACSF (in mM): 124 NaCl, 26 NaHCO₃, 11 glucose, 3 KCl, 2 CaCl₂, 1.3 MgCl₂, and 1.4 NaH₂PO₄; at 32-34°C for recordings.

Electrophysiological recordings were performed as previously described (Moseley *et al.*, 2025) using a Multiclamp 700B amplifier (Molecular Devices, San Jose, CA) and acquired using the pClamp software suite (Molecular Devices). Recordings were discarded if series resistance changed by more than 20% during the recording or reached 25 MΩ. Recording pipettes were

pulled from borosilicate glass (open tip resistance 3-5 M Ω ; King Precision Glass Co., Claremont, CA). Whole cell-patch clamp recordings were obtained from ventral DGCs (vDGCs) identified by location in the granule cell layer and morphology. Recordings were obtained from mCherry expressing inhibitory interneurons.

To examine interneuron mediated inhibition to vDGCs, we used mice expressing pAAV-mDlx-Ch2-mCherry to optogenetically stimulate interneurons while recording vDGCs with 1 mM kynurenic acid (Sigma-Aldrich). The optogenetic experiments were performed using a pipette recording solution which contained (in mM) 140 Cs-gluconate, 10 HEPES, 1 NaCl, 2 MgATP, 1 MgCl₂, 1 CaCl₂, and 5 EGTA; pH adjusted to 7.2 with CsOH with an osmolarity of 290-295 mOsm. While recording vDGCs, in a stereotyped fashion mCherry expressing interneurons were activated using blue light centered on the soma of the cell through the 40x objective. To limit the stimulation of more than one mCherry expressing interneuron and the dendrites or axons of other interneurons, the field stop was closed to prevent stray blue light from activating a larger diameter and ensures light activation of a diameter of ~30 μ m. Evoked inhibitory post-synaptic currents (eIPSCs) were measured at 0 mV. To evaluate a successful connection between an interneuron and vDGCs, 5 light stimulations (10 ms duration, inter-stimulus interval of 1 sec) were delivered to an interneuron while recording a vDGCs. A successful connection was defined as at least 3 eIPSCs per 5 stimulations as previously described (Hunt *et al.*, 2010a). The average eIPSC amplitude was calculated for only successful connections across sequential stimulations. To determine short-term plasticity of GABAergic synapses from connecting interneurons and vDGCs, interneurons were stimulated with high-frequency light stimuli trains (10 light stimulations, 2 ms duration, 10 ms interstimulus interval at 50 Hz). Percent change in eIPSC amplitude was measured using Clampfit where evoked

IPSCs with an amplitude greater than 30 pA were included as evoked responses. In addition, intrinsic properties and efficacy of optogenetic activation of mCherry expressing interneurons were measured in current clamp using a recording pipette solution that contained (in mM) 130 K-gluconate, 10 HEPES, 1 NaCl, 2 MgATP, 1 MgCl₂, 1 CaCl₂, and 5 EGTA; pH adjusted to 7.2 with KOH with an osmolarity of 290-295 mOsm. Action potentials were evoked in mCherry-expressing interneurons from -60 mV by 5 light stimuli (10 ms duration, 1 second interval). Intrinsic properties were measured from voltage responses to 1 sec hyperpolarizing current steps (-200 pA to 300 pA) from -60 mV holding potential.

Excitatory synaptic input to mCherry expressing interneurons was measured in voltage clamp using a pipette solution of Cs-gluconate. Spontaneous excitatory post synaptic currents (sEPSCs) were recorded at a holding potential of -70 mV. Recordings were analyzed using Mini Analysis (version 6.0.7, Synaptosoft Inc.). Synaptic current amplitude was required to be greater than three times root mean square noise level to be included in sEPSCs frequency and amplitude analyses.

Immunostaining

Ventral hippocampal sections used for electrophysiology recordings were fixed in 4% paraformaldehyde overnight at 4°C. After fixation, slices were rinsed in PBS and cryoprotected in 30% sucrose in PBS overnight until they sunk. Slices were then re-sectioned at 50 µm for immunostaining. For immunostaining, sections were permeabilized for 1 hour using 0.3% Triton X-100 in PBS then blocked in 10% Normal Goat Serum in 0.1% Triton X-100 for 1 hour at room temperature. After blocking, sections were incubated in primary antibody (2% Normal Goat Serum in 1% Triton X-100 in PBS) overnight at 4°C. After washing with PBS, sections were

incubated in secondary antibody (2% Normal Goat Serum in 0.02% Triton X-100 in PBS then washed and mounted using DAPI Mounting Medium (Vectashield). Primary antibodies were mouse anti-GAD67 (1:1000; Millipore, MAD5406) and rabbit anti-mCherry (1:1500; Thermo Fisher, PA5-34974). Secondary antibodies were goat anti-mouse 680 (1:1000, Thermo Fisher) and goat anti-rabbit 594 (1:1000, Thermo Fisher).

For identification of stimulated mCherry expressing inhibitory interneurons 2 mg/ml of biocytin was dissolved in K-gluconate internal solution. Cells were recorded in current clamp with injected current steps to allow for diffusion of biocytin through the axons and dendrites of the recorded cell. After filling, slices were placed in 4% paraformaldehyde overnight prior to washing in 1x phosphate buffered saline (PBS) 3 times for 10 minutes each. To visualize recorded cells, slices were either incubated in AMCA (Rockland; 1:1000) or Fluorescein (Vector Laboratories; 40 mg/ml) overnight at 4°C. Slices were then rinsed in 1x PBS then went through a passive clearing protocol. After biocytin labeling, slices were incubated in Delipidation Buffer (LifeCanvas Technologies) for 2 hours at room temperature then multiple washes in PBS prior to incubation in 50% and 100% of EasyIndex solution (LifeCanvas Technologies) for 30 minutes sequentially. Slices were mounted on conclave slides in 100% Easy Index.

Statistical Analysis

Statistical measures were performed with Prism (GraphPad, San Diego, CA). Electrophysiological data were analyzed using one or two-way ANOVA with Tukey test for multiple comparisons. Data are presented as mean $\pm$ SEM and statistical significance was set to $p < 0.05$ for all tests. All statistical tests are shown in the Statistics Table.

Results

Experimental design

To measure GABAergic synapses in the ventral dentate gyrus, we bilaterally injected an AAV with ChR2 fused to mCherry (AAV9-mDlx-ChR2-mCherry) into the ventral dentate gyrus in the same surgery as the IHK (Fig. 5.1A). The expression is driven under the Dlx promoter which allows for viral transfection in GABAergic interneurons with no obvious specificity for interneuron sub-type (Dimidschstein *et al.*, 2016) (Fig.5.1B). We found no obvious difference in mCherry expression across genotypes or treatments with approximately 14-18 mCherry positive interneurons per horizontal slice.

To assess how development of TLE modifies inhibitory synapses on vDGCs in the dentate gyrus, we took advantage of the ChR2 fusion to mCherry expressing interneurons to selectively activate interneurons and record evoked inhibitory post-synaptic currents (eIPSCs) in the presence of 1 mM kynurenic acid to prevent feedback excitation and overall excitatory synaptic effects, isolating GABAergic synapses from stimulated interneurons. We optogenetically stimulated ChR2-mCherry positive interneurons located near the granule cell layer or hilar interneurons to assay overall GABAergic synapses from many different interneuron subtypes. The effective light intensity minimally required to evoke 1-2 action potentials when stimulating and recording ChR2-mCherry positive interneurons was used to measure successful connections to vDGCs (Fig.5.2C-D). Differences in light evoked action potentials were not observed across genotypes or treatment, suggesting similar excitability of interneurons and efficacy of ChR2 stimulation. We determined that a light stimulation of at least 10 ms was the minimal duration required to elicit reliable and repeatable evoked action potentials in interneurons. A successful connection between stimulated interneurons and vDGC was determined by the light evoked

IPSCs (eIPSCs) in vDGCs (Fig. 5.1C-D). Similar to light evoked evoked APs, recordings of current evoked action potentials did not reveal any obvious difference in excitability in inhibitory interneurons. It is possible that differences in excitability were not detected due to varying interneuron subtypes located in the dentate gyrus.

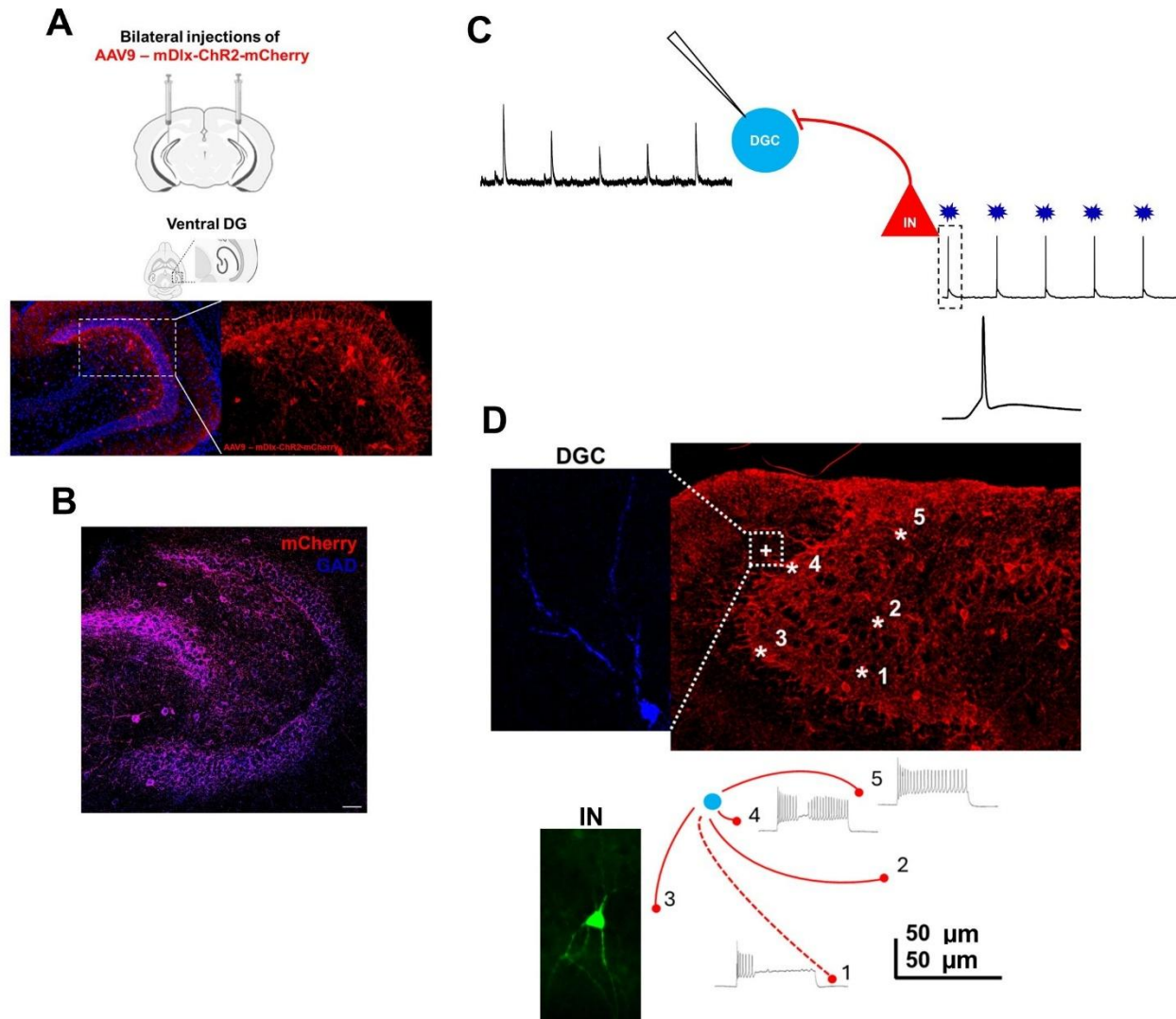


Figure 5.1. Experimental design of optogenetic stimulation of ChR2-mCherry expressing interneurons. (A) Bilateral injections of AAV9-mDlx-ChR2-mCherry virus into the ventral dentate gyrus (DG) results. Representative image of viral expression and DAPI. (B) Representative immunostaining of mCherry virus expression and GAD67. (C) Optogenetic stimulation of interneurons and recording DGCs. 5 blue light stimulations (10 ms duration, 1 sec intervals) elicits action potentials in ChR2 expressing interneurons. Successful connections between stimulated interneuron and DGC results in evoked IPSCs corresponding to action potentials. (D) Experimental design. (a) Biocytin filled DGC, recorded at + positive on slice. (b) Representative image of ventral DG slice with identified stimulated interneurons (*). (c) Schematic of actual micron distance of recorded DGC (blue) and stimulated interneurons (red: 1-5) represented in (b). Solid red line represents successful connections (2-5). Dashed red line represents unsuccessful connection (1). Recorded evoked action potentials in stimulated interneurons (2,4,5) with current injections of 240 pA. Image of biocytin filled (3) interneuron.

Development of TLE does not alter excitatory synaptic input to inhibitory interneurons in the ventral dentate gyrus

Our previous work suggests that development of TLE in mice lacking tau expression is associated with increased inhibitory synaptic input to DGCs (Moseley *et al.*, 2025). Studies have shown in rodent models of TLE that inhibitory interneurons can receive increased excitatory input from sprouted synapses or boutons from DGCs (Buckmaster & Jongen-Relo, 1999; McNeill *et al.*, 2003; White *et al.*, 2004; Halabisky *et al.*, 2010). Therefore, we wanted to test the hypothesis that the observed increase in inhibitory synaptic input to vDGCs after development of epilepsy in mice lacking tau expression, may be in part due to recurrent excitation to inhibitory interneurons.

Using AAV-Dlx-ChR2-mCherry expression in all GABAergic interneuron subtypes, we recorded spontaneous excitatory post-synaptic currents (sEPSCs) in mCherry labelled cells (Fig. 5.2). Interestingly, our assay of sEPSCs in wildtype and tau^{-/-} demonstrated no significant changes in sEPSC frequency or amplitude in interneurons (Fig. 5.2 D-E). This suggests that tau expression nor the development of epilepsy, modifies excitatory synaptic input to interneurons, indicating that tau protein does not play a role in excitatory synaptic transmission from DGCs, unlike previous studies have suggested (Shao *et al.*, 2022a). Therefore, it is likely that the modified inhibitory synaptic input to vDGCs after development of TLE is due to changes in the nature of the GABAergic synapses onto vDGCs, rather than excitatory input upstream from either vDGCs or surviving mossy cells. Even so, future studies are required to determine potential excitatory synaptic input to specific interneuron subtypes.

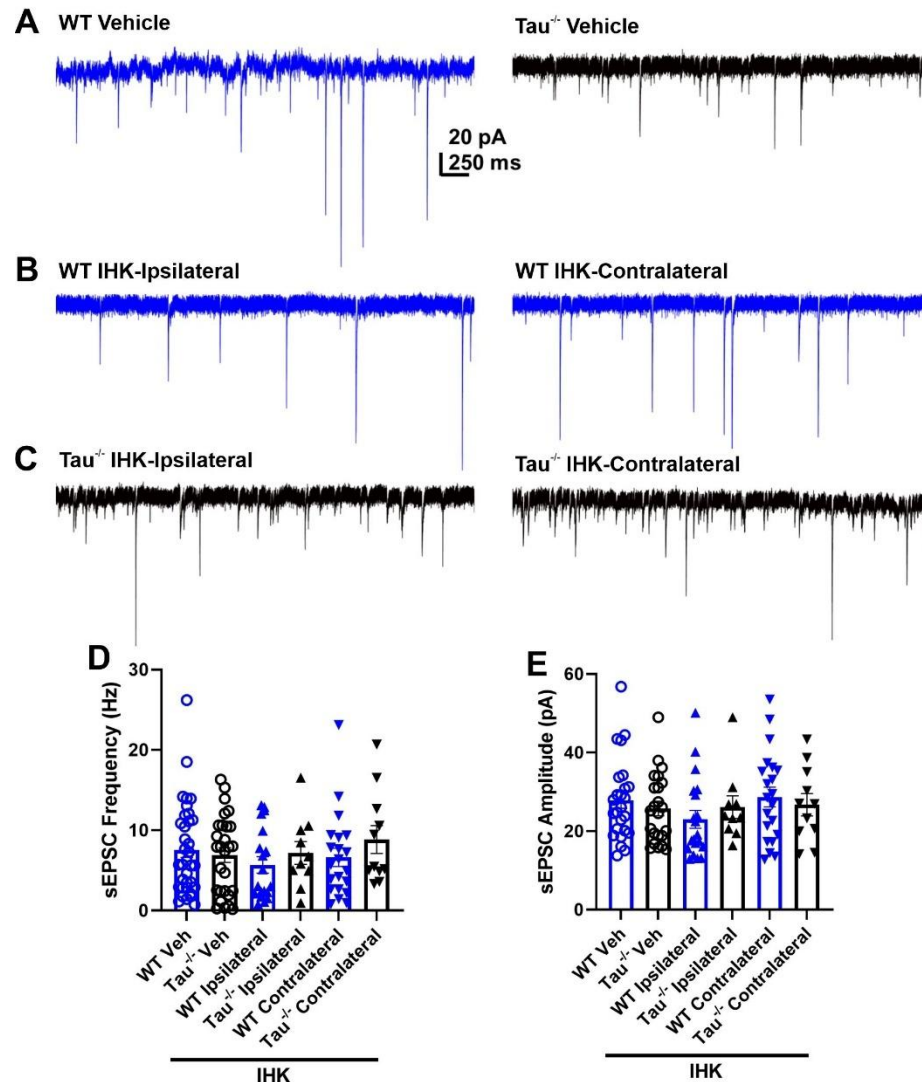


Figure 5.2. Development of TLE does not alter excitatory synaptic input to inhibitory interneurons in the ventral dentate gyrus (A) Representative traces showing sEPSCs recorded in interneurons from vehicle-treated wildtype (WT) and $\tau^{-/-}$ mice. (B) Representative sEPSC traces from interneurons ipsilateral and contralateral to IHK injection in IHK-treated wildtype mice. (C) Representative sEPSC traces from interneurons ipsilateral and contralateral to IHK injection in IHK-treated $\tau^{-/-}$ mice. (D) sEPSC frequency from vehicle and IHK-treated wildtype and $\tau^{-/-}$ mice ($p > 0.05$, Two-way ANOVA). (E) Amplitude of sEPSC events from vehicle and IHK-treated wildtype and $\tau^{-/-}$ mice ($p > 0.05$, Two-way ANOVA). WT Vehicle: N=9, n=35; $\tau^{-/-}$ Vehicle: N=7, n=26; WT IHK: N=5, ipsilateral: n=19, contralateral: n=21 ; $\tau^{-/-}$ IHK N=6, ipsilateral: n=10, contralateral: n=11).

After development of TLE in wildtype mice, optogenetic stimulation of inhibitory interneurons results in less evoked inhibition to vDGCs.

Excitatory input to inhibitory interneurons appears to remain unchanged after development of epilepsy, suggesting altered inhibition in the dentate gyrus is localized to GABAergic synapses at sites distal to the kainate injection after spontaneous seizures development. Mice expressing murine tau (i.e., wildtype mice) exhibit reduced action potential independent inhibitory synaptic events while overall inhibitory input is unchanged, potentially masked by normalized excitability of interneurons. We took advantage of the ChR2 fusion to mCherry expressing interneurons to selectively activate interneurons and record evoked inhibitory post-synaptic currents (eIPSCs) as previously described. We hypothesized that the decreased inhibitory synaptic input to vDGCs after development of TLE in wildtype mice is a result of reduced number of synaptic contacts from inhibitory interneurons and reduced synaptic strength.

Optogenetic stimulation (10 ms duration, 5 stimulations, 1 sec interstimulus interval) of inhibitory interneurons expressing ChR2 resulted in reduced average eIPSC amplitude ipsilateral to IHK injection to vDGCs compared to vehicle-treated controls and contralateral to IHK injection (Fig. 5.3 A-D). Even so, there was no significant difference in the number of successful connections between stimulated interneurons and vDGCs ipsilateral or contralateral in IHK treated wildtype mice compared to controls (Fig. 5.3 A-C,E). Next, we were interested in examining the functionality of successful inhibitory connections, so we challenged known positive connections with high-frequency stimulation (10 ms duration, 10 stimulations, 10 ms interstimulus interval). Analysis of the percent change in the evoked amplitude from the first stimulation revealed less percent change in amplitude of eIPSCs in contralateral connections in

IHK-treated wildtype mice. This suggests a potential change in short-term plasticity involving presynaptic mechanisms, the GABAergic synapses contralateral to IHK but not ipsilateral (Fig. 5.3 F-I). Notably, there was no difference detected in the number of evoked action potentials in interneurons associated with light stimulations between interneurons from vehicle and IHK-treated wildtype mice and suggesting similar ChR2 expression and activity in interneurons. Together, this suggests that after development of TLE, ipsilateral to focal injection but still at a distant site in the ventral hippocampus, there may be a loss of GABAergic synapses onto vDGCs with no change in the number of interneurons connected to a particular granule cell.. Differently, interneurons contralateral to the kainate lesion do not demonstrate any difference in the average eIPSC amplitude compared to vehicle controls but exhibit sustained evoked GABA release onto connected vDGCs across high frequency patterns of activity. Together, this indicates a loss of inhibition in the ipsilateral ventral dentate gyrus and compensatory synaptic plasticity modifications in the contralateral ventral dentate gyrus.

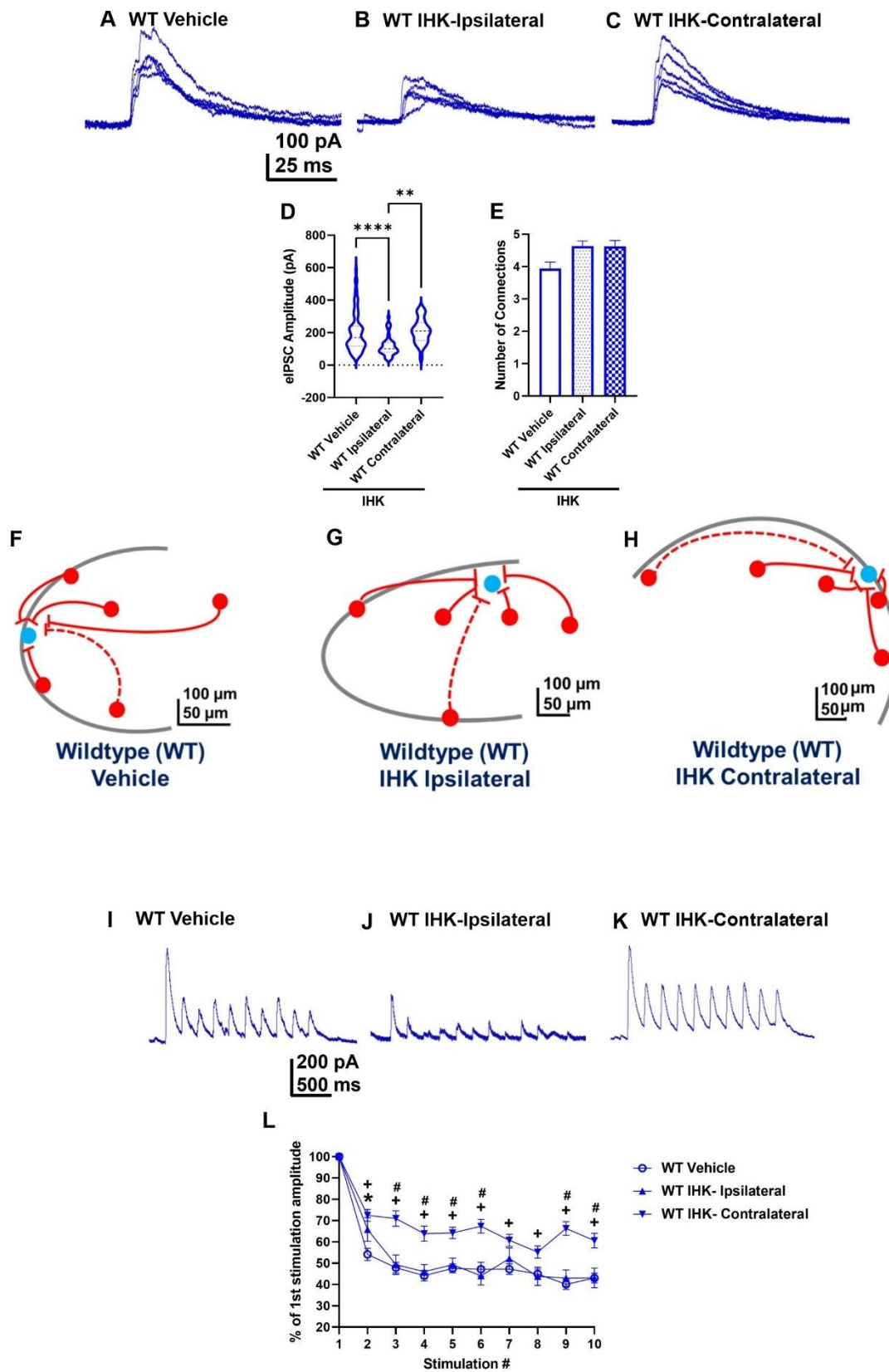


Figure 5.3. After development of TLE in wildtype mice, optogenetic stimulation of inhibitory interneurons results in less evoked inhibition to vDGCs. (A) Representative trace showing evoked IPSCs (eIPSCs) from stimulated interneurons recorded in vDGCs from vehicle-treated wildtype (WT) mice using the stimulation protocol (5 stimulations, 10 ms duration, 1 sec interstimulus interval). The 5 eIPSCs from one stimulation train were overlaid. (B) Representative eIPSC trace from interneurons ipsilateral IHK injection in IHK-treated wildtype mice. (C) Representative eIPSC trace from interneurons contralateral to IHK injection in IHK-treated wildtype mice. Vehicle: N=10, n=123 interneurons; WT IHK: N=6, ipsilateral: n=44 interneurons, contralateral: n=26 interneurons). (D) Box and whisker plot of the peak average eIPSC amplitude across 5 stimulations from vehicle and IHK-treated wildtype mice (**** $p<0.0001$, ** $p<0.01$, One-way ANOVA). (E) Number of successful interneuron-vDGC connections observed from vehicle and IHK-treated wildtype mice ($p>0.05$, One-way ANOVA). Vehicle: N=10, n=33 vDGCs; WT IHK: N=6, ipsilateral: n=11 vDGCs, contralateral: n=8 vDGCs) The max number of interneuron connections possible for one vDGCs was 5. (F) Representative schematic of connections between 5 interneurons and a vDGC from a wildtype vehicle slice, (G) IHK-treated ipsilateral slice, and (H) IHK-treated contralateral slice. Solid lines represent successful connections, and dashed lines represent no connection. (I) Representative trace showing evoked IPSCs (eIPSCs) from stimulated interneuron recorded in vDGCs from vehicle-treated wildtype (WT) mice using the stimulation protocol (10 stimulations, 10 ms duration, 10 ms interstimulus interval). (J) Representative eIPSC trace from interneuron ipsilateral IHK injection in IHK-treated wildtype mice. (K) Representative eIPSC trace from interneuron contralateral to IHK injection in IHK-treated wildtype mice. (L) Summary of percent of 1st pulse amplitude from connected interneuron and vDGCs challenged with high frequency stimulation. * vehicle vs. IHK ipsilateral, + vehicle vs. IHK contralateral, # IHK ipsilateral vs. IHK contralateral. Vehicle: N=9, n=27 interneurons ($p<0.05$, Two-way ANOVA). WT IHK: N=5, ipsilateral: n=44 interneurons, contralateral: n=34 interneurons).

Development of TLE in mice lacking tau expression, increases evoked inhibition and more synaptic connections between inhibitory interneurons and vDGCs

Previous work has suggested an increase in inhibitory synaptic input to vDGCs after development of TLE in the absence of tau expression in mice. We hypothesized that the increase in inhibitory tone may be due to increased connectivity of interneurons and vDGCs and strength of those GABAergic synapses. Stimulation of Chr2-mCherry expressing interneurons from confirmed positive connections to vDGCs (10 ms duration, 5 stimulations, 1 sec interstimulus interval), revealed significantly greater average peak eIPSC amplitude in interneurons ipsilateral

and contralateral to IHK injection in tau^{-/-} mice that developed TLE compared to vehicle controls (p<0.05) and a larger amplitude in contralateral connections compared to ipsilateral (Fig. 5.4 A-D; p<0.05). Additionally, there were significantly more successful connections between interneurons and vDGCs ipsilateral and contralateral to IHK injections compared to vehicle controls (Fig. 5.4E; p<0.05). Together, suggesting the strength of the synapses between interneurons and vDGCs is greater after the development of TLE in mice lacking tau expression which could be a result of an increase in the number of interneurons forming synapses onto a single vDGC. High frequency stimulation (10 ms duration, 10 stimulations, 10 ms interstimulus interval) did not result in observed differences in the change in eIPSC amplitude across the stimulations, although stimulation of interneurons in vehicle treated tau^{-/-} mice demonstrated significantly less percent change in amplitude in the second and third stimulations compared to ipsilateral and contralateral stimulations (Fig. 5.4F-I). It is possible that this observed change in plasticity is because of reduced eIPSC amplitude, that initial overall lower presynaptic GABA release onto vDGCs in response to stimulation affects the release in sequential stimulations. Like in wildtype mice, there was no observed difference in evoked action potentials in stimulated interneurons between IHK-treated mice and vehicle-treated mice. Together these results indicate that development of TLE in mice lacking tau expression is associated with strengthened connectivity of inhibitory synapses onto vDGCs distant from the focal injection of kainate, that is not observed in wildtype mice that develop TLE.

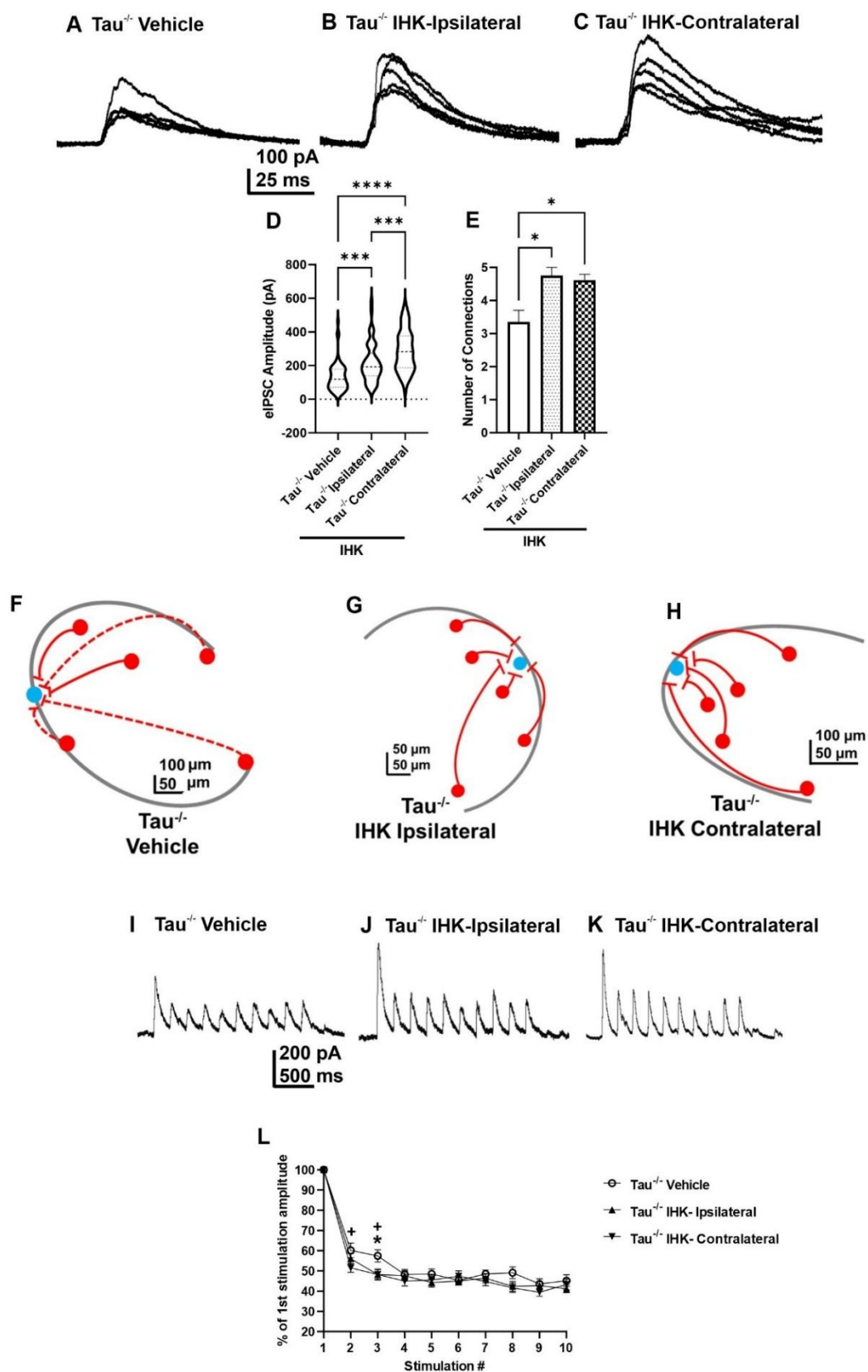


Figure 5.4. After development of TLE in wildtype mice, optogenetic stimulation of inhibitory interneurons results in less evoked inhibition to vDGCs. (A) Representative trace showing evoked IPSCs (eIPSCs) from stimulated interneurons recorded in vDGCs from vehicle-treated tau^{-/-} mice using the stimulation protocol (5 stimulations, 10 ms duration, 1 sec interstimulus interval). The 5 eIPSCs from one stimulation train were overlayed. (B) Representative eIPSC trace from interneurons ipsilateral IHK injection in IHK-treated tau^{-/-} mice. (C) Representative eIPSC trace from interneurons contralateral to IHK injection in IHK-treated tau^{-/-} mice. Vehicle: N=10, n=64 interneurons; Tau^{-/-} IHK: N=6, ipsilateral: n=58 interneurons, contralateral: n=59 interneurons). (D) Box and whisker plot of the peak average eIPSC amplitude across 5 stimulations from vehicle and IHK-treated tau^{-/-} mice (****p<0.0001, ***p<0.001, One-way ANOVA). (E) Number of successful interneuron-vDGC connections observed from vehicle and IHK-treated tau^{-/-} mice (*p<0.05, One-way ANOVA). Vehicle: N=10, n=20 vDGCs; Tau^{-/-} IHK: N=6, ipsilateral: n=8 vDGCs, contralateral: n=13 vDGCs) The max number of interneuron connections possible for one vDGCs was 5. (F) Representative schematic of connections between 5 interneurons and a vDGC from a tau^{-/-} vehicle slice, (G) IHK-treated ipsilateral slice, and (H) IHK-treated contralateral slice. Solid lines represent successful connections, and dashed lines represent no connection. (I) Representative trace showing evoked IPSCs (eIPSCs) from stimulated interneurons recorded in vDGCs from vehicle-treated tau^{-/-} mice using the stimulation protocol (10 stimulations, 10 ms duration, 10 ms interstimulus interval). (J) Representative eIPSC trace from interneurons ipsilateral IHK injection in IHK-treated tau^{-/-} mice. (K) Representative eIPSC trace from interneurons contralateral to IHK injection in IHK-treated tau^{-/-} mice. (L) Summary of percent of 1st pulse amplitude from connected interneuron and vDGCs challenged with high frequency stimulation. * vehicle vs. IHK ipsilateral, + vehicle vs. IHK contralateral, # IHK ipsilateral vs. IHK contralateral. Vehicle: N=9, n=50 interneurons (p<0.05, Two-way ANOVA). Tau^{-/-} IHK: N=7, ipsilateral: n=40 interneurons, contralateral: n=47 interneurons).

Discussion

Studies examining the effect of microtubule associated tau protein expression on evoked seizures suggest a protective effect of lack of tau expression on the outcome of seizures. Our previous study demonstrated a differential effect of lack of tau expression on seizures and epileptogenesis in the IHK model of TLE (Moseley *et al.*, 2025). It was found that mice with genetic ablation of tau expression exhibit resistance to evoked seizures during status epilepticus and develop spontaneous seizures at a lower rate with reduced epileptiform activity during the

ictal phase, suggesting tau protein plays a role in the cellular and synaptic mechanisms involved in the epileptogenic process. Assays of excitatory and inhibitory synaptic transmission of DGCs in the dorsal hippocampus revealed increased inhibitory synaptic input to dorsal DGCs contralateral to the kainate injection in tau^{-/-} mice that developed TLE (Moseley *et al.*, 2025). Another study demonstrated that the increase in contralateral input in DGCs in the dorsal hippocampus is also observed in both hemispheres (i.e., ipsilateral and contralateral) in the ventral hippocampus in mice lacking tau expression after epileptogenesis. Together, these results indicate a role of tau protein in inhibitory synaptic transmission within the dentate gyrus and highlight modified inhibitory input to DGC at sites distant to the focal injury as a potential mechanism by which lack of tau expression confers resistance and reduced severity of spontaneous seizures.

The functions of tau protein have been extensively studied for its role in microtubule stabilization and axonal transport, and its expression is crucial for synaptic plasticity. However, the role of tau protein in synaptic transmission is still not well defined. Ablation of tau expression has been shown to reduce excitability of excitatory pyramidal cells in the cortex and promotes action potential dependent inhibitory input to pyramidal cells (Chang *et al.*, 2021a). Another study found minimal effects of tau ablation on excitatory and inhibitory synaptic transmission to DGCs (Roberson *et al.*, 2011b). Differently, our assays of inhibitory synaptic transmission suggest lower inhibitory input to DGCs in the dorsal and ventral hippocampus (Moseley *et al.*, 2025). Together, the opposing effects of lack of tau expression in synaptic transmission in the hippocampus make it difficult to delineate the role of tau protein but may indicate a differential role of tau protein in hippocampal circuits.

Mechanisms by which genetic deletion of tau protein may confer protection against evoked seizures and epileptogenesis are also unclear. It has been shown that selective ablation of tau expression in excitatory neurons but not inhibitory neurons counteract network hyperactivity reduce epileptiform activity comparable to global knockout of tau in mice after PTZ administration, suggesting excitatory cells being the critical cell type in which tau protects against evoked seizure expression (Shao *et al.*, 2022b). While genetic deletion of tau in mice appears to suppress evoked seizures and lower the rate of spontaneous seizure development and severity, mice still undergo epileptogenesis with development of TLE in the IHK model that is associated with significant increases in synaptic inhibition at sites distant to the focal injection (Moseley *et al.*, 2025). This would suggest that the positive effects of lack of tau expression on seizures and epileptogenesis are likely through inhibitory synaptic mechanisms rather than changes to excitatory synaptic transmission.

As we have previously shown, changes in inhibitory input to DGCs at distal sites from the focal kainate injection after development of TLE in mice lacking tau expression may suggest inhibitory synaptic reorganization as a mechanism for tau's role in epileptogenesis. Specifically, results indicate a potential loss of functional GABAergic synapses in wildtype mice expressing murine tau and an increase in GABAergic synapses to DGCs in the ventral dentate gyrus in tau^{-/-} mice after development of TLE. Therefore, we were interested in further investigating changes in inhibitory synapses onto vDGCs after development of TLE in wildtype and tau^{-/-} mice.

Here we used AAV with ChR2-mCherry fusion that drives selective expression in forebrain GABAergic interneurons under the control of the *Dlx* promoter. This AAV has been shown to be selectively expressed in all interneurons subtypes in the hippocampus and cortex (Dimidschstein *et al.*, 2016). To date, there are no studies indicating a differential role of tau

protein in specific interneuron subtypes (Shao *et al.*, 2022b). Therefore, using an AAV that allows for expression in all interneuron subtypes allowed for unbiased examination of inhibitory input to DGCs in the ventral dentate gyrus. Injection of the AAV in the ventral dentate gyrus, resulted in robust expression in all mice (wildtype and tau^{-/-}) with no obvious differences detected between vehicle-treated and IHK-treated mice, suggesting no effect of tau expression or epilepsy on the interneuron population in the ventral dentate gyrus.

Excitatory synaptic reorganization has been well described in rodent models and patients with TLE. It has been shown that the increase in excitatory synapses also make contact with inhibitory interneurons in the hilus of the dentate gyrus, thereby increasing their output onto DGCs (Sloviter, 1992; Kotti *et al.*, 1997; Wenzel *et al.*, 2000; Buckmaster *et al.*, 2002; Cavazos *et al.*, 2003; Sloviter *et al.*, 2006). Even so, our results of sEPSC frequency in inhibitory interneurons from vehicle and IHK-treated wildtype and tau^{-/-} mice did not result in any observed changes in excitatory synaptic input to interneurons. This is a similar result to previous work that did not identify changes in excitatory synaptic input to parvalbumin (PV) positive interneurons in the ventral dentate gyrus after development of TLE using the IHK model (Lee *et al.*, 2024a). Together, this suggests that the observed changes in inhibitory synaptic signaling to vDGCs in tau^{-/-} mice after the development of epilepsy is not upstream of the GABAergic synapses and due to increased excitatory input to inhibitory interneurons.

Previous results indicate potential inhibitory synaptic changes associated with epileptogenesis at distant sites from the kainate lesion in wildtype and tau^{-/-} mice. Studies have demonstrated a reduction in inhibitory synaptic input to vDGCs and pyramidal cells in the CA1 after development of TLE in the IHK model (Kang *et al.*, 2021a; Lee *et al.*, 2024a). Our results differ in that wildtype mice exhibit reduced action-potential independent inhibitory synaptic

events in vDGCs after development of TLE, while overall inhibitory synaptic transmission was intact which could be due to different ages and time post-IHK. Even so, these results suggest a potential loss of GABAergic synapses after development of TLE. In the context of tau expression, mice lacking tau expression exhibit increased action-potential independent inhibitory synaptic events after development of TLE, indicating potential changes in the number of GABAergic synapses on vDGCs.

Here, we investigated the synaptic strength and connectivity between inhibitory interneurons and vDGCs in mice expressing murine tau and mice lacking tau expression after development of TLE using the IHK model. Results of direct optogenetic stimulation of inhibitory interneurons demonstrated a decrease in the average eIPSC amplitude in vDGCs ipsilateral to IHK injection, which could suggest a lower probability of GABA release or reduced number of functional GABAergic synapses, supporting our previous findings. Even so, we did not observe significant differences in the number successful connections between activated interneurons and vDGCs. It is possible that the lack of observed differences in interneuron-vDGCs connections in wildtype mice could be due to the lack of spatial resolution of this direct optogenetic approach, where the stimulation of 5 interneurons per vDGCs is not high-throughput enough to detect differences in connectivity. Nevertheless, optogenetic stimulation of inhibitory interneurons in tau^{-/-} mice resulted in significantly increased average eIPSC amplitude ipsilateral and contralateral to the IHK injection with increased number of successful connections between interneurons and recorded vDGCs. Together, these results indicate a potential mechanism for tau protein in synaptogenesis. Reactive synaptogenesis of surviving interneurons has been proposed as a compensatory mechanism for the initial loss of interneurons that restores at least some part of inhibition that is lost. Studies of human tissue and rodent models of TLE examining fibers of

GABAergic terminals like somatostatin-expressing interneurons that the dendrites of DGCs have shown transient immunoreactivity throughout the molecular layer of the dentate gyrus that is associated with initial decrease shortly following status epilepticus and an increase at later time points throughout epileptogenesis that persists well into the expression of spontaneous seizures (Davenport *et al.*, 1990; deLanerolle *et al.*, 1992a; deLanerolle *et al.*, 1992b; Mathern *et al.*, 1995; Houser & Esclapez, 1996; Buckmaster & Dudek, 1997a; Mathern *et al.*, 1997; Andre *et al.*, 2001; Kobayashi & Buckmaster, 2003b). In human epileptic tissue, other interneurons like parvalbumin expressing and cholecystokinin expressing interneurons that provide perisomatic inhibition to DGCs have shown to increase axon basket formation surrounding DGCs and their axon initial segments (Arellano *et al.*, 2004). Moreover, cholecystokinin expressing interneurons in models of TLE have shown not only increased synapses onto axon initial segments of DGCs but also synapse presence in the molecular layer of the dentate gyrus (Gruber *et al.*, 1993; Wittner *et al.*, 2001). Our results suggest the possibility of synaptogenesis occurring in mice lacking tau expression that develop TLE where the process of epileptogenesis results in increased synaptic connections of interneurons on DGCs where more interneurons synapse onto DGCs that normally are not innervating DGCs in naïve tau^{-/-} mice. It is possible that the lower rate of epileptogenesis resulting in TLE development in mice lacking tau expression could be due to increased inhibitory synapse formation into DGCs after an insult like status epilepticus.

In addition to modified inhibitory input to DGCs and reactive inhibitory synaptogenesis, modulation of inhibitory synaptic plasticity has also associated with TLE in the dentate gyrus. Here, we measured short-term plasticity of GABAergic synapses through high frequency optogenetic stimulation of inhibitory interneurons. First, we found that high frequency stimulations in interneurons contralateral the IHK injection demonstrated minimal depression of

evoked responses recorded in vDGCs compared to ipsilateral and vehicle-controls, suggesting altered synaptic plasticity of contralateral GABAergic synapses after development of epilepsy. Even so, there were no differences detected in plasticity ipsilateral to IHK injection compared to vehicle controls other than the fact that high frequency stimulation resulted in an increase in failures. Together with previous results demonstrating a reduction in the average eIPSC amplitude in vDGCs and a potential functional loss of GABAergic synapses, with surviving synapses be more likely to fail in response to high frequency stimulations, suggests that development of TLE is associated with loss of inhibitory synapses to vDGCs but that surviving synapses maintain their short-term plasticity even if they are less reliable. Differently, contralateral connections between inhibitory interneurons and vDGCs demonstrate a potential loss in GABAergic synapses that is likely functionally compensated by preserved strength and sustained inhibition at surviving synapses that is accompanied by an alteration in short-term plasticity that could be due to presynaptic release mechanisms. In contrast, high frequency optogenetic stimulations in mice lacking tau expression demonstrated no change in synaptic plasticity between vehicle and IHK-treated tau^{-/-}, suggesting short-term plasticity and release probability of GABA remains intact after development of epilepsy in mice lacking tau expression. Combined with the observed increase in evoked IPSC responses and increased in interneuron connectivity, it is more likely that development of epilepsy in mice lacking tau expression is associated with significant synaptic reorganization of inhibitory interneurons at distant sites to the initial focal kainate injection.

Conclusions

This study highlights potential inhibitory synaptic mechanisms by which genetic deletion of tau may cause differential effects on seizures and epileptogenesis in a mouse model of acquired TLE. Using optogenetic stimulation of ChR2-expressing interneurons in the ventral dentate gyrus, we were able to elucidate key features of epileptogenesis associated with inhibitory neuroplasticity, where development of TLE is associated with reduced synaptic strength of GABAergic synapses but compensatory contralateral alterations to synaptic plasticity. Further, we identified a role for tau protein in GABAergic synaptogenesis where development of epilepsy in mice lacking tau expression results in increased inhibition to vDGCs likely from an increase in GABAergic synapses from inhibitory interneurons. Further studies are required to elucidate modifications of specific receptor mediated synaptic plasticity.

CHAPTER 6: DISCUSSION

Summary of Findings

The work in this dissertation focused on examining the effect of lack of tau expression on the epileptogenesis of acquired TLE using the IHK rodent model. The first principal finding is that genetic deletion of tau suppresses individual seizures but does not protect against the process of epileptogenesis, which has not been demonstrated previously. This finding sent me on a mission to devise and perform experiments aimed to test how tau expression may be modifying the cellular and synaptic mechanisms associated with epileptogenesis. Using whole-cell patch clamp electrophysiology, I found that development of TLE in mice lacking tau expression is associated with increased inhibitory contralateral input to DGCs in the dorsal hippocampus. This finding that there is potentially inhibitory synaptic reorganization distal to the initial insult, led me to ask the question if this is also occurring at sites more widespread in the ventral hippocampus. Unfortunately for me this required me to repeat the previous electrophysiological experiments in the ventral dentate gyrus. Nevertheless, I persevered. My examination of synaptic transmission in the ventral dentate gyrus identified neuroplastic changes associated with epileptogenesis in the IHK model. Specifically, the key findings were that inhibitory synaptic input to vDGCs is reduced in wildtype mice but increased synaptic input to vDGCs occurs in mice lacking tau expression after the development of TLE. I hypothesized that this increase was likely due to increased connectivity between inhibitory interneurons and vDGCs. Therefore, in my final experiment I wanted to further investigate the nature of the inhibitory synaptic connections to vDGCs. Using an optogenetic approach, I found not only a loss of functional inhibitory synapses ipsilateral but compensatory synaptic plasticity contralateral in wildtype

mice. Further, epileptogenesis in mice with genetic deletion of tau results in an increase in inhibitory synapses on vDGCs that are likely to result in a net strengthening of inhibition. Together, these findings point to widespread inhibitory synaptic reorganization being the key mechanism by which lack of tau expression modifies epileptogenesis in acquired TLE. Throughout these experiments, I was also able to identify a potential role of microtubule-associated tau protein in the hippocampal circuit.

Differential effect of lack of tau expression on seizures and epileptogenesis

Previous studies had identified a protective role of tau deletion on seizures outcomes in animal models of genetic epilepsies and chemoconvulsant models of evoked seizures (Roberson *et al.*, 2007; DeVos *et al.*, 2013; Holth *et al.*, 2013; Gheyara *et al.*, 2014; Li *et al.*, 2014b; Putra *et al.*, 2020; Shao *et al.*, 2022a). Even so, prior to the studies in this dissertation, tau's role in acquired TLE was unexplored. This was important to investigate because of the difference in etiologies between genetic and acquired epilepsies. While genetic epilepsies are a consequence of mutations in voltage-gated channels, spontaneous seizures in acquired epilepsies develop after a delay following a precipitating injury (e.g., status epilepticus, traumatic brain injury) that triggers cortical circuits to be rewired, resulting in an increased probability of unprovoked spontaneous seizures. Although lack of tau expression had been shown to confer resistance to evoked seizures, whether this translated to altered epileptogenesis was not clear. Using the IHK model, I found that lack of tau expression doesn't prevent evoked seizures or the subsequent development of TLE. The finding that genetic deletion of tau expression conferred resistance to convulsive seizures during status epilepticus was not surprising as this supported prior studies. What was interesting was that tau^{-/-} mice still exhibited convulsive seizures during the induction

period after IHK, which had not been demonstrated prior. Nevertheless, tau^{-/-} mice that reached status epilepticus in the induction period exhibited the minimum number of seizures and significantly less than wildtype controls, supporting prior studies examining genetic deletion of tau using evoked seizures models (i.e, PTZ). Even so, there was no significant difference in the epileptiform activity during the ictal phase of the convulsive seizures but likely alterations in the power spectral density, indicating different contributions of specific cortical neuron subtype recruited during seizure generation. Together, this suggests that the protective effects of lack of tau expression on evoked seizures are only on the threshold to generate seizures rather than the severity of individual seizures. The novel findings on the effect of lack of tau expression on acquired TLE development, was somewhat surprising. I hypothesized that lack of tau expression would suppress epileptogenesis and prevent the development of spontaneous seizures, even after the expression of status epilepticus. Differently, I found that mice lacking tau expression can develop TLE spontaneous seizures but do so at a lower rate than wildtype mice. This was the first documentation on the effect of tau deletion on epileptogenesis. Additionally, unlike evoked seizures, lack of tau expression did not alter the burden of spontaneous seizures once TLE developed. Even so, it was interesting to find that the spontaneous seizures were less severe in mice lacking tau expression and exhibited a potential shift in spectral power density. Altogether, genetic deletion of tau suppresses individual seizures but does not protect against the process of epileptogenesis. Notably, these results highlight a possible role of microtubule associated tau protein in the epileptogenic process and likely in the cellular and synaptic mechanisms that contribute to epileptogenesis and the eventual presentation of epilepsy.

It is important to consider the possibility of compensatory microtubules associated proteins since microtubules associated-tau protein is genetically ablated embryonically in the

mice used here. Anti-sense oligonucleotides effectively knockdown the tau protein level by 80% postnatally. Even so, it was shown that postnatal ablation of tau expression does not significantly alter seizure activity compared embryonic deletion (DeVos *et al.*, 2013), opposing the possibility of compensatory mechanisms being responsible for altered seizures and epileptogenesis. Further, it has been shown that there are minimal developmental effects of the absence of tau expression in which there is no upregulation of other microtubule associated proteins, therefore, the differential effect of lack of tau expression on seizures and epileptogenesis is more likely due to the loss of tau function (Voskobiynyk *et al.*, 2024).

Lack of tau expression modifies epileptogenesis associated neuroplasticity in the dentate gyrus

Since I had determined the effects of lack of tau expression on epileptogenesis of acquired TLE, I began to hypothesize potential alterations to epileptogenesis associated neuroplasticity and how tau protein might play a role in the hippocampal circuit. I focused on the dentate gyrus and DGCs of the hippocampus because of its importance in TLE and involvement in epileptogenesis. Cellular neuropathology in the dentate gyrus associated with TLE Synaptic mechanisms involved in epileptogenesis include hippocampal sclerosis and dispersion, axonal sprouting of DGCs, and selective neuron loss (Scharfman, 2019). The network changes resulting from excitatory synaptic reorganization and inhibitory synaptic loss that is associated with epileptogenesis have been well described (Buckmaster & Dudek, 1997a; Buckmaster *et al.*, 2002; Winokur *et al.*, 2004a). My initial investigation into modified synaptic transmission in the dentate gyrus was in the dorsal hippocampus, near the unilateral kainate injection. Results demonstrated a similar increase in excitatory input to DGCs in both wildtype and tau^{-/-} mice after

development of TLE, suggesting that tau expression does not alter excitatory synaptic reorganization among DGCs observed with TLE development in this model (Fig. 6.1). Differently, inhibitory synaptic input to DGCs was similarly ablated near the kainate injection in both genotypes, consistent with either a loss of inhibitory neurons or a reduction in their ability to release GABA in the dentate gyrus. Interestingly, inhibitory input to DGCs was increased in the dorsal dentate gyrus contralateral to the kainate injection. I hypothesized that the increase in inhibition could be due to axon sprouting or synaptic reorganization of surviving interneurons, which has been described in rodent models of TLE (Buckmaster & Wen, 2011; Kang *et al.*, 2022a; Lee *et al.*, 2024a) or DGCs forming new connections with interneurons after TLE (Buckmaster *et al.*, 2002; Halabisky *et al.*, 2010; Butler, 2017; Kang *et al.*, 2022a). Together, with the finding that DGCs receive less inhibitory input to DGCs in naïve tau^{-/-} mice, it implies that tau protein normally plays a role in regulating local inhibitory synaptic transmission in the dentate gyrus and when tau is not expressed, there is a restorative increase in synaptic inhibition. These findings in the dorsal hippocampus, near the kainate injection, allowed me to hypothesize if neuroplastic changes associated with TLE development are detectable at sites distant to the focal injection.

initial injury. I hypothesized that lack of tau expression contributes to widespread synaptic reorganization of inhibitory circuits after epileptogenesis. Unlike in the dorsal dentate gyrus where there was an increase in excitatory synaptic input to DGCs ipsilateral to IHK injection in both genotypes, epileptogenesis associated changes in excitatory synaptic input to DGCs in the ventral dentate gyrus were undetected (Fig. 6.2). This suggests that excitatory synaptic reorganization that is associated with TLE development is limited to the epileptogenic foci in the IHK model and is not detectable at distant regions. An interesting finding was that vDGCs naïve, vehicle-treated tau^{-/-} mice normally receive less excitatory input, which was not observed in the dorsal dentate gyrus. This is likely due to differences between the dorsal and ventral hippocampus in glutamatergic inputs via the entorhinal cortex. Even so, it is possible that less excitatory network activity in the ventral dentate gyrus in mice lacking tau expression may also contribute to the modified TLE development and individual spontaneous seizures expression. In wildtype mice it was found that there is likely a functional loss of inhibitory synapses onto vDGCs, while overall inhibitory input is maintained. This indicates that there are widespread inhibitory synaptic changes associated with TLE development in the IHK model, although it is more likely that the significant loss of inhibition near the epileptogenic foci is more responsible for triggering the epileptogenic process. Differently, tau^{-/-} mice appear to start out with less inhibitory input but development of TLE results in increased inhibitory synaptic input to vDGCs. This increase in inhibition is likely to be due to an increase in functional inhibitory synapses from sprouted axons of inhibitory interneurons. It is important to note that the increase in overall inhibitory synaptic input after development of TLE in tau^{-/-} mice restored inhibition to a similar level as naïve wildtype mice. This suggests that the increase in inhibition that's observed in tau^{-/-} mice is not enough to prevent the epileptogenic process from occurring but may be responsible

for the lower rate of TLE development and reduced individual seizure expression. More specifically, it is likely the increase in inhibitory tone in the tau^{-/-} mice may be responsible for the numerical increase in power spectra coming from increased activity from cortical interneurons. Future studies are required to elucidate power spectra density changes in individual seizure expression in specific regions like hippocampus in tau^{-/-} mice using depth EEG electrodes which would allow for more temporal resolution of neural activity and a more correlative explanation for the inhibitory synaptic changes associated with epilepsy development. Even so, these findings allowed me to hypothesize and further examine the nature of inhibitory synapses in the ventral dentate gyrus.

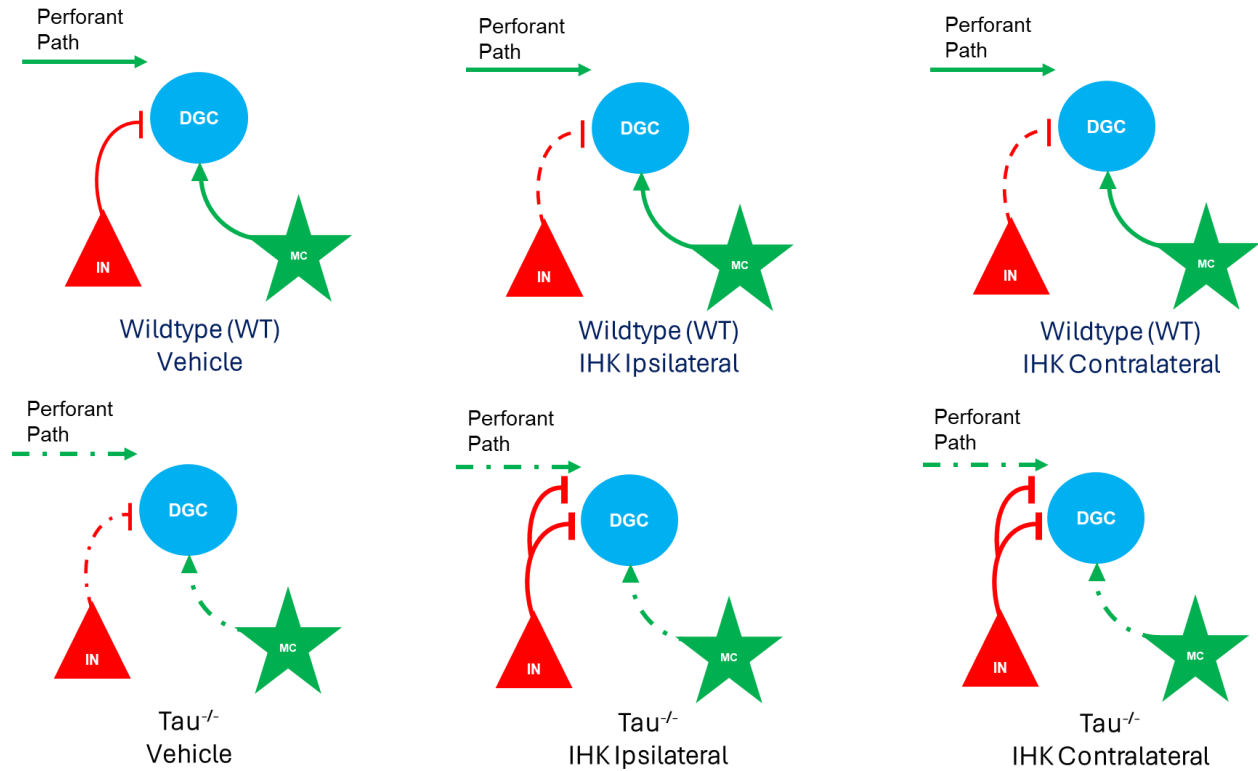
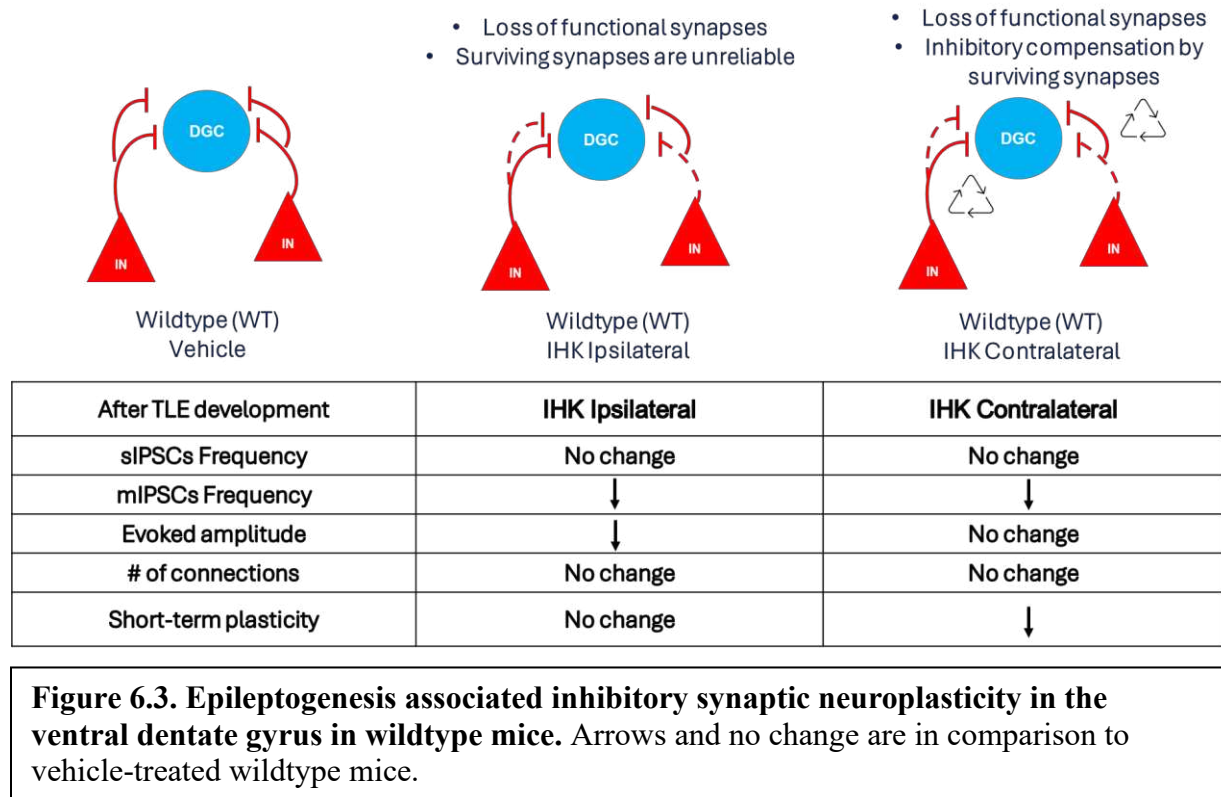


Figure 6.2. Widespread epileptogenesis associated synaptic neuroplasticity in the ventral dentate gyrus circuit. Top row: schematic of local dorsal dentate gyrus circuit in vehicle-treated and IHK-treated wildtype mice. Middle: ipsilateral to IHK injection there is a significant loss of inhibitory input to DGCs likely from inhibitory interneuron cell loss and significant increase in excitatory input from the perforant path and/or aberrant mossy fiber axon sprouting from other DGCs. Glutamatergic input from hilar mossy cells is unlikely due to susceptibility to insult. Right: contralateral to IHK injection there is no overt changes to excitatory input but some loss of inhibitory input to DGCs. Bottom row: schematic of local dorsal dentate gyrus circuit in vehicle-treated and IHK-treated tau^{-/-} mice. Left: DGCs in vehicle-treated, naïve tau^{-/-} mice receive less inhibitory input normally. Middle: ipsilateral to IHK injection there is a significant loss of inhibitory input to DGCs likely from inhibitory interneuron cell loss and significant increase in excitatory input from the perforant path and/or aberrant mossy fiber axon sprouting from other DGCs. Right: contralateral to IHK injection there is no change in excitatory input but a significant increase in inhibitory contralateral input in tau^{-/-} mice after development of TLE.

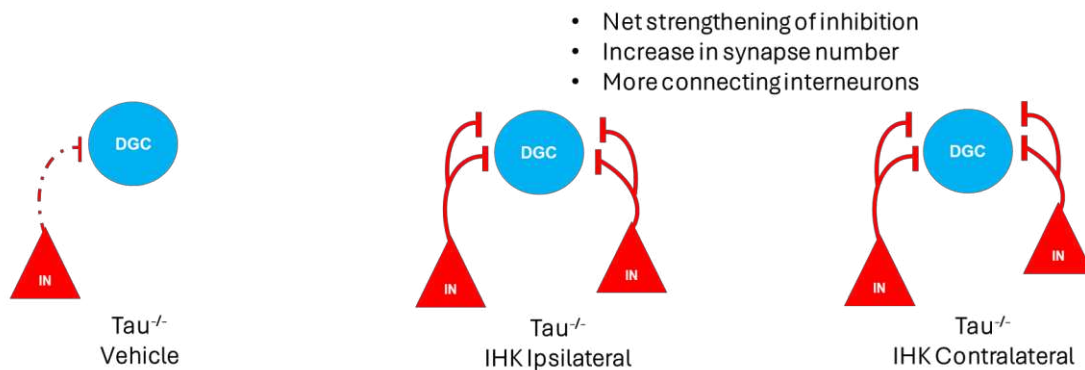
Using an optogenetic approach, I examined if the development of TLE in mice lacking tau expression was associated with an increase in strength of inhibitory synapses onto vDGCs. I found that development of epilepsy in wildtype mice is associated with a reduced inhibitory evoked response in vDGCs ipsilateral but not contralateral to IHK injection. Even so, there was no change in the number any given interneurons synapsing on vDGCs. While, results indicated a loss of functional inhibitory synapses previously, there was no change in the number of any given interneuron forming synaptic connections on vDGCs. This likely suggests that although the number of interneurons synapsing on vDGCs is unchanged, there is still a loss of some of their synapses, assuming an interneuron forms multiple synapses normally (Fig. 6.3). There are a few possible explanations for the reduction in the evoked amplitude response such as a reduced presynaptic release probability, change in short-term plasticity, or modified post-synaptic receptor function and/or expression. Together, with my previous results of reduced inhibitory event frequency but no change in amplitude of inhibitory post-synaptic currents, I hypothesized that this is likely a dysfunction on the presynaptic side of the interneuron-vDGC synapse. Therefore, I was interested in examining the functionality of the connecting synapses more in depth. I used a high-frequency stimulation protocol for positive interneuron-vDGC connections (i.e., 50 Hz) to elucidate changes in GABA release over time that could be due to altered release or a change in the releasable pool of vesicles. There was no obvious change in short-term plasticity of interneuron-vDGCs synapses ipsilateral to IHK injection. Surprisingly, these synapses contralateral to the IHK injection demonstrated endurance of GABA release during high frequency optogenetic stimulations (Fig. 6.3). This suggests that after development of epilepsy, there is a loss of functional synapses, as indicated by reduced action potential independent events, but a compensatory increase in the presynaptic vesicle pool dynamics occurs

contralaterally. While it is unlikely from these results that altered evoked IPSC amplitudes are due to post-synaptic changes in GABA receptor expression or function differential expressions of GABA receptors (i.e., GABA_A and GABA_B receptors) have been shown in rodent models of seizures and humans. Studies using the electrical kindling model have shown an increase in the expression of different subunits of GABA_A receptors, which could partially explain changes in evoked amplitudes (Kamphuis *et al.*, 1995). While other studies have demonstrated an initial loss of GABA_B receptors in the dentate gyrus after kainic acid induced status epilepticus and a long-term upregulation in expression (Furtinger *et al.*, 2003; Straessle *et al.*, 2003). Therefore, differential changes in GABA receptor expression, either post-synaptically or pre-synaptically, could be responsible for synaptic plasticity changes contralateral to IHK injection observed in wildtype mice after development of TLE. However, it is unlikely changes in postsynaptic receptor expression or function in DGCs due to no observed change in the amplitude of action potential independent or dependent inhibitory events (i.e., s/m IPSCs) in this current study.



Differently, development of TLE in mice lacking tau expression resulted in an increase in overall inhibitory input, including an increase in action potential independent inhibitory events, with no change in event amplitude that suggests an increase in the number of functional GABAergic synapse formation during epileptogenesis (Fig. 6.4). Further, optogenetic stimulation revealed an increase in the number of interneurons connecting to a given vDGC, which could explain the increase in the evoked inhibition observed in the amplitude. It was found that genetic deletion of tau expression likely is associated with a reduction in the number of inhibitory synapses normally (Fig. 6.4). It is conceivable to think that fewer interneurons are normally connected to vDGCs in mice lacking tau expression and development of epilepsy causes sprouting of inhibitory interneuron axons to form new synapses with vDGCs that would not normally be connected. Therefore, tau protein functions in inhibitory interneuron synapse

formation and/or function normally, which identifies a novel role of tau protein that has not been described before. Overall, these results suggest an increase in GABAergic synapses on vDGCs, throughout the epileptogenic process, which has been described (Maglócsy *et al.*, 2000; Bausch, 2005). Therefore, it is likely that the differential effect of tau protein on seizures and epileptogenesis is due its function in inhibitory synaptic transmission within the hippocampus.



After TLE development	IHK Ipsilateral	IHK Contralateral
sIPSCs Frequency	↑	↑
mIPSCs Frequency	↑	↑
Evoked amplitude	↑	↑
# of connections	↑	↑
Short-term plasticity	No change	No change

Figure 6.4. Epileptogenesis associated inhibitory synaptic neuroplasticity in the ventral dentate gyrus in tau^{-/-} mice. Arrows and no change are in comparison to vehicle-treated tau^{-/-} mice.

Final Thoughts

It is possible that the increase in widespread inhibition in mice lacking tau expression is responsible for lack of tau's protective effects on seizures and epileptogenesis. Being that microtubule associated tau protein has been shown to regulate synapses through microtubule stability, protein translation, and activity-dependent remodeling (Chang *et al.*, 2021b; Thomas *et*

al., 2025), it is possible that when tau is not present to act as a “brake” the homeostatic constraints that exist in situations of excitability allow inhibitory synapses to form more readily in conditions of excitability. The finding that interneurons and inhibition appear to be more affected by the loss of tau was somewhat puzzling as it conflicted with previous work which highlighted that knockout of tau in all excitatory neurons responsible for reducing seizure activity and not inhibitory neurons (Shao *et al.*, 2022a). It is possible that DGCs are not affected by the loss of tau as other excitatory neurons are since DGCs are unique in their slow firing during activation compared to other excitatory cell types in the hippocampus like pyramidal cells. With the output regulation of the dentate gyrus being tightly controlled by inhibition, it is comprehensible to think that a lack of tau expression may affect circuit activity primarily through the inhibitory pathway. Together, I find that tau protein gets more complicated the more I learn through my own experiments and reading of what others have discovered. It is likely that tau protein wears many different hats for different cell types and tailors its role depending on the circuit. However, my work has identified tau’s role in inhibitory synaptic transmission within the local dentate gyrus network, which may be responsible for its differential effects on seizures and epileptogenesis.

The studies in my dissertation were aimed at answering questions that followed up a very big question which is, does lack of tau expression play any role in the epileptogenesis of acquired TLE. I think the results in this study allow me to answer that question simply: yes. I think if tau protein did not play a role in acquired TLE, I would not be writing this now. Based on the previous literature describing genetic deletion of tau as this answer to curing epilepsy and what I know now, I would say, “No ablating tau from a human or mouse is not going to prevent seizures”. I would also add that ablation of tau after an injury like status epilepticus or a

traumatic brain injury will not prevent the epileptogenic process from occurring. I began thinking of this project not as a mission to cure epilepsy (because that would be crazy), but as information that adds to a story that may one day lead to finding a more optimal treatment or therapy. At the end of the day we cannot prevent people from riding a motorcycle without a helmet or somehow predict when someone is about to exhibit their first seizure. The main take away from this work is that microtubule associated tau protein functions in the hippocampal circuit, specifically in inhibitory synaptic transmission and because of this lack of tau expression provides positive effects on individual seizures and epileptogenesis. It is likely that microtubule associated tau protein regulates axon maintenance and synaptogenesis of inhibitory neurons normally, so when tau is not expressed and the network undergoes induced excitability, axon sprouting of inhibitory interneurons is a positive consequence on the process of epileptogenesis. The work in this dissertation identified a novel function of tau protein in inhibitory synaptic transmission in the dentate gyrus

Future Directions

If I were to continue working on this project, there are a couple of things I would like to pursue. First, I would be interested in altering the timeline of the experimental design. Previous work in the lab identified an age-dependent effect of tau protein on excitatory input to DGCs, where excitatory input is lower at 1.5 months of age compared to wildtype mice but normalizes to similar levels of wildtype mice by 4 months of age (Cloyd *et al.*, 2021). This finding suggested that tau protein plays a role in excitatory synaptic input in the dorsal dentate gyrus circuit. It was hypothesized that global tau ablation reduces excitability to DGCs that may underlie resistance to evoked seizures during status epilepticus after kainate injection.

Nevertheless, my finding of lower inhibitory input to DGCs in tau^{-/-} mice at 16-18 weeks (~3.5-4 months) of age compared to wildtype mice was perplexing. Therefore, I recorded preliminary data of sIPSCs in DGCs at the time of the IHK surgery (i.e., 1.5 months, 6-8 weeks of age) (Fig.6.5). Much to my surprise, the results revealed more inhibitory input to DGCs compared to age-matched wildtype mice a (6-8 weeks of age) and tau^{-/-} mice at 16-18 weeks of age. This does not only suggest a transient role of tau protein on synaptic transmission in the dentate gyrus but also provides evidence of the role of tau expression on the inhibitory circuit that may be responsible for evoked seizures resistance and modified epileptogenesis combined with previous results of lower excitatory synaptic input. Therefore, one future direction is to change the age of IHK surgery to 16-18 weeks of age, when inhibitory input is lower in tau^{-/-} mice but excitatory input is intact. I hypothesize that mice lacking tau expression will be more likely to exhibit convulsive seizures during SE with modified epileptiform activity during the ictal phase of individual seizures compared to tau^{-/-} mice at 8-10 weeks of age. Further, I would expect tau^{-/-} mice to develop spontaneous seizures at a similar rate or even more than wildtype mice. Results would clearly identify tau's role in inhibitory synaptic transmission as a mechanism for altered epileptogenesis.

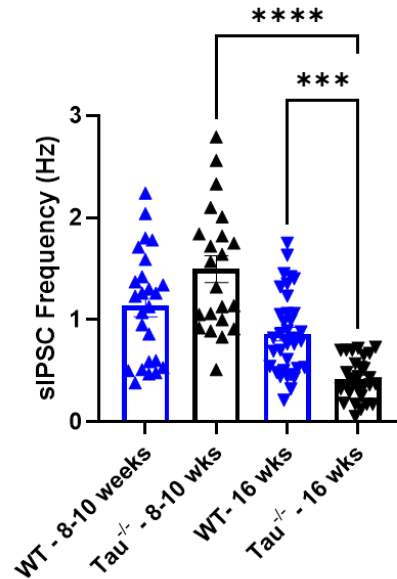


Figure 6.5. Inhibitory synaptic input to dorsal DGCs is transiently reduced throughout adulthood in tau^{-/-} mice. Whole-cell patch clamp recordings of dorsal DGCs in wildtype and tau^{-/-} mice at 8-10 weeks (age of IHK/saline injection) and 16 weeks of age (age after saline injection). WT 8-10 weeks vs. tau^{-/-} 8-10 weeks: $p = 0.0513$, *** $p < 0.001$, **** $p < 0.0001$; Two-way ANOVA.

Another future direction would be to use the CCI model of traumatic brain injury (TBI) to investigate lack of tau's effect on post-traumatic epilepsy. Unlike the IHK model, where the initial precipitating injury is status epilepticus that triggers the epileptogenic process, the CCI model utilizes a focal TBI to the dorsal hippocampus that acts as the trigger. I hypothesize that mice lacking tau expression will be less likely to develop post-traumatic epilepsy in the CCI model. Results from this proposed study would identify targeting the function of tau protein in epileptogenesis of another precipitating injury. Further, by examining the function of tau in epileptogenesis of post-traumatic epilepsy may identify key cellular and synaptic mechanisms involved in the development after brain trauma. This is important because the epileptogenesis of post-traumatic epilepsy remains unexplored.

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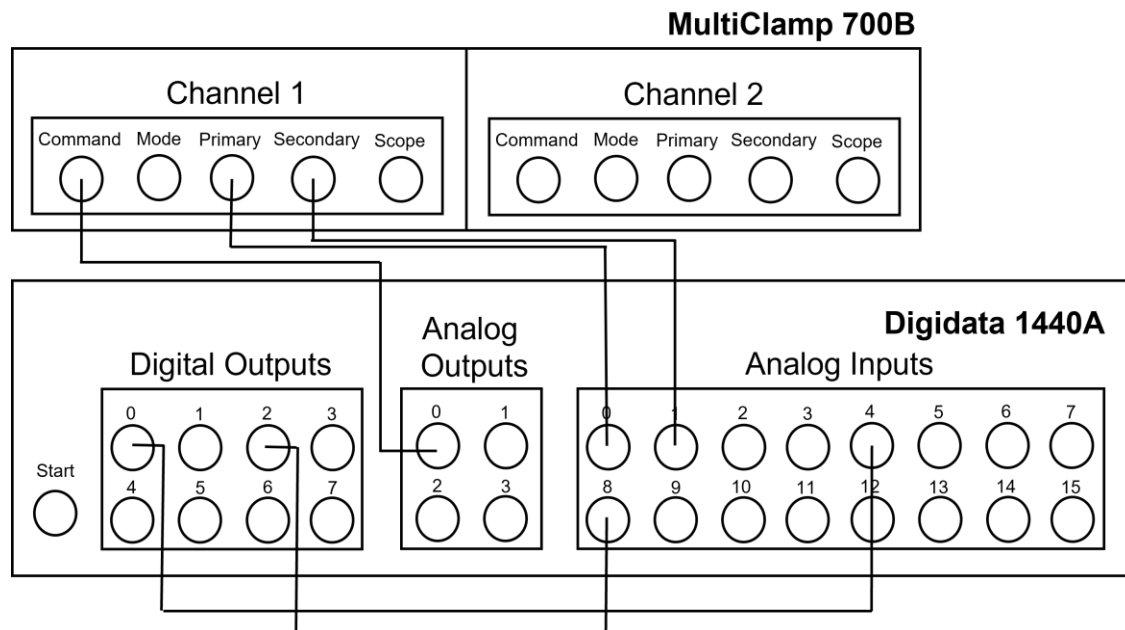
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APPENDICES

APPENDIX 1 ELECTROPHYSIOLOGY RECORDING SETUP



APPENDIX 2 SOLUTIONS USED FOR ELECTROPHYSIOLOGY

A2.1 SUCROSE CUTTING SOLUTION

Compound	Molecular weight (g/mol)	Concentration (mM)	Grams for 1L (5x)
NaCl	58.44	85	24.820
Sucrose	342.3	75	128.36
KCl	74.55	2.5	0.9325
D-Glucose	180.156	25	22.525
NaH ₂ PO ₄	137.99	1.25	0.862
MgCl ₂	203.30	4	4.066
CaCl ₂	147.01	0.5	0.3675
NaHCO ₃	84.01	24	10.081

A2.2 RECORDING SOLUTION

Compound 10X Stock (without NaHCO ₃)	Molecular weight (g/mol)	Concentration (mM)	Grams for 1L (10x)
KCl	74.55	3	2.2365
D-Glucose	180.156	11	19.8165
NaCl	58.44	124	72.4656
NaH ₂ PO ₄	137.99	1.4	1.9319
MgCl ₂	203.30	1.3	2.6429
CaCl ₂	147.01	2	2.9402

10x Bicarbonate	Molecular weight (g/mol)	Concentration (mM)	Grams for 1L (10x)
NaHCO ₃	84.01	26	21.845

A2.3 WHOLE CELL INTERNAL SOLUTIONS

Compound	in 100 ml	Units	Concentration (mM)
K-gluconate	3.0446	g	130
HEPES	0.2383	g	10
NaCl	100	μL (of 1M stock)	1
MgCl ₂	100	μL (of 1M stock)	1
CaCl ₂	100	μL (of 1M stock)	1
EGTA	0.19024	g	5
Mg-ATP	0.5	g	2

- Adjust to pH 7.2 with KOH before adding EGTA and titrate again
- Adjust osmolarity to 290-295 with MilliQ water
- Aliquot to 50 μL and store at -20°C

Compound	in 100 ml	Units	Concentration (mM)
Gluconic acid	4.405	mL	130
HEPES	0.2383	g	10
NaCl	100	μ L (of 1M stock)	1
MgCl ₂	100	μ L (of 1M stock)	1
CaCl ₂	100	μ L (of 1M stock)	1
EGTA	0.19024	g	5
Mg-ATP	0.5	g	2

- Adjust to pH 7.2 with CsOH before adding EGTA and titrate again
- Adjust osmolarity to 290-295 with MilliQ water
- Aliquot to 50 μ L and store at -20°C

APPENDIX 3 STATISTICS TABLES FOR EACH CHAPTER

A3.1 CHAPTER 3 STATISTICS TABLE

Figure	Structure	Type of test	Results																		
Fig. 3.1A	Nonparametric	Chi-square	$X^2 = 9.085$ $p = 0.0026$																		
Fig. 3.1B	Normal	Unpaired t test (two tailed)	$t = 2.490$, $df = 16$ $p = 0.0241$																		
Fig. 3.1C	Normal	Unpaired t test (two tailed)	$t = 0.362$, $df = 7$ $p = 0.497$																		
Fig. 3.1F	Normal	Unpaired t test (two tailed)	$t = 1.705$, $df = 74$ $p = 0.0923$																		
Fig. 3.1G	Normal	Unpaired t test (two tailed)	$t = 1.221$, $df = 104$ $p = 0.2250$																		
Fig. 3.1H	Normal	Unpaired t test (two tailed) Two-way ANOVA	Periodogram (AUC) $t = 1.818$, $df = 52$ $p = 0.0749$ Power Bands Sidak's multiple comparisons test WT vs. Tau^{-/-} Delta, $p < 0.0001$ Theta, $p = 0.0094$ Alpha, $p = 0.7398$ Sigma, $p = 0.6885$ Beta, $p = 0.9686$ Gamma, $p = 0.9717$ ANOVA table <table><thead><tr><th></th><th>SS</th><th>DF</th><th>MS</th><th>F</th><th>P-value</th></tr></thead><tbody><tr><td>Row</td><td></td><td></td><td>1.96e-17</td><td>F (1, 294) =</td><td></td></tr><tr><td>Factor</td><td>1.96e-17</td><td>1</td><td>-17</td><td>45.86</td><td>$p < 0.0001$</td></tr></tbody></table>		SS	DF	MS	F	P-value	Row			1.96e-17	F (1, 294) =		Factor	1.96e-17	1	-17	45.86	$p < 0.0001$
	SS	DF	MS	F	P-value																
Row			1.96e-17	F (1, 294) =																	
Factor	1.96e-17	1	-17	45.86	$p < 0.0001$																

			Column Factor Residual 1.58e-17 1.23e-16 5 294 1.58e-17 4.28e-19 F (5, 294) = 36.86 p < 0.0001
Fig. 3.2A	Nonparametric	Chi-square	t = 0.2250, df = 104 p = 0.2250
Fig. 3.2B	Nonparametric	Chi-square	X² = 0.003 p = 0.9583
Fig. 3.2C	Normal	Two-way ANOVA	ANOVA table SS DF MS F P-value Interaction 9.675 1 9.675 F (1, 54) = 1.298 p=0.2596 Row Factor 5.615 1 5.615 F (1, 54) = 0.7533 p=0.3893 Column Factor 0.02839 1 0.0283 9 0.003809 F (1, 54) = p=0.9510 Residual 402.5 54 7.454 Tukey's multiple-comparisons test WT:No SE : SRS vs. WT:SE : SRS, p = 0.8897 WT:No SE : SRS vs. Tau-/-:No SE : SRS, p = 0.6909 WT:No SE : SRS vs. Tau-/-:SE : SRS, p = 0.9539 WT:SE : SRS vs. Tau-/-:No SE : SRS, p = 0.8058 WT:SE : SRS vs. Tau-/-:SE : SRS, p = 0.9874 Tau-/-:No SE : SRS vs. Tau-/-:SE : SRS, p = 0.7588
Fig. 3.4C	Normal	Unpaired t test (two tailed)	t = 4.828, df = 17 p = 0.0002
Fig. 3.4D	Normal	Unpaired t test (two tailed)	t = 2.340, df = 17 p = 0.0317
Fig. 3.4E	Normal	Unpaired t test (two tailed)	Periodogram (AUC) t = 2.382, df = 19 p = 0.028 Power Bands Sidak's multiple comparisons test WT vs. Tau-/- Delta, p>0.9999 Theta, p>0.9999

			Alpha, p=-0.1712 Sigma, p>0.9999 Beta, p>0.9999 Gamma, p>0.9999					
			ANOVA table	SS	DF	MS	F	P-value
			Row Factor	2.21e-19	1	2.21e-19	F (1, 114) = 0.8105	p=0.3699
			Column Factor	2.47e-18	5	4.94e-19	F (5, 114) = 1.810	P=0.1163
			Residual	3.11e-17	114	2.73e-19		
Fig. 3.5D	Normal	Two-way ANOVA	ANOVA table	SS	DF	MS	F	P-value
			Row Factor	4.443	45	0.0987	F (45, 123) = 1.298	p=0.9074
			Column Factor	7.709	5	1.542	F (45, 123) = 11.04	p < 0.0001
			Residual	17.17	123	0.1396		
			Tukey's multiple-comparisons test Vehicle WT vs. Vehicle Tau ^{-/-} , p = 0.9777 Vehicle WT vs. Ipsilateral WT, p < 0.0001 Vehicle WT vs. Ipsilateral Tau ^{-/-} , p < 0.0001 Vehicle WT vs. Contralateral WT, p = 0.2891 Vehicle WT vs. Contralateral Tau ^{-/-} , p = 0.4066 Vehicle Tau ^{-/-} vs. Ipsilateral WT, p < 0.0001 Vehicle Tau ^{-/-} vs. Ipsilateral Tau ^{-/-} , p = 0.0004 Vehicle Tau ^{-/-} vs. Contralateral WT, p = 0.5946 Vehicle Tau ^{-/-} vs. Contralateral Tau ^{-/-} , p = 0.7583 Ipsilateral WT vs. Ipsilateral Tau ^{-/-} , p = 0.9952 Ipsilateral WT vs. Contralateral WT, p = 0.2259 Ipsilateral WT vs. Contralateral Tau ^{-/-} , p = 0.0512 Ipsilateral Tau ^{-/-} vs. Contralateral WT, p = 0.4843 Ipsilateral Tau ^{-/-} vs. Contralateral Tau ^{-/-} , p = 0.1830 Contralateral WT vs. Contralateral Tau ^{-/-} , p = 0.993					
Fig. 3.5E	Normal	Two-way ANOVA	ANOVA table	SS	DF	MS	F	P-value
			Row Factor	882.6	45	19.61	F (45, 123) = 1.645	p = 0.0173

			F (45, 123) = <table><tr><td>Column</td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>Factor</td><td>203.5</td><td>5</td><td>40.70</td><td>3.414</td><td>p = 0.0064</td></tr><tr><td>Residual</td><td>1431</td><td>123</td><td>0.1396</td><td></td><td></td></tr></table> Tukey's multiple-comparisons test Vehicle WT vs. Vehicle Tau^{-/-}, p = 0.0534 Vehicle WT vs. Ipsilateral WT, p = 0.9994 Vehicle WT vs. Ipsilateral Tau^{-/-}, p = 0.9802 Vehicle WT vs. Contralateral WT, p = 0.0989 Vehicle WT vs. Contralateral Tau^{-/-}, p = 0.1264 Vehicle Tau^{-/-} vs. Ipsilateral WT, p = 0.1549 Vehicle Tau^{-/-} vs. Ipsilateral Tau^{-/-}, p = 0.4352 Vehicle Tau^{-/-} vs. Contralateral WT, p = 0.9910 Vehicle Tau^{-/-} vs. Contralateral Tau^{-/-}, p = 0.9997 Ipsilateral WT vs. Ipsilateral Tau^{-/-}, p = 0.9987 Ipsilateral WT vs. Contralateral WT, p = 0.1729 Ipsilateral WT vs. Contralateral Tau^{-/-}, p = 0.2261 Ipsilateral Tau^{-/-} vs. Contralateral WT, p = 0.3389 Ipsilateral Tau^{-/-} vs. Contralateral Tau^{-/-}, p = 0.4391 Contralateral WT vs. Contralateral Tau^{-/-}, p = 0.9996						Column						Factor	203.5	5	40.70	3.414	p = 0.0064	Residual	1431	123	0.1396														
Column																																						
Factor	203.5	5	40.70	3.414	p = 0.0064																																	
Residual	1431	123	0.1396																																			
Fig. 3.6D	Normal	Two-way ANOVA	<table><tr><td>ANOVA table</td><td>SS</td><td>DF</td><td>MS</td><td>F</td><td>P-value</td></tr><tr><td>Row</td><td></td><td></td><td></td><td>F (34, 91) =</td><td></td></tr><tr><td>Factor</td><td>4.211</td><td>34</td><td>0.1239</td><td>1.385</td><td>p = 0.0878</td></tr></table> Column <table><tr><td>Factor</td><td>11.50</td><td>5</td><td>2.299</td><td>F (5, 91) = 29.10</td><td>p < 0.0001</td></tr><tr><td>Residual</td><td>7.825</td><td>91</td><td>0.086</td><td></td><td></td></tr></table> Tukey's multiple-comparisons test Vehicle WT vs. Vehicle Tau^{-/-}, p < 0.0001 Vehicle WT vs. Ipsilateral WT, p < 0.0001 Vehicle WT vs. Ipsilateral Tau^{-/-}, p < 0.0001 Vehicle WT vs. Contralateral WT, p = 0.0391 Vehicle WT vs. Contralateral Tau^{-/-}, p = 0.9997 Vehicle Tau^{-/-} vs. Ipsilateral WT, p = 0.0253 Vehicle Tau^{-/-} vs. Ipsilateral Tau^{-/-}, p = 0.0151 Vehicle Tau^{-/-} vs. Contralateral WT, p = 0.4987 Vehicle Tau^{-/-} vs. Contralateral Tau^{-/-}, p < 0.0001 Ipsilateral WT vs. Ipsilateral Tau^{-/-}, p > 0.9999 Ipsilateral WT vs. Contralateral WT, p = 0.0003 Ipsilateral WT vs. Contralateral Tau^{-/-}, p < 0.0001						ANOVA table	SS	DF	MS	F	P-value	Row				F (34, 91) =		Factor	4.211	34	0.1239	1.385	p = 0.0878	Factor	11.50	5	2.299	F (5, 91) = 29.10	p < 0.0001	Residual	7.825	91	0.086		
ANOVA table	SS	DF	MS	F	P-value																																	
Row				F (34, 91) =																																		
Factor	4.211	34	0.1239	1.385	p = 0.0878																																	
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Residual	7.825	91	0.086																																			

			Ipsilateral Tau ^{-/-} vs. Contralateral WT, p = 0.0002 Ipsilateral Tau ^{-/-} vs. Contralateral Tau ^{-/-} , p < 0.0001 Contralateral WT vs. Contralateral Tau ^{-/-} , p = 0.1036						
Fig. 3.6E	Normal	Two- way ANOV A	ANOVA						
			table	SS	DF	MS	F	P-value	
							F (34,		
			Row				91) =		
			Factor	4160	34	122.4	1.225	p = 0.2215	
			Column				F (5, 91)		
			Factor	1733	5	346.5	= 3.470	p = 0.0064	
			Residual	9187	91	99.85			
						Tukey's multiple-comparisons test Vehicle WT vs. Vehicle Tau ^{-/-} , p = 0.6096 Vehicle WT vs. Ipsilateral WT, p = 0.0517 Vehicle WT vs. Ipsilateral Tau ^{-/-} , p = 0.0057 Vehicle WT vs. Contralateral WT, p = 0.7179 Vehicle WT vs. Contralateral Tau ^{-/-} , p = 0.0789 Vehicle Tau ^{-/-} vs. Ipsilateral WT, p = 0.5832 Vehicle Tau ^{-/-} vs. Ipsilateral Tau ^{-/-} , p = 0.1861 Vehicle Tau ^{-/-} vs. Contralateral WT, p < 0.9999 Vehicle Tau ^{-/-} vs. Contralateral Tau ^{-/-} , p = 0.7527 Ipsilateral WT vs. Ipsilateral Tau ^{-/-} , p = 0.9899 Ipsilateral WT vs. Contralateral WT, p = 0.7847 Ipsilateral WT vs. Contralateral Tau ^{-/-} , p = 0.9992 Ipsilateral Tau ^{-/-} vs. Contralateral WT, p = 0.3987 Ipsilateral Tau ^{-/-} vs. Contralateral Tau ^{-/-} , p = 0.9118 Contralateral WT vs. Contralateral Tau ^{-/-} , p = 0.9114			

A3.2 CHAPTER 4 STATISTICS TABLE

Figure	Structure	Type of test	Results					
Fig. 4.1B	Normal	Two-way ANOVA	ANOVA table	SS	DF	MS	F	P-value

			Interaction 3732 20 186.6 F (20, 300) = 2.287 p=0.0016 Row Factor 4416 4 1104 F (4, 300) = 13.53 p<0.0001 Column Factor 8182 5 1636 F (5, 300) = 20.06 p<0.0001 Residual 24473 300 81.58 Tukey's multiple-comparisons test WT Vehicle vs. Tau^{-/-} Vehicle, p = 0.3481 WT Vehicle vs. WT IHK-Ipsilateral, p <0.0001**** WT Vehicle vs. Tau^{-/-} IHK-Ipsilateral p <0.0001**** WT Vehicle vs. WT IHK-Contralateral p = 0.9597 WT Vehicle vs. Tau^{-/-} IHK-Contralateral p = 0.9043 Tau^{-/-} Vehicle vs. WT IHK-Ipsilateral, p <0.0001**** Tau^{-/-} Vehicle vs. Tau^{-/-} IHK-Ipsilateral, p =0.0005 Tau^{-/-} Vehicle vs. WT IHK-Contralateral, p = 0.9562 Tau^{-/-} Vehicle vs. Tau^{-/-} IHK-Contralateral, p = 0.1192 WT IHK-Ipsilateral vs. Tau^{-/-} IHK-Ipsilateral, p = 0.4500 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p < 0.0001**** WT IHK-Ipsilateral vs. Tau^{-/-} IHK-Contralateral, p < 0.0001**** Tau^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p < 0.0001**** Tau^{-/-} IHK-Ipsilateral vs. Tau^{-/-} IHK-Contralateral, p < 0.0001**** WT IHK-Contralateral vs. Tau^{-/-} IHK-Contralateral, p =0.5842			
Fig. 4.1C	Normal	Two-way ANOVA	ANOVA table SS DF MS F P-value Row Factor 671.2 20 33.56 F (20, 34) = 1.007 p=0.4793 Column Factor 498.0 5 99.60 F (5, 34) = 1.007 p=0.0243 Residual 1133 34 33.33 Tukey's multiple-comparisons test			

			WT Vehicle vs. Tau ^{-/-} Vehicle, p = 0.7351 WT Vehicle vs. WT IHK-Ipsilateral, p >0.9999 WT Vehicle vs. Tau ^{-/-} IHK-Ipsilateral p =0.9936 WT Vehicle vs. WT IHK-Contralateral p =0.1636 WT Vehicle vs. Tau ^{-/-} IHK-Contralateral p =0.9996 Tau ^{-/-} Vehicle vs. WT IHK-Ipsilateral, p =0.8192 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p =0.6358 Tau ^{-/-} Vehicle vs. WT IHK-Contralateral, p =0.0069** Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral, p =0.8203 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.9977 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.3725 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p >0.9999 Tau ^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.7903 Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p >0.9999 WT IHK-Contralateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.7417					
Fig. 4.1D	Normal	Two-way ANOVA	ANOVA table SS DF MS F P-value Row Factor 32724 20 1636 F (20, 34) = 0.8327 p=0.6604 Column Factor 268307 5 53661 F (20, 34) = 27.31 p<0.0001 Residual 58947 34 1965 Tukey's multiple-comparisons test WT Vehicle vs. Tau ^{-/-} Vehicle p = 0.9992 WT Vehicle vs. WT IHK-Ipsilateral, p < 0.0001 WT Vehicle vs. Tau ^{-/-} IHK-Ipsilateral p < 0.0001 WT Vehicle vs. WT IHK-Contralateral p = 0.9533 WT Vehicle vs. Tau ^{-/-} IHK-Contralateral p = 0.9961 Tau ^{-/-} Vehicle vs. WT IHK-Ipsilateral, p < 0.0001 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p < 0.0001 Tau ^{-/-} Vehicle vs. WT IHK-Contralateral, p = 0.9961 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral, p =0.9998 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.2623 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p < 0.0001 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p < 0.0001 Tau ^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p < 0.0001 Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.0002 WT IHK-Contralateral vs. Tau ^{-/-} IHK-Contralateral, p > 0.9999					

Fig. 4.1E	Normal	Two-way ANOVA	ANOVA table SS DF MS F P-value Row Factor 83781 20 4410 F (20, 34) = 1.440 p=0.1710 Column Factor 272841 5 54568 F (5, 34) = 0.7242 p<0.0001 Residual 107170 35 3062				
			Tukey's multiple-comparisons test WT Vehicle vs. Tau^{-/-} Vehicle, p = 0.8755 WT Vehicle vs. WT IHK-Ipsilateral, p < 0.0001**** WT Vehicle vs. Tau^{-/-} IHK-Ipsilateral p = 0.0003*** WT Vehicle vs. WT IHK-Contralateral p = 0.9998 WT Vehicle vs. Tau^{-/-} IHK-Contralateral p = 0.2091 Tau^{-/-} Vehicle vs. WT IHK-Ipsilateral, p < 0.0001**** Tau^{-/-} Vehicle vs. Tau^{-/-} IHK-Ipsilateral, p < 0.0001**** Tau^{-/-} Vehicle vs. WT IHK-Contralateral, p = 0.7422 Tau^{-/-} Vehicle vs. Tau^{-/-} IHK-Contralateral, p = 0.6070 WT IHK-Ipsilateral vs. Tau^{-/-} IHK-Ipsilateral, p > 0.9999 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p < 0.0001**** WT IHK-Ipsilateral vs. Tau^{-/-} IHK-Contralateral, p < 0.0001**** Tau^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.0005*** Tau^{-/-} IHK-Ipsilateral vs. Tau^{-/-} IHK-Contralateral, p < 0.0001**** WT IHK-Contralateral vs. Tau^{-/-} IHK-Contralateral, p = 0.1491				
Fig. 4.3D	Normal	Two-way ANOVA	ANOVA table SS DF MS F P-value Row Factor 8.019 26 0.3084 F (26, 104) = 1.348 p=0.1470 Column Factor 9.487 5 1.897 F (5, 104) = 8.294 p<0.0001 Residual 23.79 104 0.2288				
			Tukey's multiple-comparisons test				

			WT Vehicle vs. Tau ^{-/-} Vehicle, p = 0.0168* WT Vehicle vs. WT IHK-Ipsilateral, p = 0.8703 WT Vehicle vs. Tau ^{-/-} IHK-Ipsilateral p = 0.3840 WT Vehicle vs. WT IHK-Contralateral p = 0.0967 WT Vehicle vs. Tau ^{-/-} IHK-Contralateral p = 0.4631 Tau ^{-/-} Vehicle vs. WT IHK-Ipsilateral, p = 0.2889 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.8991 Tau ^{-/-} Vehicle vs. WT IHK-Contralateral, p < 0.0001**** Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral, p = 0.7663 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.9464 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.0039** WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.9796 Tau ^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.0004** Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p > 0.9999 WT IHK-Contralateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.0004***																													
Fig. 4.3E	Normal	Two-way ANOVA	<table><tr><td>ANOVA table</td><td>SS</td><td>DF</td><td>MS</td><td>F</td><td>P-value</td></tr><tr><td>Row Factor</td><td>222.2</td><td>26</td><td>8.887</td><td>F (26, 104) = 0.9175</td><td>p=0.5813</td></tr><tr><td>Column Factor</td><td>135.7</td><td>5</td><td>27.14</td><td>F (5, 104) = 2.802</td><td>p=0.0206</td></tr><tr><td>Residual</td><td>978.3</td><td>104</td><td>9.686</td><td></td><td></td></tr></table> Tukey's multiple-comparisons test WT Vehicle vs. Tau^{-/-} Vehicle, p =0.7213 WT Vehicle vs. WT IHK-Ipsilateral, p = 0.9940 WT Vehicle vs. Tau^{-/-} IHK-Ipsilateral p = 0.9971 WT Vehicle vs. WT IHK-Contralateral p = 0.2794 WT Vehicle vs. Tau^{-/-} IHK-Contralateral p = 0.9760 Tau^{-/-} Vehicle vs. WT IHK-Ipsilateral, p =0.3877 Tau^{-/-} Vehicle vs. Tau^{-/-} IHK-Ipsilateral, p = 0.9693 Tau^{-/-} Vehicle vs. WT IHK-Contralateral, p = 0.0083** Tau^{-/-} Vehicle vs. Tau^{-/-} IHK-Contralateral, p = 0.3244 WT IHK-Ipsilateral vs. Tau^{-/-} IHK-Ipsilateral, p = 0.9289 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.6156 WT IHK-Ipsilateral vs. Tau^{-/-} IHK-Contralateral, p >0.9999 Tau^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.1693 Tau^{-/-} IHK-Ipsilateral vs. Tau^{-/-} IHK-Contralateral, p = 0.8616						ANOVA table	SS	DF	MS	F	P-value	Row Factor	222.2	26	8.887	F (26, 104) = 0.9175	p=0.5813	Column Factor	135.7	5	27.14	F (5, 104) = 2.802	p=0.0206	Residual	978.3	104	9.686		
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Row Factor	222.2	26	8.887	F (26, 104) = 0.9175	p=0.5813																											
Column Factor	135.7	5	27.14	F (5, 104) = 2.802	p=0.0206																											
Residual	978.3	104	9.686																													

			WT IHK-Contralateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.8084				
Fig. 4.3I	Normal	Two-way ANOVA	ANOVA table SS DF MS F P-value Row Factor 0.2045 17 0.0120 F (17, 63) = 0.4594 p=0.9618 Column Factor 0.3615 5 0.0723 F (5, 63) = 2.760 p=0.0256 Residual 1.650 63 0.0261				
			Tukey's multiple-comparisons test WT Vehicle vs. Tau ^{-/-} Vehicle, p = 0.3282 WT Vehicle vs. WT IHK-Ipsilateral, p = 0.9981 WT Vehicle vs. Tau ^{-/-} IHK-Ipsilateral p = 0.9492 WT Vehicle vs. WT IHK-Contralateral p > 0.9999 WT Vehicle vs. Tau ^{-/-} IHK-Contralateral p = 0.4207 Tau ^{-/-} Vehicle vs. WT IHK-Ipsilateral, p = 0.2721 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.0754 Tau ^{-/-} Vehicle vs. WT IHK-Contralateral, p = 0.5466 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral, p > 0.9999 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.9992 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.9978 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.2986 Tau ^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.9612 Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.0911 WT IHK-Contralateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.5755				
Fig. 4.3J	Normal	Two-way ANOVA	ANOVA table SS DF MS F P-value Row Factor 74.01 17 4.354 F (17, 63) = 1.838 p=0.0423 Column Factor 4.619 5 0.9238 F (5, 63) = 0.3900 p=0.8539 Residual 149.2 63 2.369				
			Tukey's multiple-comparisons test WT Vehicle vs. Tau ^{-/-} Vehicle, p = 0.9993				

			WT Vehicle vs. WT IHK-Ipsilateral, p > 0.9999 WT Vehicle vs. Tau ^{-/-} IHK-Ipsilateral p > 0.9999 WT Vehicle vs. WT IHK-Contralateral p > 0.9999 WT Vehicle vs. Tau ^{-/-} IHK-Contralateral p = 0.8035 Tau ^{-/-} Vehicle vs. WT IHK-Ipsilateral, p > 0.9999 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p > 0.9999 Tau ^{-/-} Vehicle vs. WT IHK-Contralateral, p > 0.9999 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral, p = 0.9396 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Ipsilateral, p > 0.9999 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p > 0.9999 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.9299 Tau ^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p > 0.9999 Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p =0.8936 WT IHK-Contralateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.9237																																								
Fig. 4.5D	Normal	Two-way ANOVA	<table><tr><td>ANOVA table</td><td>SS</td><td>DF</td><td>MS</td><td>F</td><td>P-value</td></tr><tr><td>Row</td><td></td><td></td><td></td><td>F (25, 83)</td><td></td></tr><tr><td>Factor</td><td>54.06</td><td>25</td><td>2.163</td><td>= 0.7898</td><td>p=0.7434</td></tr><tr><td>Column</td><td></td><td></td><td></td><td>F (5, 83)</td><td></td></tr><tr><td>Factor</td><td>64.78</td><td>5</td><td>12.96</td><td>=4.732</td><td>p=0.0007</td></tr><tr><td>Residual</td><td>227.3</td><td>83</td><td>2.738</td><td></td><td></td></tr></table> Tukey's multiple-comparisons test WT Vehicle vs. Tau^{-/-} Vehicle, p =0.0005*** WT Vehicle vs. WT IHK-Ipsilateral, p > 0.9999 WT Vehicle vs. Tau^{-/-} IHK-Ipsilateral p = 0.9741 WT Vehicle vs. WT IHK-Contralateral p = 0.9955 WT Vehicle vs. Tau^{-/-} IHK-Contralateral p = 0.9438 Tau^{-/-} Vehicle vs. WT IHK-Ipsilateral, p =0.0009*** Tau^{-/-} Vehicle vs. Tau^{-/-} IHK-Ipsilateral, p = 0.0110* Tau^{-/-} Vehicle vs. WT IHK-Contralateral, p = 0.0058** Tau^{-/-} Vehicle vs. Tau^{-/-} IHK-Contralateral, p = 0.0143* WT IHK-Ipsilateral vs. Tau^{-/-} IHK-Ipsilateral, p = 0.9868 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.9984 WT IHK-Ipsilateral vs. Tau^{-/-} IHK-Contralateral, p = 0.9673 Tau^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.9999 Tau^{-/-} IHK-Ipsilateral vs. Tau^{-/-} IHK-Contralateral, p > 0.9999 WT IHK-Contralateral vs. Tau^{-/-} IHK-Contralateral, p = 0.9991					ANOVA table	SS	DF	MS	F	P-value	Row				F (25, 83)		Factor	54.06	25	2.163	= 0.7898	p=0.7434	Column				F (5, 83)		Factor	64.78	5	12.96	=4.732	p=0.0007	Residual	227.3	83	2.738		
ANOVA table	SS	DF	MS	F	P-value																																						
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Residual	227.3	83	2.738																																								

Fig. 4.5E	Normal	Two-way ANOVA	ANOVA table	SS	DF	MS	F	P-value
			Row Factor	1672	25	66.88	F (25, 83) = 1.010	p=0.4650
			Column Factor	601.9	5	120.4	F (5, 83) = 1.818	p=0.1186
			Residual	5296	83	0.1091		
			Tukey's multiple-comparisons test					
			WT Vehicle vs. Tau ^{-/-} Vehicle, p > 0.9999					
			WT Vehicle vs. WT IHK-Ipsilateral, p = 0.7499					
			WT Vehicle vs. Tau ^{-/-} IHK-Ipsilateral p = 0.9998					
			WT Vehicle vs. WT IHK-Contralateral p = 0.4270					
			WT Vehicle vs. Tau ^{-/-} IHK-Contralateral p = 0.9645					
			Tau ^{-/-} Vehicle vs. WT IHK-Ipsilateral, p = 0.8796					
			Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.9997					
			Tau ^{-/-} Vehicle vs. WT IHK-Contralateral, p = 0.5608					
			Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral, p = 0.9691					
			WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.7508					
			WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.9864					
			WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.3554					
			Tau ^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.4129					
			Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.9974					
			WT IHK-Contralateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.1308					
Fig. 4.5I	Normal	Two-way ANOVA	ANOVA table	SS	DF	MS	F	P-value
			Row Factor	2.183	21	0.1039	F (21, 87) = 0.9529	p=0.5273
			Column Factor	4.063	5	0.8127	F (5, 87) = 7.451	p<0.0001
			Residual	9.490	87	0.1091		
			Tukey's multiple-comparisons test					
			WT Vehicle vs. Tau ^{-/-} Vehicle, p = 0.0001***					
			WT Vehicle vs. WT IHK-Ipsilateral, p = 0.0031**					
			WT Vehicle vs. Tau ^{-/-} IHK-Ipsilateral p = 0.9890					
			WT Vehicle vs. WT IHK-Contralateral p = 0.0019**					
			WT Vehicle vs. Tau ^{-/-} IHK-Contralateral p = 0.9191					

			Tau ^{-/-} Vehicle vs. WT IHK-Ipsilateral, p = 0.9438 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.0119* Tau ^{-/-} Vehicle vs. WT IHK-Contralateral, p = 0.9835 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral, p = 0.0114* WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.0968 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p >0.9999 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.1113 Tau ^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.0649 Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p =0.9998 WT IHK-Contralateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.0727					
Fig. 4.5J	Normal	Two- way ANOV A	ANOVA table	SS	DF	MS	F	P-value
			Row				F (21, 87)	
			Factor	340.5	21	16.21	= 1.243	p=0.2382
			Column				F (5, 87)	
			Factor	177.1	5	35.42	=2.714	P=0.0251
			Residual	1135	87	13.05		
			Tukey's multiple-comparisons test					
			WT Vehicle vs. Tau ^{-/-} Vehicle, p =0.9993 WT Vehicle vs. WT IHK-Ipsilateral, p = 0.9838 WT Vehicle vs. Tau ^{-/-} IHK-Ipsilateral p = 0.7078 WT Vehicle vs. WT IHK-Contralateral p = 0.0645 WT Vehicle vs. Tau ^{-/-} IHK-Contralateral p >0.9999 Tau ^{-/-} Vehicle vs. WT IHK-Ipsilateral, p = 0.9076 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.5171 Tau ^{-/-} Vehicle vs. WT IHK-Contralateral, p = 0.0258* Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral, p = 0.9980 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.9564 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p =0.2735 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.9951 Tau ^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.9166 Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p =0.7891 WT IHK-Contralateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.1238					

A3.3 CHAPTER 5 STATISTICS TABLE

Figure	Structure	Type of test	Results					
Fig. 5.2D	Normal	Two-way ANOVA	ANOVA table	SS	DF	MS	F	P-value
			Row				F (36, 82)	
			Factor	1007	36	27.98	= 1.065	p=0.3971
			Column				F (5, 82)	
			Factor	221.3	5	44.26	=1.685	p=0.1473
			Residual	2153	82	26.26		
			Tukey's multiple-comparisons test					
			WT Vehicle vs. Tau ^{-/-} Vehicle, p > 0.0009					
			WT Vehicle vs. WT IHK-Ipsilateral, p = 0.1126					
			WT Vehicle vs. Tau ^{-/-} IHK-Ipsilateral p = 0.8332					
			WT Vehicle vs. WT IHK-Contralateral p = 0.7106					
			WT Vehicle vs. Tau ^{-/-} IHK-Contralateral p = 0.9989					
			Tau ^{-/-} Vehicle vs. WT IHK-Ipsilateral, p = 0.1905					
			Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.8790					
			Tau ^{-/-} Vehicle vs. WT IHK-Contralateral, p = 0.7849					
			Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral, p = 0.9992					
WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.9765								
WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.8680								
WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.5966								
Tau ^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p > 0.0009								
Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p =0.9729								
WT IHK-Contralateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.9892								
Fig. 5.2E	Normal	Two-way ANOVA	ANOVA table	SS	DF	MS	F	P-value
			Row				F (36, 82)	
			Factor	3690	36	108.5	= 1.106	p=0.3533
			Column				F (5, 82)	
			Factor	337.3	5	67.45	=0.6876	p=0.6344
			Residual	6867	82	98.09		
			Tukey's multiple-comparisons test					

			WT Vehicle vs. Tau ^{-/-} Vehicle, p > 0.0009 WT Vehicle vs. WT IHK-Ipsilateral, p = 0.6782 WT Vehicle vs. Tau ^{-/-} IHK-Ipsilateral p = 0.9998 WT Vehicle vs. WT IHK-Contralateral p > 0.0009 WT Vehicle vs. Tau ^{-/-} IHK-Contralateral p > 0.0009 Tau ^{-/-} Vehicle vs. WT IHK-Ipsilateral, p = 0.7952 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.9997 Tau ^{-/-} Vehicle vs. WT IHK-Contralateral, p > 0.0009 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral, p > 0.0009 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.9656 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.5996 WT IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.8473 Tau ^{-/-} IHK-Ipsilateral vs. WT IHK-Contralateral, p =0.9979 Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p =0.9996 WT IHK-Contralateral vs. Tau ^{-/-} IHK-Contralateral, p > 0.0009																														
Fig. 5.3D	Normal	One-way ANOVA	ANOVA Summary F 11.80 P value <0.0001 R squared 0.1110 ANOVA table <table><tr><td></td><td>SS</td><td>DF</td><td>MS</td><td>F</td><td>P-value</td></tr><tr><td></td><td></td><td></td><td></td><td>F (2, 189)</td><td></td></tr><tr><td>Treatment</td><td>265892</td><td>2</td><td>132946</td><td>= 11.80</td><td>p<0.0001</td></tr><tr><td>Residual</td><td>2128960</td><td>189</td><td>11264</td><td></td><td></td></tr><tr><td>Total</td><td>2394852</td><td>191</td><td></td><td></td><td></td></tr></table> Tukey’s multiple-comparisons test WT Vehicle vs. WT IHK-Ipsilateral, p < 0.0001 WT Vehicle vs. WT IHK-Contralateral p = 0.9584 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p < 0.0013		SS	DF	MS	F	P-value					F (2, 189)		Treatment	265892	2	132946	= 11.80	p<0.0001	Residual	2128960	189	11264			Total	2394852	191			
	SS	DF	MS	F	P-value																												
				F (2, 189)																													
Treatment	265892	2	132946	= 11.80	p<0.0001																												
Residual	2128960	189	11264																														
Total	2394852	191																															
Fig. 5.3E	Normal	One-way ANOVA	ANOVA Summary F 3.057 P value = 0.0561 R squared 0.1109 ANOVA table <table><tr><td></td><td>SS</td><td>DF</td><td>MS</td><td>F</td><td>P-value</td></tr></table>		SS	DF	MS	F	P-value																								
	SS	DF	MS	F	P-value																												

			F (2, 49) =			
			Treatment	5.778	2	2.889 3.057 p=0.0561
			Residual	46.30	49	0.9449
			Total	52.08	51	
			Tukey's multiple-comparisons test			
			WT Vehicle vs. WT IHK-Ipsilateral, p = 0.1090			
			WT Vehicle vs. WT IHK-Contralateral p = 0.1836			
			WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.9997			
Fig. 5.3I	Normal	Two-way ANOVA	ANOVA table	SS	DF	MS F P-value
			Row Factor	243716	9	27080 F (9, 1320) = 66.91 p<0.0001
			Column Factor	66023	2	33012 F (5, 1320) =81.56 p<0.0001
			Residual	534245	1320	404.7
			Tukey's multiple-comparisons test			
			Row 1			
			WT Vehicle vs. WT IHK-Ipsilateral, p>0.9999			
			WT Vehicle vs. WT IHK-Contralateral p>0.9999			
			WT IHK-Ipsilateral vs. WT IHK-Contralateral, p>0.9999			
			Row 2			
			WT Vehicle vs. WT IHK-Ipsilateral, p = 0.0271			
			WT Vehicle vs. WT IHK-Contralateral p < 0.0001			
			WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.4026			
			Row 3			
			WT Vehicle vs. WT IHK-Ipsilateral, p = 0.9520			
			WT Vehicle vs. WT IHK-Contralateral, p < 0.0001			
			WT IHK-Ipsilateral vs. WT IHK-Contralateral, p < 0.0001			
			Row 4			
			WT Vehicle vs. WT IHK-Ipsilateral, p = 0.9107			
			WT Vehicle vs. WT IHK-Contralateral, p < 0.0001			
			WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.0017			
			Row 5			

			WT Vehicle vs. WT IHK-Ipsilateral, p = 0.9250 WT Vehicle vs. WT IHK-Contralateral, p = 0.0002 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.0113 Row 6 WT Vehicle vs. WT IHK-Ipsilateral, p = 0.7729 WT Vehicle vs. WT IHK-Contralateral, p < 0.0001 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p < 0.0001 Row 7 WT Vehicle vs. WT IHK-Ipsilateral, p = 0.5210 WT Vehicle vs. WT IHK-Contralateral, p = 0.0034 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.2205 Row 8 WT Vehicle vs. WT IHK-Ipsilateral, p = 0.9665 WT Vehicle vs. WT IHK-Contralateral, p = 0.0372 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.0725 Row 9 WT Vehicle vs. WT IHK-Ipsilateral, p = 0.7969 WT Vehicle vs. WT IHK-Contralateral, p < 0.0001 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p < 0.0001 Row 10 WT Vehicle vs. WT IHK-Ipsilateral, p > 0.9999 WT Vehicle vs. WT IHK-Contralateral, p < 0.0001 WT IHK-Ipsilateral vs. WT IHK-Contralateral, p = 0.0021
Fig. 5.4D	Normal	One-way ANOVA	ANOVA Summary F 32.41 P value <0.0001 R squared 0.2669 ANOVA table SS DF MS F P-value Treatment 748995 2 374498 F (2, 178) p<0.0001 Residual 2057053 178 11556 Total 2806048 180 Tukey's multiple-comparisons test Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.0001 Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral p < 0.0001

			Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.0009					
Fig 5.4E	Normal	One-way ANOVA	ANOVA Summary					
			F 6.152 P value 0.0048 R squared 0.2446					
			ANOVA					
			table	SS	DF	MS	F	P-value
							F (2, 38) =	
			Treatment	17.85	2	8.924	6.152	p=0.0048
			Residual	55.13	38	1.451		
			Total	72.98	40			
			Tukey's multiple-comparisons test					
			Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p = 0.0225					
			Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral p = 0.0147					
			Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p = 0.9665					
Fig. 5.4I	Normal	Two-way ANOVA	ANOVA					
			table	SS	DF	MS	F	P-value
							F (9, 1340) =	
			Row Factor	364391	9	40488	156.3	p<0.0001
							F (2, 1340)	
			Column Factor	4254	2	2127	=8.213	p=0.0003
			Residual	347036	1340	259.0		
			Tukey's multiple-comparisons test					
			Row 1					
			Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Ipsilateral, p>0.9999					
			Tau ^{-/-} Vehicle vs. Tau ^{-/-} IHK-Contralateral p>0.9999					
			Tau ^{-/-} IHK-Ipsilateral vs. Tau ^{-/-} IHK-Contralateral, p>0.9999					
			Row 2					
			WT Vehicle vs. WT IHK-Ipsilateral, p = 0.4457					
			WT Vehicle vs. WT IHK-Contralateral p = 0.0220					

			WT IHK-Ipsilateral vs. WT IHK-Contralateral, $p = 0.3904$ Row 3 WT Vehicle vs. WT IHK-Ipsilateral, $p = 0.0201$ WT Vehicle vs. WT IHK-Contralateral, $p = 0.0141$ WT IHK-Ipsilateral vs. WT IHK-Contralateral, $p > 0.9999$ Row 4 WT Vehicle vs. WT IHK-Ipsilateral, $p = 0.9801$ WT Vehicle vs. WT IHK-Contralateral, $p = 0.5739$ WT IHK-Ipsilateral vs. WT IHK-Contralateral, $p = 0.7272$ Row 5 WT Vehicle vs. WT IHK-Ipsilateral, $p = 0.4160$ WT Vehicle vs. WT IHK-Contralateral, $p = 0.6194$ WT IHK-Ipsilateral vs. WT IHK-Contralateral, $p = 0.9292$ Row 6 WT Vehicle vs. WT IHK-Ipsilateral, $p = 0.9938$ WT Vehicle vs. WT IHK-Contralateral, $p = 0.8109$ WT IHK-Ipsilateral vs. WT IHK-Contralateral, $p = 0.7708$ Row 7 WT Vehicle vs. WT IHK-Ipsilateral, $p = 0.8061$ WT Vehicle vs. WT IHK-Contralateral, $p = 0.4940$ WT IHK-Ipsilateral vs. WT IHK-Contralateral, $p = 0.8931$ Row 8 WT Vehicle vs. WT IHK-Ipsilateral, $p = 0.1195$ WT Vehicle vs. WT IHK-Contralateral, $p = 0.0578$ WT IHK-Ipsilateral vs. WT IHK-Contralateral, $p = 0.9745$ Row 9 WT Vehicle vs. WT IHK-Ipsilateral, $p = 0.9656$ WT Vehicle vs. WT IHK-Contralateral, $p = 0.4169$ WT IHK-Ipsilateral vs. WT IHK-Contralateral, $p = 0.6129$ Row 10 WT Vehicle vs. WT IHK-Ipsilateral, $p = 0.4651$ WT Vehicle vs. WT IHK-Contralateral, $p = 0.8357$ WT IHK-Ipsilateral vs. WT IHK-Contralateral, $p = 0.8065$
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