The Effects of Tau Expression on Glial Mitochondrial Morphology in *Drosophila melanogaster*

by

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Abstract

Tauopathies are a branch of neurodegenerative disorders characterized by the phosphorylation of the protein tau. These diseases are one of the leading causes of dementia, currently have no known cure, and are frequently heartbreaking to affected individuals and their families. While neurodegenerative diseases are poorly understood, studies have shown that hyperphosphorylated tau causes mitochondrial dysfunction and results in a decrease of ATP production during cellular respiration, as well as an increase in the toxic byproduct ROS, leading to apoptosis. While the effects of tau on mitochondria is established in neurons, this relationship has not been thoroughly examined in the "other" cells of the brain, known as glial cells. Glia support neuronal cellular respiration and synaptic functioning, making them potentially susceptible to mitochondrial tauopathy. This research examined whether the morphology and quantity of glial mitochondria are disrupted in the presence of tau in *Drosophila melanogaster*. It found that the quantity of glial mitochondria is significantly reduced in the presence of tau, but that tau had no effect on mitochondrial volume, surface area, and sphericity. These findings suggest that glial mitochondria are disrupted by tauopathy in a unique manner, and highlights their importance in future studies as therapeutic targets for neurodegenerative diseases. Given their roles in metabolic support, we further quantified age and sex differences in astrocytic mitochondria, finding that day three flies possess fewer mitochondria than days 10 and 30. These studies were employed with the goal of establishing control conditions for future studies aimed at determining astrocytic-specific effects of tau expression.

Chapter 1- Neurodegenerative Diseases and Tauopathies

Due to their prevalence in the United States as the most common neurodegenerative diseases, substantial research has gone into understanding the biochemical basis of Alzheimer's Disease (AD) and other types of dementia. Estimates show that roughly seven million people in the US are living with some type of dementia, and in 2014 this accounted for 1.6% of the population. With an increasingly aging population, this number is set to grow considerably and double by 2060 (Thorpe, Levy, and Thomas, 2021). There is no known cure for these types of diseases, and very limited treatment options. Common treatments largely focus on slowing neurodegeneration and treating negative behavioral effects of the disease, but can do nothing to stop or reverse its progression (NHS, 2023). These dementias represent a significant public health issue, and affect many elderly individuals and their families each year.

Decades of research fueled by a desire to treat the millions of people affected by AD have uncovered several possible methods of action for disease progression, but many aspects of Alzheimer's and other dementias are still poorly understood. Previous research has shown that the protein tau, a microtubule associated protein, is involved in the pathology and progression of several types of dementias, known as "tauopathies" (Bravo et al, 2024). Common tauopathies include Alzheimer's, frontotemporal dementia, chronic traumatic encephalopathy, Pick's disease, and progressive subnuclear palsy. Each of these diseases is characterized by the misfolding and aggregation of the protein tau, which is implicated in causing inflammation in cells, DNA damage, and eventually cell death (Metaxas and Kempf, 2016). In Alzheimer's, neurons in the hippocampus are most vulnerable to the effects of this protein, leading patients to progressively lose their memory as their cells die off, with damage to other areas of the brain in later stages (Rao et al, 2022).

Healthy Functions of Tau Protein

The protein tau functions in healthy cells to bind to and support microtubules, a major component of the cytoskeleton. The cytoskeleton is a highly dynamic scaffold that creates internal cellular structure, consisting of a series of filaments that proteins can be transported along. It functions similar to a cellular "highway" that motor proteins carry "cargo" down. This cargo can include cellular vesicles containing nutrients and cellular building blocks, proteins and

organelles, and mitochondria (Alberts et al, 2002). The cytoskeleton also facilitates mitosis, neuronal outgrowth, and muscle contraction, and is essential for cellular structure and function.

Normal tau promotes the cytoskeleton's ability to dynamically change (Stringham et al, 2012). In its natural form, tau can bind directly to microtubules and induce polymerization, or dissociate from the cytoskeleton to trigger depolymerization. This allows for continuous reorganization according to cellular needs (Figure 1). Tau is therefore important for cellular stability and cytoskeletal dynamics, and facilitates easy transport of proteins from one part of the cell to another (Bravo et al, 2024).

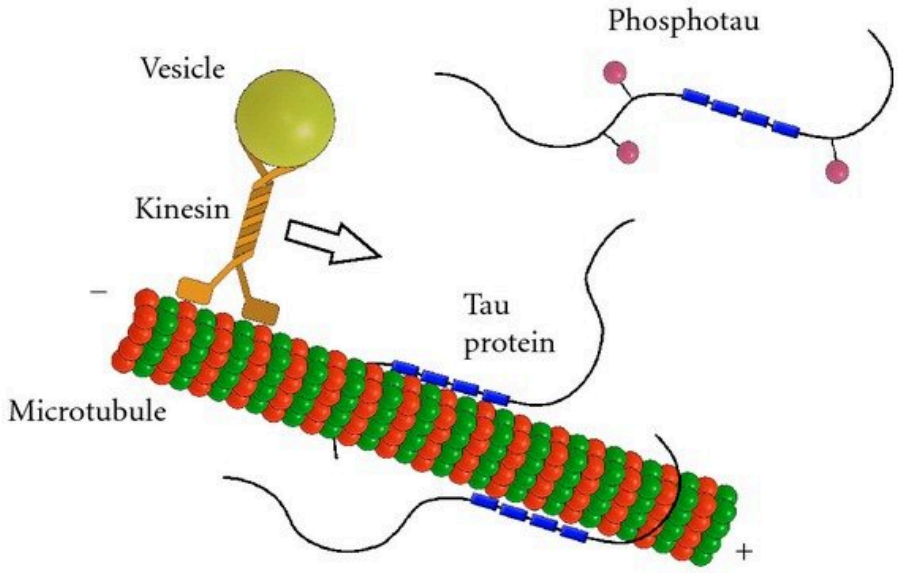


Figure 1. Tau facilitates microtubule stability and transport. Binding of tau protein to the microtubules is maintained in equilibrium by coordinated actions of kinases and phosphatases. The phosphorylation of tau (pink balls) regulates its activity to bind to microtubules and can affect axonal transport. Adapted from Kolorova et al (2012).

Tau Isoforms and Genetics

Alzheimer's Disease is associated with different isoforms (or types) of tau that are produced during mRNA splicing after DNA transcription (Bueé et al, 2000). Splicing involves the removal of unnecessary components of an RNA transcript called introns, but this process can be highly variable, as is the case with tau. Tau is encoded by the *MAPT* gene, named aptly the "microtubule associated protein tau." When this gene is translated from mRNA to protein, the exons in the pre-RNA 2, 3, and 10 are alternatively spliced together, meaning that six different

isoforms of the protein can occur (Figure 2) (Bueé et al, 2000). Removal of introns results in the creation of different forms of tau that have a varied number of N and R domains, or regions where the protein can interact with other molecules. These areas interact with microtubules and can undergo post-translational modifications (Bueé et al, 2000). The most common isoforms of tau in neurodegenerative diseases are known as "3R" and "4R," which have three and four binding regions, respectively. Depending on an individual's genetics, they may be more prone to developing these pathological isoforms of tau.

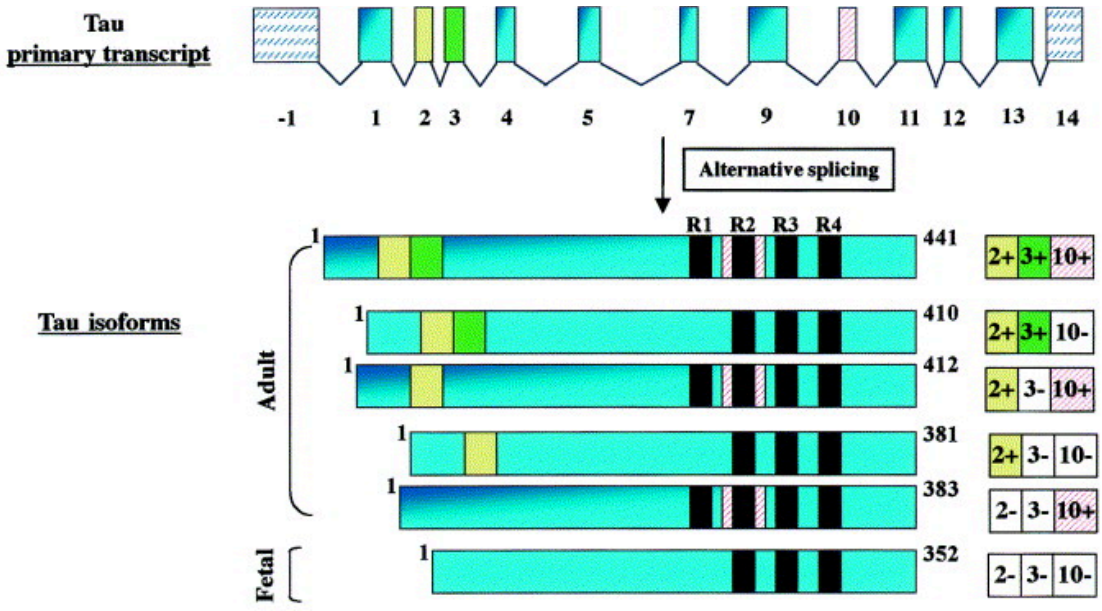


Figure 2. Alternative splicing of introns during tau protein translation. Introns 2 (yellow), 3 (green), and 10 (red) are alternatively spliced. Splicing of intron 10 in tau results in either three or four R regions, with some isoforms lacking the R1 region. Tau is characterized as "3R" or "4R" based on the number of microtubule binding repeat (R) regions it has. Introns 2 and 3 are amino acid sequences known as "N terminal inserts." Each type of tau can have between 0-2 of these inserts depending on the way they are spliced. Adapted From Bueé et al (2020).

Tau and Hyperphosphorylation

Once translated from mRNA to protein, tau can be subjected to post-translational modifications, including phosphorylation (the addition of a phosphate group). Phosphorylation causes tau to dissociate from microtubules by inducing conformational changes in its structure. In a healthy cell, phosphorylation and dephosphorylation are balanced, allowing tau to exist in

multiple different forms depending on cytoskeletal dynamics (Bueé et al, 2000). However, in neurodegenerative diseases, tau becomes hyperphosphorylated. When hyperphosphorylated, tau dissociates from microtubules and begins to bind to itself, creating toxic aggregates called neurofibrillary tangles (NFTs) (Nerkhe et al, 2021).

Neurofibrillary tangles have been shown to cause major cellular disruptions in the cytoskeleton, impacting motor protein's ability to transport important molecules and organelles to different parts of the cell. Not only does hyperphosphorylated tau fail to stabilize microtubules and lead to their dissolution, but NFTs can form blockages within the cell that prevent vesicular transport (Metaxas and Kempf, 2016). This prevents important neurotransmitters, metabolites, and proteins from reaching target destinations (particularly synapses). NFTs also incite substantial inflammation by triggering intracellular immune pathways. Increased inflammation exacerbates cellular localization issues and is frequently cytotoxic, leading to apoptosis (cell suicide) (Chen and Yu, 2023). NFTs can thus contribute directly to neurodegeneration.

Several studies have revealed that other forms of tau, in addition to aggregates, can induce cellular toxicity (Szabo, 2020; Nerkhe et al, 2021). One such form is known as "small oligomeric tau," which comprises water-soluble chains made up of many protein monomers. Like neurofibrillary tangles, tau oligomers can exhibit abnormal post-translational modifications (PTMs), including phosphorylation (Bueé et al, 2000). Hyperphosphorylated tau oligomers are thought to be a precursor to the formation of neurofibrillary tangles, but may in fact be far more damaging than their later-stage aggregate form due to their size and solubility (Figure. 3). This grants them the ability to cross cell membranes and enter important organelles, the mitochondria, and the nucleus. Tau oligomers have been shown to incite DNA and protein damage, further contributing to neurodegeneration (Bueé et al, 2000).

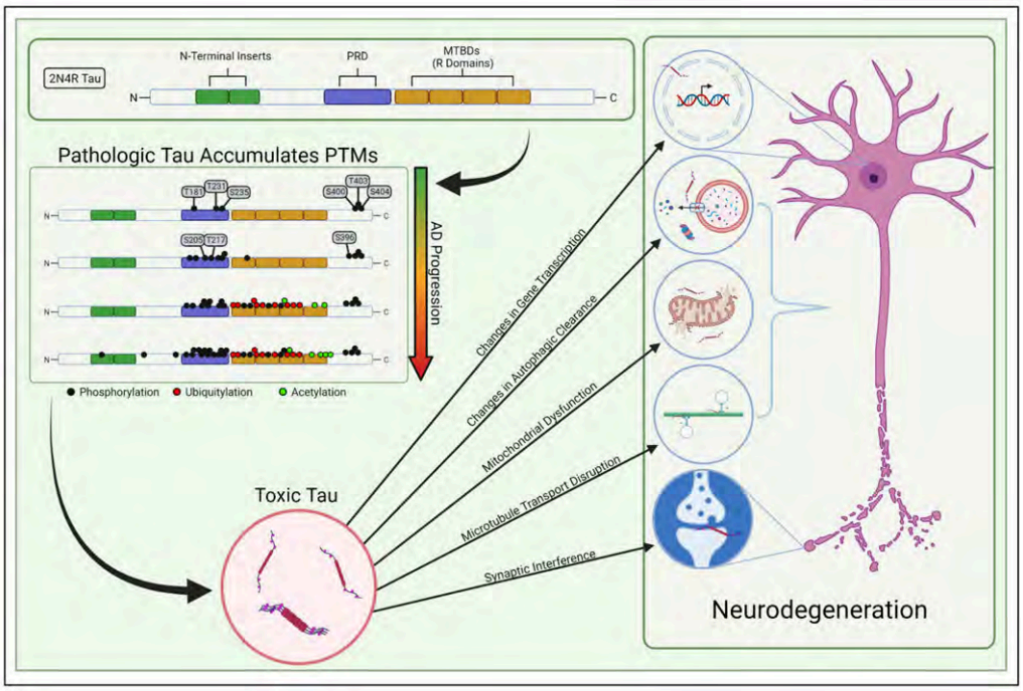


Figure 3. Tau accumulates post-translational modifications, which worsen as the disease progresses. Tau can be modified through phosphorylation, ubiquitination, and acetylation. Toxic tau leads to down-stream changes in gene transcription, autophagy, mitochondrial dysfunction, microtubule disruptions and synaptic interference. Adapted from Nerkhe et al (2021).

Glial Cells and Tauopathy

In Alzheimer's Disease and other tauopathies, hyperphosphorylated tau is predominantly found in neurons. However it has also been shown to be present in the "other" cells of the brain, known as glial cells. Glial tauopathy frequently occurs alongside neuronal disease and plays a role in tau toxicity and neurodegeneration. Depending on the type of tauopathy, glial cells can be impacted by tau on an even greater level than neurons (Kahlson and Colodner, 2016). The effects of glial tau pathology can be greatly varied due to the wide variety of functions that these cells typically perform, according to their different cell types.

Glial cells consist of several subtypes that each function to maintain a different aspect of neuronal health (Figure 4). Wrapping glia include oligodendrocytes and Schwann cells, and they function to form the myelin sheath surrounding neuronal processes. This sheath is composed of fatty membrane that acts as an axonal insulator, allowing for faster signal transduction and more coordinated brain activity (Bradl and Lassman, 2009). Microglia function as immune cells that clear toxins from the brain and destroy bacteria, viruses, and diseased tissue through

phagocytosis. Astrocytes are a highly versatile glial subtype that provides crucial homeostatic support: they regulate neurotransmitter and ion levels at synapses, control the flow of oxygen to nearby neurons, form the blood-brain barrier, and provide metabolites to neurons (Dellwo, 2023). Because of their myriad of important roles, glial disruption in neurodegenerative diseases has the potential to induce a wide range of toxic effects. Glia are generally understudied, but a mounting body of research has identified some of the possible ways that they are impacted in tauopathy and the effects that glial dysfunction has on disease progression.

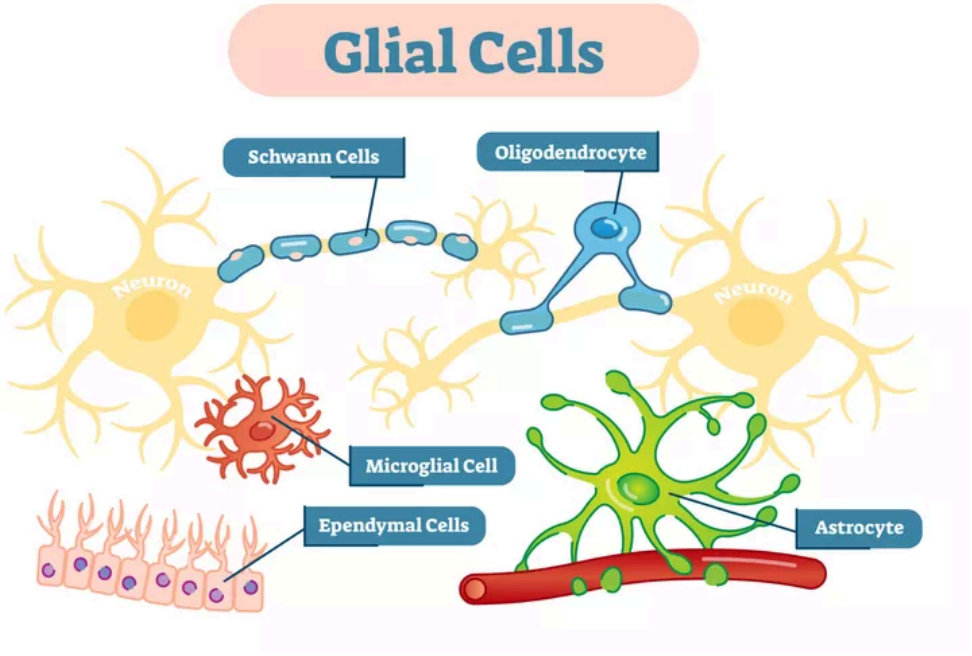


Figure 4. Diagram of different glial subtypes and the ways that they support neuronal functioning. Oligodendrocytes and schwann cells (blue) surround neurons (yellow) to form the myelin sheath. Astrocytes (green) connect with capillaries (red cylinder) and neurons. Microglia (red) move through brain tissue to destroy pathogens and other toxins. Ependymal cells (pink) wave flagella to circulate cerebrospinal fluid throughout the brain. Adapted From Dellwo (2023).

Glial cells including astrocytes, oligodendrocytes, and microglia have been found to exhibit tau pathology (Kahlson and Colodner, 2016). Glial tauopathy largely parallels neuronal disease progression, however it displays certain distinctions according to cell subtypes and depending on the tauopathy implicated. Astrocytes and microglia in healthy brain tissue appear to possess small amounts of tau protein, but it is not believed to be a major component of

cytoskeletal stabilization for these cells. However, in tauopathies, astrocytes and oligodendrocytes form tau inclusions, plaques, and tangles that greatly impact their morphology and functions (Ferrer et al, 2014; Kahlson and Colodner, 2016).

While tau pathology is frequently characterized by impacts to neurons, several types of tauopathy have been linked to glial cells. In Alzheimer's Disease, glial tau pathology is not considered to be a major hallmark of disease, however glial tauopathy is a common feature in other tauopathies like progressive subnuclear palsy (PSP), frontotemporal dementia (FTD), and corticobasal degeneration (CBD) (Kahlson and Colodner, 2016). Glial tau inclusions in both astrocytes and oligodendrocytes have been reported across diseases with several tauopathies defined by unique tau inclusions and morphologies. PSP is characterized by the formation of tufted astrocytes, which exhibit distinctive fibrous tau "tufts" on astrocytic processes, and corticobasal degeneration displays unique ring-shaped astrocytic tau plaques (Ferrer et al, 2014). Both of these diseases are further characterized by the formation of argyrophilic tau threads along the myelinating processes of oligodendrocytes (Kahlson and Colodner, 2016).

Glial tauopathies can further differ based upon the tau isoform that is most prevalent within the disease. Pick's disease displays predominantly 3R tau pathology (where tau has three repeat binding domains) while frontotemporal dementia, corticobasal degeneration, and progressive subnuclear palsy all express 4R tauopathy (Shahpasand-Kroner et al, 2022). Alzheimer's disease is characterized by a combination of these two isoforms. While not much is known about the pathogenic properties of these different isoforms, both have been shown to cause substantial deleterious effects in both neurons and glia.

Several pathological tau morphologies have been shown to alter glial behavior throughout the brain. Glia rely on microtubules to maintain their structure and transport important molecules and organelles, as well as make contact with neurons and synapses. Oligodendrocytes, in particular, rely on tau's stabilization of microtubules in order to extend their processes and form the myelin sheath around axonal tracts (Kahlson and Colodner, 2016). When tau is hyperphosphorylated, it disrupts this process and causes an overall reduction in myelination, reducing the speed and efficacy of synaptic transmission in the central nervous system.

Glial tau pathology can lead to further deleterious effects for both affected glia and overall brain health, including blood brain barrier (BBB) integrity. In transgenic tau expressing

mouse and *Drosophila* models, tau clusters in astrocytic end processes which make contact with capillaries to form the BBB. This leads to disruption of the BBB, making the brain susceptible to pathogens and toxins (Komori, 1999). Additionally, astrocytic end feet associate with neurons to maintain synaptic homeostasis, and localized tau clusters have been implicated in glutamate transporter dysfunction. Astrocytic tau is thus linked to deleterious effects for neurons including excitotoxicity, and has been shown to induce neuronal death (Kahlson and Colodner, 2016).

Additionally, the extracellular behavior of both astrocytes and microglia can be altered in the presence of tau through reactive gliosis. When infected neurons undergo apoptosis, the neurofibrillary tangles within them are released into extracellular space. Astrocytes and microglia then release cytokines to clear these NFT accumulations. (Metaxas and Kempf, 2016; Kahlson and Coldner, 2016). Unfortunately, the release of cytokines can damage nearby cells and contribute to neurodegeneration. This strong immune response results in maladaptive, clustered glia that surround neurofibrillary tangles, but fail to eliminate aggregates. While the mechanisms underlying reactive gliosis in tauopathies are not yet known, astrocytes and microglia may be affected by tau toxicity, leading to hindered immune abilities (Metaxas and Kempf 2016). Additionally, there may be too high a burden of neurofibrillary tangles for them to clear at later stages of disease progression.

Despite the clear presence of glial tau, and several pathological implications for its presence, it is not yet known how glial tauopathy develops. Tau may be transcribed directly by glia, however tau inclusions may also be explained by glial tau uptake. Astrocytes, microglia, and oligodendrocytes have been shown to transport tau from extracellular space, potentially to clear and break down toxic aggregates (Amro et al, 2021). Unfortunately, this transport process may be highly implicated in the spread of tauopathy: both astrocytes and microglia have been shown to release pathological tau at later stages of disease progression. This hyperphosphorylated tau exhibits prion-like properties, meaning it can infect healthy protein by inducing it to misfold and aggregate. This leads local healthy neurons and glia to become diseased (Holmes and Diamond, 2014; Amro et al, 2021).

Chapter 2- Neuronal Energy Use and Mitochondrial Functions

Mitochondria and Cellular Respiration

Mitochondria are known colloquially as the "powerhouse of the cell" due to their significance in the production of cellular energy from carbohydrates, fats, and protein. They do this through cellular respiration, a process that produces units of stored energy called adenosine triphosphate (ATP) from the breakdown of hydrocarbon molecules. This energy can later be accessed by breaking one of ATP's phosphate bonds, releasing free energy which can be used to power all cellular processes (Chu et al, 2022). ATP is utilized to promote chemical reactions throughout the cell and phosphorylate necessary proteins including enzymes, channels, and pumps.

Cellular respiration involves three stages and is characterized by oxidation/reduction reactions. These stages are glycolysis, the citric acid cycle, and the electron transport chain (Figure 5). In glycolysis, each molecule of glucose is broken down into two pyruvate molecules. Pyruvate is then imported into the mitochondria where it is further oxidized in the citric acid cycle, which comprises a series of chemical reactions that creates NADH and FADH2 molecules. During the final stage of the electron transport chain, electrons are donated by NADH and FADH2 to actively pump protons across the mitochondrial membrane (Szabo, 2020). This causes a mitochondrial proton gradient to be established, where the outer membrane contains many charged protons, while the outer portion of the membrane is less energized. This electrical potential and concentration gradient across the membrane is used to catalyze the reaction that converts ADP (adenosine diphosphate) to ATP (adenosine triphosphate) through the addition of a phosphate group (Szabo, 2020).

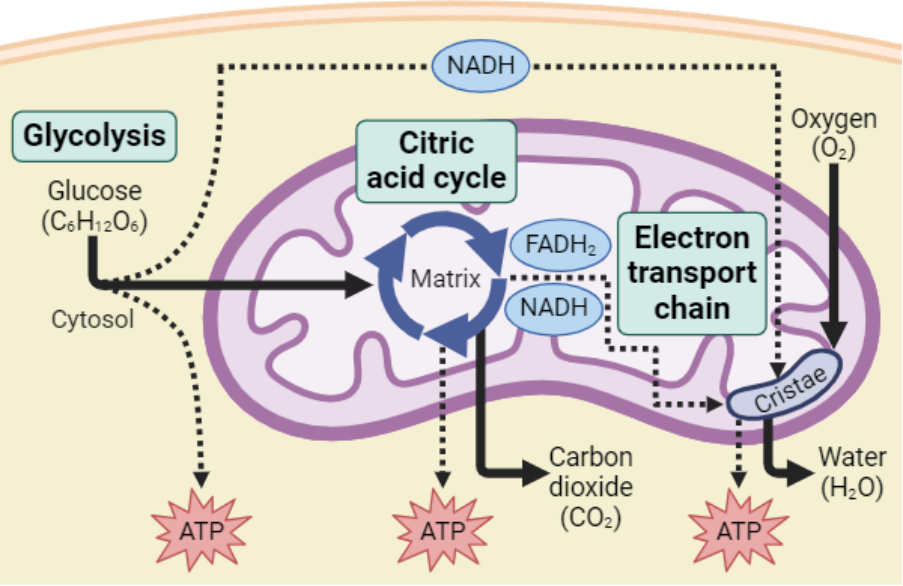


Figure 5. Glycolysis, the citric acid cycle, and the electron transport chain work together to release chemical energy from glucose and store it in ATP. Glycolysis uses glucose to produce ATP, pyruvate and NADH. Then, the citric acid cycle uses pyruvate to produce carbon dioxide, ATP, NADH, and FADH2. Finally, the oxygen dependent electron transport chain uses NADH and FADH2 to make ATP. Adapted from Khan Academy (2024).

The process of cellular respiration is critical for neural functioning due to the huge energy demand of synaptic transmission. While the brain accounts for only 5% of the body's biomass, it uses over 20% of available oxygen to produce ATP and fuel activity (Mink et al, 2021). Roughly 65% of this energy alone is used to fuel the sodium potassium pump, which uses ATP in order to pump potassium ions into the cell and sodium out of the cell, creating the resting membrane potential (Harris et al, 2012). The resting membrane potential is crucial for a neuron's ability to send signals: it creates a powerful electrical driving force which leads positively charged ions to enter the neuron and flow down the length of the axon during action potentials.

Cellular respiration is also particularly important for the neuron's ability to release synaptic vesicles following an action potential. In this process, calcium binds to proteins in the SNARE complex, which triggers them to release vesicles containing neurotransmitters. These vesicles can then fuse with the cell membrane and release their contents into the synapse (Ramakrishnan et al, 2013) The cell's calcium pumps and channels are regulated by ATP, allowing calcium ions to enter the neuron at precise times to activate neurotransmission (Harris et al, 2012). These neurotransmitters are essential for the nervous system to function and induce

a variety of key processes throughout the brain and body, including triggering heartbeat and respiration, digestion, brain activity, sleep/wakefulness, sensory information, emotion, and learning.

Importantly, neuronal use of ATP can affect synaptic plasticity and memory. The metabolic activity of neurons leads directly to the formation and strengthening of synaptic connections through long-term potentiation (LTP), allowing new memories to be encoded (Alexandrov and Pletnikov, 2022). ATP can induce LTP by acting as a neurotransmitter or through triggering synaptic activity by phosphorylating (activating) essential receptors. This process speeds up cognition, pattern recognition, communication, and sensory systems by reinforcing neuronal pathways. Thus, ATP can have protective effects against memory loss and cognitive decline, and its healthy functioning can in turn prevent neurodegeneration.

Overall, due to its considerable importance in virtually every aspect of life and cellular homeostasis, the production of ATP through cellular respiration is critical. ATP is utilized by neurons at a disproportionately high rate due to the energy demands of action potentials and synaptic transmission. Moreover, it serves to closely regulate protein and DNA activity. Any disruptions to the production of ATP contribute to deleterious effects and, depending on their severity, result in cellular death.

Non-Respiratory Mitochondrial Functions

While mitochondria are most known for their role in cellular respiration, they have been shown to perform a wide array of functions, including regulating metal and ion levels, contributing the long-term potentiation, and potentially sequestering and degrading misfolded proteins (Rizzuto et al, 2012; Raun et al, 2017; Spinelli and Haigas, 2018). While many functions of mitochondria remain a mystery, it is clear that these highly versatile organelles are critical for cellular survival.

Mitochondria aid in cellular homeostasis by regulating ion concentrations through the absorption of calcium and other metals (Rizzuto et al, 2012). When calcium levels become too high during synaptic transmission, excess calcium is sequestered into mitochondria through the mitochondrial uniporter complex (MCUC). While neurotransmitter activity is necessary, when too much calcium is present, excessive numbers of vesicles will be released. This leads to excitotoxicity and neuronal damage. Mitochondria’s ability to sequester calcium reduces these

toxic effects and regulates the intensity and duration of neurotransmission. This regulation, like ATP, is implicated in neuronal long-term potentiation and long-term depression (the weakening of synapses). The slow release of calcium at proper times leads to crucial changes in synaptic strength. Thus, mitochondria are incredibly important to ensure healthy brain development and synaptic change (Zhang et al, 2022).

While it is currently unconfirmed in humans, mitochondria from yeast species have been shown to degrade small, soluble proteins found in the cytosol and maintain protein homeostasis. When protein import through the mitochondrial uniporter complex was blocked in yeast, it substantially reduced the speed and efficacy of protein degradation (Raun et al, 2017). Some evidence suggests that humans may undergo this process as well: there is a high incidence of small, misfolded protein oligomers (including tau protein) found in mitochondria, suggesting they may be stored and possibly broken down within the mitochondrial matrix. If this is a common occurrence as this research suggests, then mitochondria are particularly important for helping prevent neurodegenerative disease. In tauopathies, mitochondria may absorb tau to engage in this process, however this is unconfirmed (Nerkhe et al, 2021).

Glial Mitochondria: Neuronal Metabolic Support, Functions, and Differences

Glial mitochondria appear to function similarly to neuronal mitochondria and their ATP usage is largely conserved across cell types. In glia, ATP regulates DNA transcription and translation, protein activity, and fuels chemical reactions. The majority of ATP, however, is used to maintain the sodium potassium pump and to regulate membrane bound ion channels (Kim et al, 2013). Notably, astrocytes and oligodendrocytes rely on the negative membrane potential created by this pump in order to regulate gliotransmission, or the process of releasing neurotransmitter type molecules onto synapses. Interestingly, ATP can be released directly as a gliotransmitter that regulates neuronal excitatory and inhibitory receptors. Generally, this ATP signaling results in reduced synaptic excitability, preventing excitotoxicity in the brain (Lezmy, 2023).

Perhaps in order to maintain gliotransmission and synaptic homeostasis, astrocytes have been shown to possess specialized small mitochondria in their fine end processes (Harris et al, 2012; Oberheim et al, 2009). It is estimated that a single astrocyte possesses enough of these fine processes to form up to 2 million connections with synapses. These processes are about 1μm in

diameter-- substantially smaller than a typical mitochondria found in other cell types. Their specialized small mitochondria tend to cluster around sites of vesicle release, glutamate transporters, and receptor activity, and may play a role in synaptic regulation (Robinson and Jackson 2016).

While the exact purpose of small astrocytic mitochondria is unknown, their location could indicate a few possibilities. Firstly, it could suggest that the synaptic gliotransmission and glutamate transport performed by astrocytes is metabolically demanding, and requires a lot of ATP localized to those areas. This would make sense based on the previously established body of research demonstrating that neuronal mitochondria localize to the most metabolically active sites, such as nodes of Ranvier and at the synaptic cleft (Misgeld et al, 2007; Ohno et al, 2011).

Additionally, astrocytic mitochondria located in the end processes may aid in extracellular ion homeostasis. Astrocytes have been shown to be able to absorb excess calcium within cells through calcium transients, however they may also do this through mitochondria, which absorb calcium at neuronal synapses. Astrocytic calcium sequestration reduces excitotoxicity and regulates the synaptic activity of neurons (Zhang et al, 2022). This could be particularly important in areas with high amounts of vesicle release and activity, such as where an astrocytic end process joins with a synapse. In either case, this research suggests that the concentration of mitochondria in end processes relates to the ways in which they support neuronal gas exchange and metabolic functioning.

While astrocytes possess both specialized small mitochondria and larger, more productive ones, they appear not to utilize them as a primary energy source. Both astrocytes and microglia have been shown to predominantly perform glycolysis and fermentation to generate ATP, two processes that occur within the cytoplasm rather than mitochondria themselves (Rose et al, 2020). This method of generating ATP is faster but substantially less productive than oxidative phosphorylation (oxphos). Each round of glycolysis generates 2 ATP per glucose molecule, as compared to 28 ATP molecules generated through oxphos (Bonora et al, 2012). However, this process generates two pyruvate molecules that can be used for future cellular respiration. The majority of pyruvate generated through glycolysis is shipped from astrocytes to the extracellular space, where it is taken up by neurons for their own cellular respiration (Rose et al, 2020).

Astrocytes can supply important metabolites to neurons through a number of different mechanisms, including through monocarboxylate transporters and gap junctions (Turner and

Adamson, 2011). They have been shown to support neurons through donation of ATP, pyruvate, and lactate molecules; maintain extracellular oxygen concentrations at synapses; and regulate glucose and gas exchange through the blood brain barrier (Wei and Morrison, 2023; Cezeriat et al, 2024). Both lactate and pyruvate molecules have been shown to have neuroprotective effects and increase synaptic plasticity in memory systems (Descalzi et al, 2019).

Due to the incredibly high energy demand of synaptic transmission, oligodendrocytes (in addition to astrocytes), have been shown to metabolically support neurons. Oligodendrocytes provide ATP building blocks to neurons along their axons through the myelin sheath (Phillips and Rothstein, 2017). Similarly to astrocytes, they do this through the use of monocarboxylate transporters, which are used to shuttle lactate and pyruvate to neurons. Lactate and pyruvate are then broken down further and used in the production of ATP. This is highly important for the speed of synaptic transmission, because it allows for the maintenance of action potentials through ATP reliant ion channels and pumps along the length of the axon (Arancibia-Carcamo and Attwell, 2014). These highly metabolically active areas involved in neuronal signaling are thus supported by oligodendrocyte metabolism.

Though both oligodendrocytes and astrocytes are directly implicated in the metabolic support of neurons and support cellular respiration, far less is known about the metabolic functions of other glial subtypes. Despite this, mitochondria and ATP have been shown to play a role in microglial neuroinflammatory signaling. Dou et al (2012) found that the release of ATP from damaged neurons results in the rapid extension of microglial processes towards the site of injury. Additionally, once microglia have received this signal, they release endogenous ATP in order to maintain their immune response through the release of cytokines. Other studies have shown that microglial mitochondria may be directly involved in the maintenance of inflammatory signals through the activation of mitochondrial complex I. This activity is linked to increases in the production of reactive oxygen species (ROS), often leading to diseased cells committing apoptosis (Jametti et al, 2024). Largely, the microglial immune response generated by ATP is an adaptive cellular process, however in autoimmune diseases and tauopathy, this process may lead to further harm.

Chapter 3-Tau's Effects on Mitochondrial Dynamics and Cellular Functioning

Mitochondria are essential for life and central nervous system health, but these organelles are highly susceptible to pathological tau. In a tauopathy model, Szabo (2020) demonstrated that small oligomeric forms of tau with abnormal PTMs (largely phosphorylation) appear to be disrupting mitochondrial functions in neurons. These tau oligomers are hyperphosphorylated and water-soluble, meaning they are able to permeate the mitochondrial membrane and disrupt the concentration gradient of hydrogen that is normally formed during cellular respiration. This results in significantly less ATP production, causing neurons to undergo metabolic stress (Perez et al, 2018). In addition to this decrease in ATP, tau oligomers have been shown to increase oxidative stress through ROS production, impact fission/fusion dynamics, and cause cellular localization issues (Szabo, 2020).

During the process of ATP production, toxic byproducts known as "reactive oxygen species" (ROS) are created. The cell contains several homeostatic mechanisms to prevent ROS toxicity and convert these molecules to safe forms, however when left unchecked ROS can damage DNA, proteins, and cellular membranes (Szabo, 2020). In tauopathies, mitochondria begin producing excess ROS during respiration. The cells' normal enzymes that convert ROS into less harmful oxygen species cannot keep up, and this leads to DNA and protein damage (Szabo, 2020). Damaged DNA leads cells to initiate apoptosis, or programmed cell death. While apoptosis is a protective immune mechanism to prevent disease, and harmful mutations, humans possess a finite number of neurons and this process leads directly to neurodegeneration.

In addition to the disruption of mitochondrial membranes and ATP production, tauopathies affect the fission and fusion dynamics of mitochondria. Mitochondrial fission is the process by which a mitochondrion splits into two daughter mitochondria. This process involves the recruitment of tubules in the endoplasmic reticulum (ER) and the dynamin protein (Adebayo et al, 2021). These tubules contact the middle portion of the mitochondrion and constrict, pinching and separating it into two smaller cells. In tauopathies, this process is disrupted due to damage to proteins that contribute to fission, such as dynamin associated protein 1 (DRP-1) (Figure 6.) (Perez et al, 2018). This results in abnormally elongated mitochondria occurring at lower numbers than the cell would otherwise possess (Szabo, 2020). This loss of mitochondria contributes further to oxidative stress, effectively "starving out" neurons. Additionally,

mitochondrial elongation results in decreased efficiency and increases the amount of ROS that is produced, leading to neuronal apoptosis and neurodegeneration.

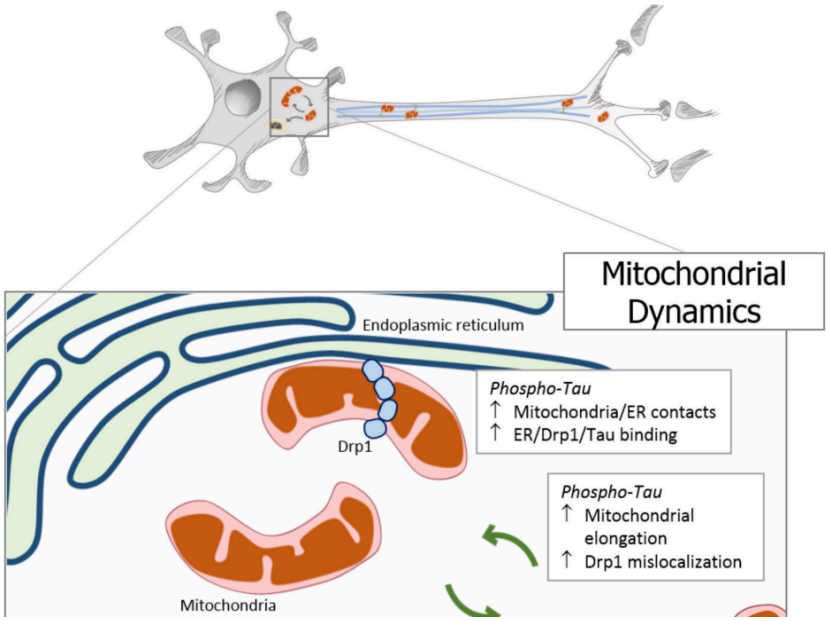


Figure 6. Pathological forms of tau affect mitochondrial fission dynamics. In neurodegenerative diseases, the accumulation of pathological forms of tau (hyperphosphorylated and cleaved) impairs the regulation of mitochondrial dynamics. The overexpression of phosphorylated tau generates an increase in mitochondrial length, a decrease in fission proteins, and an increase in DRP1 mislocalization. Adapted from Perez et al, 2018.

Finally, in tauopathies mitochondria are susceptible to cellular localization issues. Like most proteins and organelles of a cell, mitochondria are transported to different locations through motor proteins. These proteins connect with microtubules and “haul” cellular cargo. The majority of mitochondria are carried by these proteins to areas of high metabolic activity including axon terminals and dendritic spines (Askill and Kitler, 2009). When tau becomes hyperphosphorylated, the cytoskeletal system that facilitates this transport breaks down, causing mitochondria to fail to reach important destinations (Nerkhe et al, 2021). This creates an energy deficit at synapses and results in reduced signaling efficiency, disrupting the activity of other neurons in the region.

Glial Mitochondrial Impacts of Tauopathy

Though differences in mitochondria morphology are relatively well established in neurodegenerative disease models in neurons, few studies have researched the impacts of tau on glial mitochondria. This includes identifying downstream signaling effects, observing morphological changes, and determining cellular impacts. Presently, no studies thoroughly examine the mitochondrial impacts of tauopathy in several types of glia including oligodendrocytes and microglia. Despite this, a few studies have examined astrocytic mitochondria due to their importance in metabolism and have discovered interesting similarities and differences when compared with neuronal tauopathy (Perbet et al, 2023; Cezeriat et al, 2024).

Like neuronal mitochondria, astrocytic mitochondria tend to be impacted by the effects of tau through the disruption of fission/fusion, localization issues, and disruption of ATP synthesis. However, Perbet et al (2023) showed that while astrocytes take up both isoforms of tau, their mitochondria were more impacted by the 3R form of tau rather than the 4R form of tau (which is generally more deleterious to neurons). Researchers were unsure of why the differences between neuronal and astrocytic mitochondria occurred. However, they hypothesized that because 3R tau tends to be smaller, it may be more likely to form small oligomers and enter glial mitochondria. The size and binding affinity 3R tau may also influence how it interacts with the specialized small mitochondria in astrocyte end processes, making them more susceptible to its effects.

While tauopathy remains understudied in other glial subtypes, glial mitochondrial dysfunction has been studied in several cell types including oligodendrocytes, microglia, and astrocytes. Interestingly, though glial mitochondrial dysfunction induces deleterious effects, (with the exception of oligodendrocytes) this has not been linked directly to their cellular degeneration (Rose et al, 2020). This is largely because astrocytes and microglia predominantly perform glycolysis to produce ATP, rather than relying on mitochondria for oxidative phosphorylation and ATP production. Despite this, glial mitochondria are highly implicated in regulatory pathways, gliotransmission, and preventing neuronal disease progression (Rose et al, 2020). Given the effects of glial mitochondrial dysfunction and toxicity, it stands to reason that tau may induce similar impacts on glial health.

In addition to the ways that astrocytic mitochondria are affected by mitochondrial dysfunction and tauopathy, the spread of metabolites from astrocytes to neurons may be involved

in neurodegenerative disease progression. Despite their importance for neuroprotection and providing metabolites to neurons, in tauopathy models astrocytic pyruvate transporters can transfer toxic tau to neurons and infect healthy protein. In healthy astrocytes, these transporters exist on the mitochondrial membrane and pump pyruvate out so that it can be donated to nearby neurons (Cezeriat et al, 2024). However, in Alzheimer's Disease models, this pyruvate transport leads to tau and amyloid beta accumulation. While the mechanism for this process is still unknown, this may indicate that astrocytic mitochondria act as a "hub" for hyperphosphorylated tau to spread throughout the brain via pyruvate transporters (Cezeriat et al, 2024).

Chapter 4- Fruit Fly Models of Neurodegenerative Disease

While some may think it counterintuitive to study human diseases like Alzheimer's in a fruit fly, there are many reasons why this small, simplistic insect can provide valuable insights into neurodegenerative diseases. Despite possessing smaller nervous systems than humans, or even other mammals, on a cellular level fruit flies share many common features with us. They possess the same basic building blocks including neurons and glia, have mitochondria that function similarly to ours, and utilize several of the same neurotransmitters (Leyssan and Hassan, 2007). Additionally, *Drosophila* models are highly genetically manipulatable, have short reproductive periods and lifespans, and provide a large number of progeny (Jeibmann and Paulus, 2009). Researchers have been using this model organism for decades and a great deal of insights into their biology, behavior, and genetics have been revealed. This has led to the development of a variety of genetic tools and several studies to cross-reference during the research process.

Drosophila Glial Subtypes: A Comparison to Human Glial Cells

Though *Drosophila* possess different glial subtypes than humans, many of their functions overlap, making them analogous to many of our own glia. This includes wrapping glia, ensheathing glia, and cortical glia-- all of which appear to wrap around the cell bodies, axons, and dendritic processes of neurons (Figure 7). This likely functions similarly to the myelination process that oligodendrocytes and Schwann cells engage in (Freeman, 2015). Ensheathing glia, in particular, seem to create fiber tracts surrounding neuronal processes during development. These glia demarcate different brain regions through the formation of fibrous membranes in the neuropil (extracellular space) in the fruit fly brain. They also may increase speed of signal transduction and participate in the gas exchange process due to their proximity to the fruit fly tracheal network (Freeman, 2015).

Instead of using blood for gas exchange, flies possess a series of tubes known as trachea, where oxygen flows and diffuses throughout the central nervous system. These trachea are surrounded and highly innervated by both cortical glia and by "astrocyte like glia," (ALG) which appear to form a "tracheal brain barrier" and widely extend throughout the brain. This barrier functions similarly to a human blood brain barrier, which is also made up of tightly packed

astrocytes (Freeman, 2015). Instead of cerebro-spinal fluid, fruit flies use a compound called hemolymph, which also appears to be regulated by ALG. Similarly to human astrocytes, ALG cells in flies can regulate extracellular ion concentrations and closely associate with synapses (Freeman, 2015).

In addition to the cortical and astrocyte-like glial cells that fruit flies possess, they have perineurial and subperineurial glia. These cell types are relatively understudied compared to ALG, but they appear to surround the neuropil, and may form a protective barrier around the brain, as well as form a part of the neuro-muscular junction. Subperineurial glia lie below perineurial glia, which form the carbohydrate rich lamina on the outer surface of the *Drosophila* brain (Freeman, 2015). These cells establish a barrier that prevents the entry of chemicals or pathogens that circulate throughout the fly body. This barrier also functions in a similar capacity to a human's blood brain barrier, except these cells are not currently known to participate in the gas exchange process.

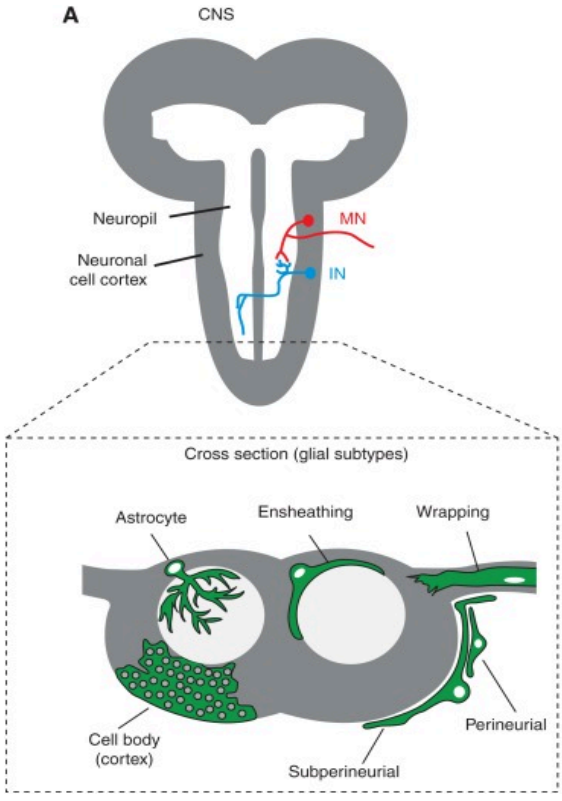


Figure 7. Subtypes, positions, and morphology of *Drosophila* glia. (Top) Overview of the *Drosophila* larval central nervous system (CNS). The neuronal cell cortex (gray) houses all neuronal and most glial cell bodies. CNS synaptic contacts between neurons are found within the

neuropil (light gray). (Bottom) cross-sectional view of glial subtypes (green). Morphological arrangement in the adult brain is similar. Adapted from Freeman (2015).

Clearly, *Drosophila* possess many cellular similarities and overlapping glial subtypes, however their brain development and central nervous system also appear to follow similar aging patterns to human ones. Davie et al (2018) found that over a fly's lifespan, neurons begin to proliferate less, matching human patterns of senescence. They further identified cell type specific differences between neurons and glia, with an increase in glial proliferation over the fly's lifespan. This is reminiscent of human aging processes, in which astrocytes and oligodendrocytes increase within hippocampal brain regions and microglia begin to proliferate throughout the brain at advanced ages (Soreq et al, 2017). Thus, while fruit fly brains clearly exhibit many differences, they follow similar cellular aging trajectories to human brains and can serve as an effective model organism.

Drosophila **Use in Tauopathy Research**

Fruit flies are highly genetically manipulatable and (in addition to sharing several cell types) possess several genetic similarities with humans. It is estimated that fruit flies possess 75% of the same genes that cause diseases in humans, including an endogenous tau protein known as "*Drosophila* tau" or "dtau" (Varte et al, 2023; Gistelinck et al, 2012). While dtau contains several differences and is less neurotoxic than human tau, when this protein is overexpressed it results in similar neurodegenerative effects to the expression of human tau (Gistelinck et al, 2012). *Drosophila* models of neurodegenerative are highly utilized, and effects can be measured through both behavioral and biochemical assays. This includes through locomotion tests, life-span analyses, assessment of learning and memory, observing vacuolization of the brain, and neuronal morphology and cell counting (Varte et al, 2023).

While flies naturally possess endogenous tau, it is possible to express human tau in any of its six isoforms using the GAL4/UAS binary system or by otherwise incorporating it into the *Drosophila* genome. This has allowed the detailed study of the effect of tau on different cell types and at different stages in fly development (Gistelinck et al, 2012). Both 0N4R and 0N3R tau models have been created in *Drosophila* to elucidate their differences in neurons and glia, further modeling glial tauopathies (Shahpasand-Kroner et al, 2022). Several stable human tau expressing lines can easily be ordered directly from stock companies, such as the Bloomington

Stock center. Several of these stocks also allow the visualization of different cell types, molecules, and structures through the use of genetically encoded fluorescent proteins. Due to their affordability and convenience, it has been possible to make several discoveries into the pathology of tau, including its effects on neuronal and glial mitochondria, using fruit fly models (Chee et al, 2005; Varte et al, 2023).

Because of their widespread use, researchers have utilized *Drosophila* models in the study of neurodegenerative disease for nearly 30 years, and many findings have contributed to elucidating mechanisms of progression and avenues for treatment. In fruit fly neurons, the expression of toxic human tau isoforms results in the abnormal elongation of mitochondria. This results in mitochondrial dysfunction and is associated with an increase in apoptosis (Varte et al, 2023). Duboff et al (2012) found that in neuronal tauopathy models of fruit flies, mitochondrial fission decreases due to the failure of (DRP1) to properly bind to mitochondria. Additionally, fruit flies (like humans) show reduced axonal transport capabilities in the presence of tau, resulting in issues with the localization of mitochondria (Varte et al, 2023).

All of these findings are consistent with studies on human tauopathies and mitochondrial impacts, indicating that fruit flies can be an incredibly useful tool to identify cellular and protein based aspects of disease which can then be tested in human tissue. This allows researchers to screen for the most useful directions of study in order to save valuable resources like human Alzheimer's brain tissue, as well as study behavioral aspects of the disease in a more ethical manner than through the use of human subjects. Due to the consistency of stocks and the ease of caring for fruit flies, *Drosophila* experiments are also highly repeatable. Overall, they are a valuable analogue for human tauopathies and can be incredibly useful for the advancement of medicine, including glial tauopathy research.

Chapter 5- Study Aims

Because of their roles in neuronal metabolism and implications in neurodegenerative disease progression, it is important to study glial mitochondrial dynamics and how they may be uniquely disrupted in the presence of tau. Prior research has demonstrated that astrocytic and oligodendritic mitochondria are highly involved in neuronal metabolic support through the donation of ATP, glucose, and metabolic building blocks (Turner and Adamson, 2011; Wei and Morrison, 2023). In addition to their direct metabolic roles, glial cells are highly varied and perform many essential roles within the brain, so the impacts of tauopathy on these cells can lead to significant changes in brain health. Glial cells are also thought to participate in the spread of neurodegenerative disease and may be uniquely impacted by tauopathy due to cell type differences (Cezeriat et al, 2024). Research into tau's impacts on glial mitochondria is therefore highly salient for the overall understanding of neurodegenerative disease.

While there are several studies linking glial cells to neurodegenerative diseases and others that outline their roles in neuronal support and metabolism, very few studies focus on the intersections between these phenomena. This thesis aimed to fill in some of the unknowns by quantifying glial mitochondria and observing morphological differences in the presence of glial tau expression, using fruit flies as a model organism. Our research model utilized dissected *Drosophila* brains to determine the quantity, size, and shape of mitochondria in the presence of human tau. This thesis will examine the total number, average volume, surface area, and sphericity of all mitochondria in each brain, comparing between the male and female and tau versus control conditions. These measurements will hopefully determine whether tau leads to any morphological changes, such as mitochondrial elongation. We hypothesized that panglial tau expression will lead to a decrease in mitochondrial counts due to imbalances in fission/fusion dynamics and mitochondrial tau toxicity. Given that tau disrupts mitochondrial fission and results in neuronal mitochondrial elongation, we further hypothesized that glial mitochondria will exhibit reduced sphericity and greater volume, indicating morphological changes in the presence of tau.

While all glia are susceptible to the detrimental effects of tau, astrocyte-like-glia are of particular interest due to the astrocyte's roles in respiration, cellular metabolism, and gas exchange. Astrocytic mitochondria remain highly understudied, despite their importance for

neuronal functioning. Thus, in addition to examining the panglial effects of tau, this research will delve further into the age and sex differences associated with astrocytic mitochondria, with the hope of establishing clear control conditions for future study. It will determine whether mitochondrial counts and the morphologies of volume, surface area, and sphericity are affected by sex or differences between ages 3, 10, and 30 days old. We hypothesized that female flies will possess more mitochondria due to large brain sizes compared with males, and that flies at days 10 and 30 will express a greater number of mitochondria than day 3 flies due to glial proliferation over the lifespan.

Through studying glial mitochondrial morphology, we hope to provide a basis for further tauopathy research in order to better understand therapeutic targets for neurodegenerative disease. If glial mitochondria are impacted differently than neuronal ones, unique treatments must be developed in order to best treat specific cell types. Moreover, the hypothesized elongation and reduction of glial mitochondria could contribute directly to the progression of tauopathy and neuronal cell death, and it is crucial to identify how and why this process occurs through further research.

Materials and Methods

The GAL4/UAS and tubulin-GAL80^{TS} Systems

Fruit flies are highly genetically manipulatable, and over the last several decades researchers have developed several tools to control their gene expression. In 1985, Giniger, Varnum, and Ptashne discovered a transcription system in yeast that would be used to revolutionise fruit fly genetics, known as the GAL4/UAS binary system. GAL4 was discovered to be a transcription factor commonly found in baker's yeast which naturally binds to "upstream activation sequences" (UAS) to drive the yeast's DNA transcription (Giniger, Varnum, and Ptashne, 1985). Brand and Perrimon (1993) utilized this system to create genetically engineered flies by injecting them with DNA plasmids containing genes that encode for the GAL4 protein or UAS promoter. Fischer et al (1988) and Brand and Perrimon (1993) demonstrated that when this GAL4 protein was present in fruit flies carrying the yeast UAS sequences, it drove target gene transcription. This has allowed researchers to induce tissue specific mutations that carry across generations. These can occur at different stages in the flies' lifespan or in response to different temperatures and environmental factors, which researchers can tightly control.

UAS sequences can be attached to any gene of interest, including those that would not normally occur in fruit flies (like human tau protein). GAL4 can in turn be attached to a different promoter sequence (such as a cell type of interest). The *repo* promoter, for example, is unique to all glial cells (Brenner et al, 1994). When GAL4 and UAS are both present in the same fly, the GAL4 protein can "turn on" the UAS promoter, which triggers the gene of interest to be transcribed. This means that with a glial cell specific GAL4 *and* a UAS-tau sequence, fruit flies will only make tau in their glial cells. However, parent flies that have only GAL4 or only UAS genes will not express tau, meaning that they can survive its toxic effects and healthy stocks can be maintained.

Once the GAL4/UAS system had been developed, researchers discovered that they could make use of naturally occurring activators and repressors of the GAL4 gene, including the GAL80 protein. This protein naturally binds to GAL4's transcription site and inhibits its activity, preventing any protein from being created. McGuire et al (2003) genetically altered the GAL80 protein to create a version that is temperature sensitive. This GAL80^{TS} construct becomes

inactive at high temperatures ranging from roughly 25-30℃. This has allowed researchers to control when a target gene will be expressed in a fly's development: when temperatures are below 25℃, GAL4's expression is blocked and a fly will not express any transgenes. When flies are heat shocked at temperatures ranging from 25-30℃, however, GAL4 activity resumes and flies begin to produce a protein of interest. This grants the ability to wait until a fly reaches adulthood to express proteins that can be potentially lethal or would interfere with normal development (such as tau).

This experiment utilized two main stocks for panglial tau mitochondrial analyses: one containing tau, and one control stock. The experimental stock (*repoGAL4, GAL80^{TS}, UAS tau*) contains a *repo-GAL4* driver that is expressed in all fly glia, as well as a *UAS-tau* sequence with a heat shock sensitive *tubulin-GAL80^{TS}* construct. Progeny were raised in 25℃ to drive moderate, but nonlethal, amounts of glial tau expression. The control stock contains the *repo-GAL4* driver and *tubulin-GAL80^{TS}*, but lacks tau. The tau variant that the experimental flies produce is human wild type 0N4R tau, which has zero n-terminal inserts and 4 binding domains (Colodner and Feany, 2010). These stocks are both crossed to a *UAS-mitoGFP* stock, which has an upstream promoter that causes the mitochondria to fluoresce, resulting in progeny that produce green mitochondria in their glial cells (Figure 8)(Chen et al, 2020).

Experiments measuring astrocytic mitochondria at ages of interest and between sexes utilized the *RG8E01(astro) GAL4, UAS-hisRFP* stock (Kang '22). This astrocyte specific driver combined to *UAS-histone RFP* labels the histone wrappings around DNA, allowing for the visualization of astrocyte nuclei (Beliharz et al, 2015). When crossed to *UAS-mitoGFP* flies, this stock indicates both total astrocytic mitochondria, and their localisation near nuclei, respectively (Figure 8). The resulting progeny from these crosses were hatched and aged in standard 25℃ conditions.

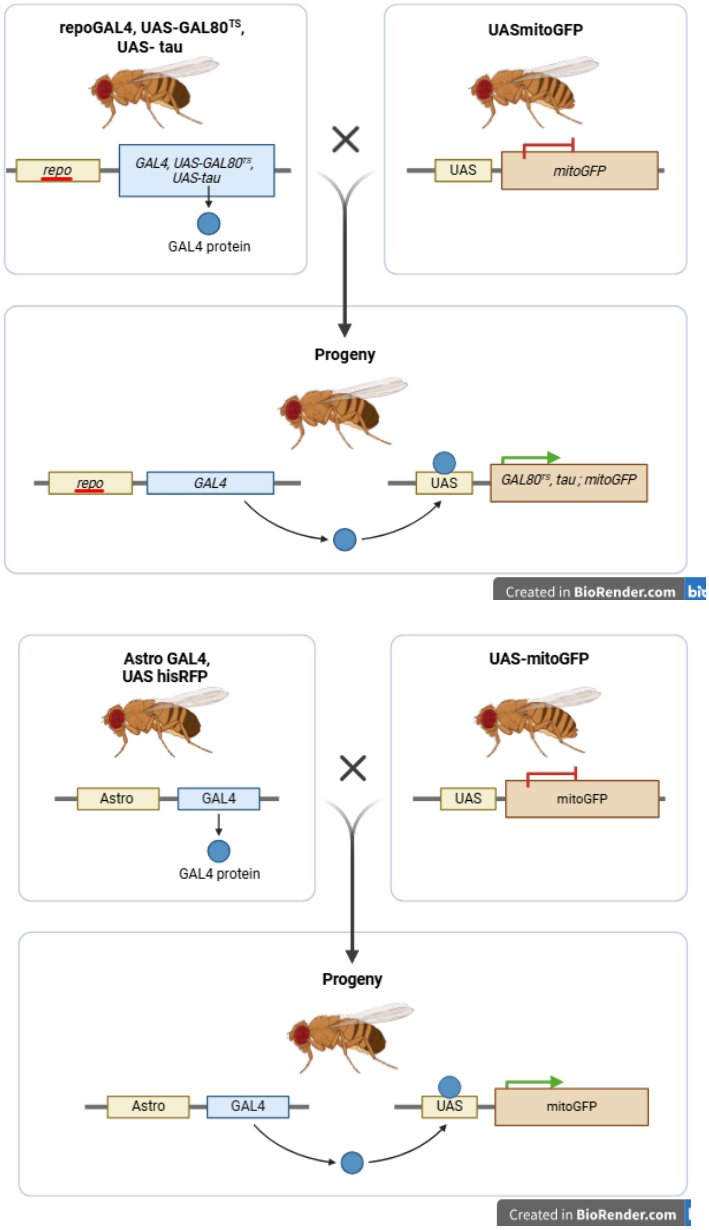


Figure 8. Genetic crossing scheme using the GAL4/UAS system. The GAL4 transcription factor binds to the UAS promoter, which triggers the transcription of the genes of interest (mitochondrial GFP) in cell types of interest in progeny. Top: panglial experimental crosses. Bottom: astrocyte-specific crosses.

Brain Dissections, Processing, and Imaging

After the creation of these fly crosses, progeny were collected and aged at 25℃. The panglial flies were aged to 10 days, while astrocyte specific experimental flies were aged to days 3, 10, and 30. At these different time points, male and female brains were dissected in phosphate

buffered saline (PBS), then fixed in 10uL of 4% paraformaldehyde (PFA) in a well of a terasaki plate. This fixation stops all biological processes and hardens and preserves each brain. The brains were then washed twice in PBS for 20 minutes each, and placed in a solution with 0.5% Triton-X in PBS (known as PBT) to make the cell membranes more permeable to dyes for an additional 20 minutes. After this, the brains were processed for 10 minutes each in 40%, 60%, and 80% glycerol solutions. Glycerol caused the brains to become more translucent and reflective, allowing for better microscopy visualization. The brains were then mounted in VectaShield antifade mounting media with DAPI (a blue fluorescent nuclear stain) on slides using a coverslip bridge to prevent damage. All slides were sealed and refrigerated at 4℃ in a standard slide box to prevent photobleaching.

Slides were imaged using a NIKON TI2 Eclipse C2 confocal microscope, using laser-scanning confocal microscopy. This process involves the use of specific wavelengths of light to excite fluorescent proteins and stained cells found within each fruit fly brain, and detecting the emitted light from the sample. Background light was minimized through the use of a pinhole aperture, which allows only emitted light from the focal plane of interest to be visualized and photographed. This focal plane can be shifted throughout each brain to retrieve an accurate focused image of each layer, creating a z-stack of images that can be compiled (Elliott et al, 2020). This experiment utilized blue (DAPI), red (histoneRFP), when applicable, and green (mitoGFP) fluorescence with the following laser and gain values: GFP- Laser 12, Gain 20; RFP-Laser 15, Gain 20; DAPI- Laser 25, Gain 20. These values were optimized to highlight the glial mitochondria and astrocyte-like glia in each brain, as well as visualize all cells with DAPI (Figure 9).

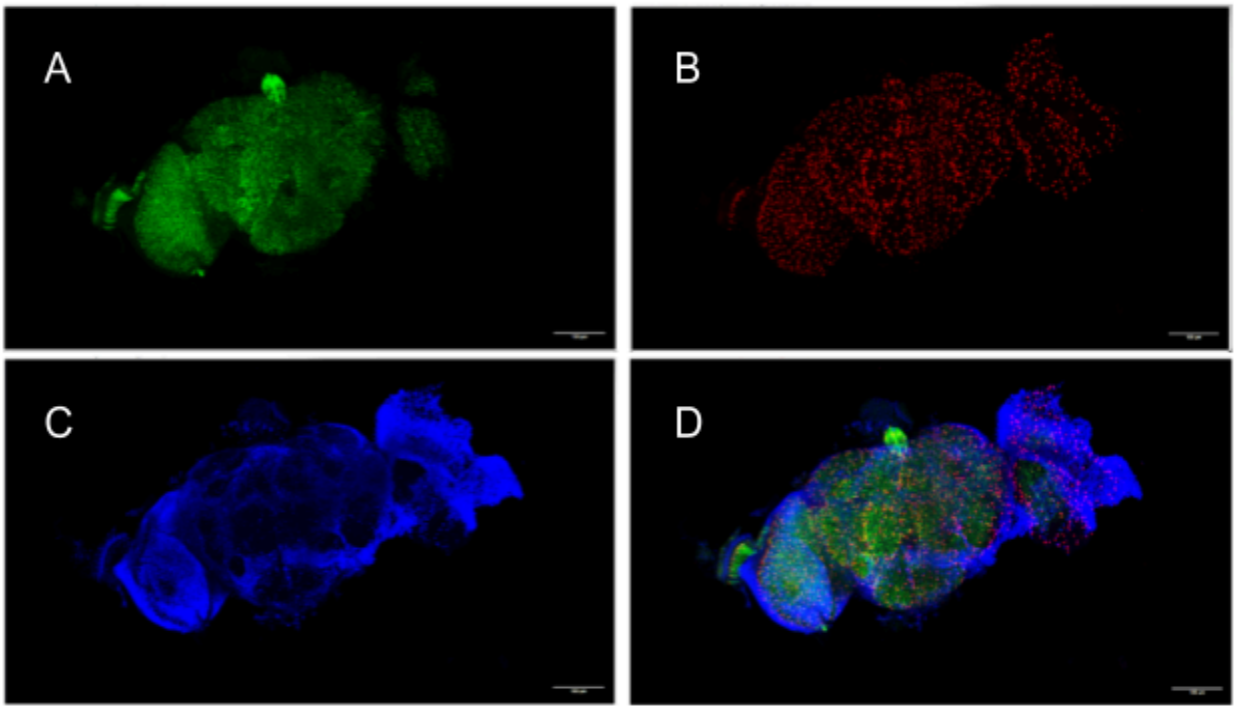


Figure 9. Day 10 female *R8GE01(astro) GAL4, UAShisRFP ;UASmitoGFP* representative brain. A. mitoGFP max intensity protection (slices 30-maximum). B. Max projection of histoneRFP showing astrocyte-like glial nuclei. C. Max projection of all cell nuclei stained with DAPI. D. Merged channels.

Morphological Analysis

Once each brain was dissected and imaged, each z stack was analyzed in order to calculate the number of mitochondria present in each brain and determine their average morphological dimensions. The mitochondrial analyzer plugin for ImageJ was used to count each individual mitochondria in each image in the stack, measuring their volume, sphericity, and surface area (Hemel et al, 2021; Chauhdry, 2021). This software uses the "3D threshold function" to decrease background signal utilizing an adaptive thresholding technique. Unlike nonadaptive thresholding, the mitochondria analyzer determines the appropriate thresholding based on the amount of GFP signal in a particular area, ensuring that overexposed sections of the brain do not prevent more dim mitochondrial puncta from being counted (Hemel et al, 2021; Chauhdry, 2021). The optimized settings for thresholding for this experiment were determined to be c=2, gamma= .6, and radius (of each mitochondria measured) equal to .75 microns. These

values were determined to allow the most mitochondrial fluorescent spots to be counted without increasing background interference (Figure 10).

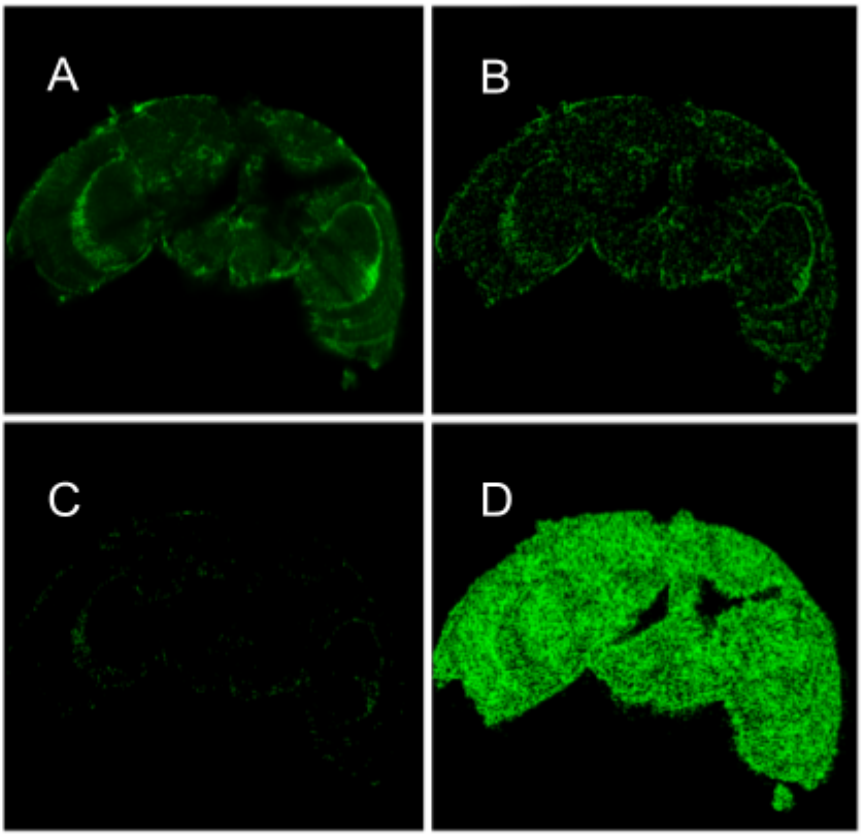


Figure 10. Determining correct settings for Mitochondrial Analyzer thresholding. A. original single confocal plane image of day 10 male *repoGAL4, GAL80^{TS}* brain. B thresholded image of brain with optimized settings of c=2, gamma = 0.6, radius = 0.75. Green puncta show counted mitochondria after background noise has been subtracted. C. High background subtraction values of c=10, gamma = 0.9, radius = 1.25, resulting in undercounting of mitochondria. D. Low background subtraction values of c=1, gamma = 0.2, radius = 0.75, resulting in overcounting of mitochondria.

Once thresholded, the software counted and analyzed each individual mitochondria by multiplying each spot's dimensions to obtain volume and surface area values in microns. The sphericity measurement analyzed how round each individual mitochondria was according to a normalized form factor equation. As the spots become more spherical, the numerical output approaches 1, with values above 1 indicating a mitochondria's elongation (Chauhdry, 2021). For

each brain analyzed, the average volume, sphericity, and surface area of mitochondria were calculated and used for statistical analyses.

Statistical Analyses and Quantification

A power analysis was conducted in R studio using preliminary data (n=3-5 brains per group) to determine the appropriate number of brains for each experimental condition. This analysis revealed that for panglial experiments, 12 brains from each condition were found to be optimal (Cohen's F= .64, power of 50% and alpha 0.05). Based on this analysis and several other *Drosophila* studies that utilized similar methods (with sample sizes ranging from n=3 to n=10), a goal of 10-12 quality brains for each sex and condition was established (Catterson et al, 2024; Bankapalli et al, 2024). For the astrocyte specific experiments only five brains were needed to achieve sufficient power (Cohen's F = .834, power level = 80%, alpha = 0.05). For these experiments, 5-10 quality brains from each age and sex were dissected prior to analysis.

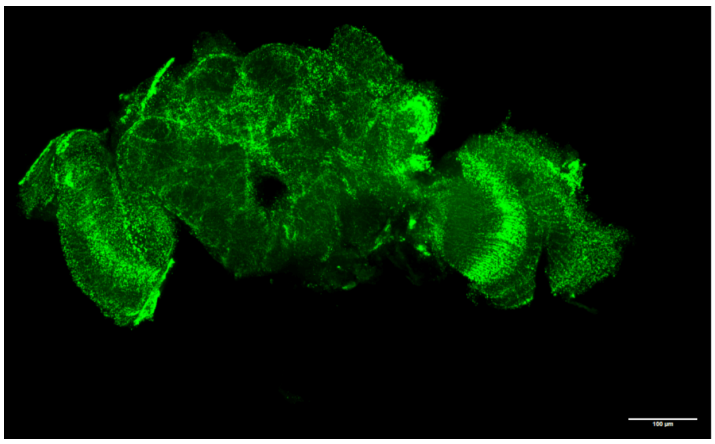
When sufficient power was reached for the panglial experiments, GraphPad Prism was used to perform two-way analyses of variance (ANOVAs) to examine differences in control and tau expressing flies, for both sexes, for each of the following variables: mitochondrial counts, volume, surface area, and sphericity. For the astrocyte specific analysis, a two-way ANOVA was conducted to determine if there were any significant differences between sexes and at each age of interest. Graphs and representative images with ROIs were generated using imageJ and GraphPad, depicting any morphological differences.

Results

This research sought to examine the potential ways that glial mitochondria are uniquely impacted by tau expression. It focused on quantifying and examining morphological differences in glial mitochondria in the presence of tau, using brains from *Drosophila melanogaster* (fruit flies). This research further focused on astrocytic mitochondria under control conditions, examining differences at different ages and between sexes. Data was quantified by doing a full count of all mitochondria in each brain using imageJ software; calculating average volume, sphericity, and surface area of all mitochondria; and observing glial morphologies of interest found in raw images.

Tau significantly reduces panglial mitochondrial quantity

To determine if tau expression in glial cells affected mitochondrial number, panglial expressing tau flies were compared to non-tau expressing control flies at day 10. Total mitochondrial counts for each sex were analyzed using a two-way analysis of variance (ANOVA) in GraphPad Prism. Panglial tau brains showed a visually reduced GFP signal (Figure 11) as compared with controls at day 10, and puncta count analysis showed a significant decrease in the number of mitochondria in the presence of tau ($p < 0.0001$) (Figure 12). Among female flies, tau led to a 35.8% decrease in total puncta, with males displaying a 53.6% decrease as compared with control. Sex had no effect on the number of mitochondria present in each brain ($p= .89$), and no interaction was found between the conditions and sex ($p=.45$).



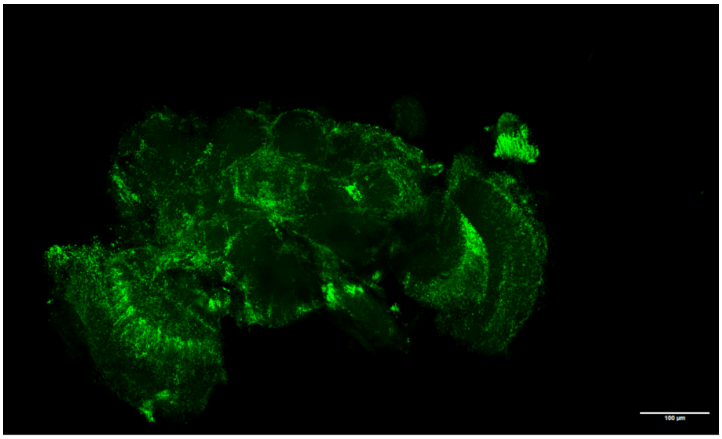


Figure 11. Tau reduces mitochondrial number and fluorescent intensity. Max fluorescent intensity projections reveal tau induces reduced fluorescent intensity and mitochondrial puncta in panglial tau expressing flies. Top: day 10 female panglial control brain. Bottom: day 10 female tau expressing brain.

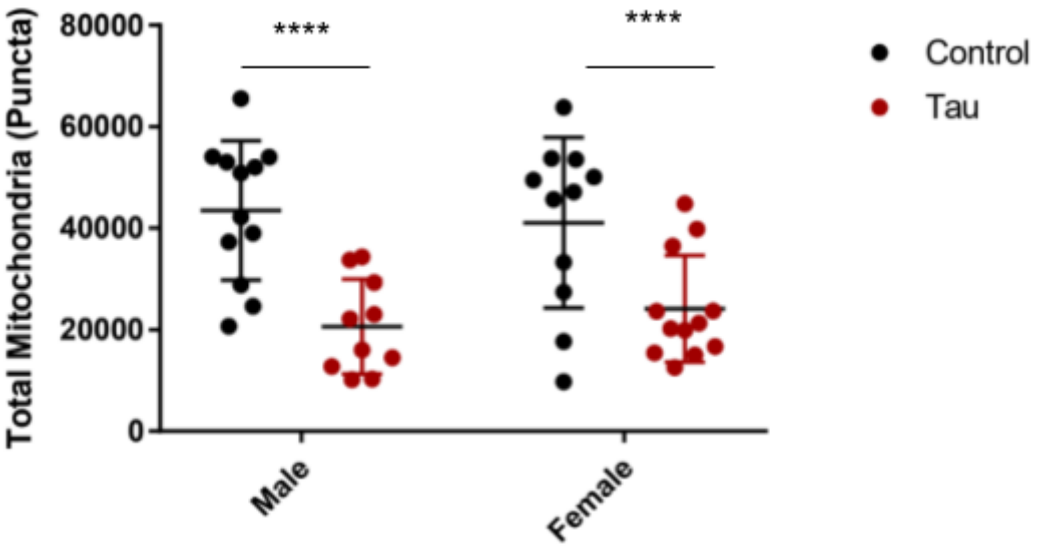


Figure 12. Tau induces reduced panglial mitochondrial counts among both sexes. Total mitochondrial puncta were quantified among panglial tau (red) versus control (black) brains (n=10-12), using all z-stack images for analysis. A two-way ANOVA and Tukey HSD post-hoc test revealed tau brains showed a highly significant difference at day 10, displaying a reduced number of puncta as compared with control (**** $p < 0.0001$).

Tau has no effect on average panglial mitochondrial volume, surface area, and sphericity measurements

To determine whether the expression of tau resulted in mitochondrial elongation or size differences, mitochondrial morphologies were analyzed using average volume, surface area, and sphericity measurements from each individual brain. Three separate two-way ANOVAs were performed to compare control versus tau conditions and between sexes, and no significant differences were found for any of these three dimensions ($p > .05$) (Figure 13).

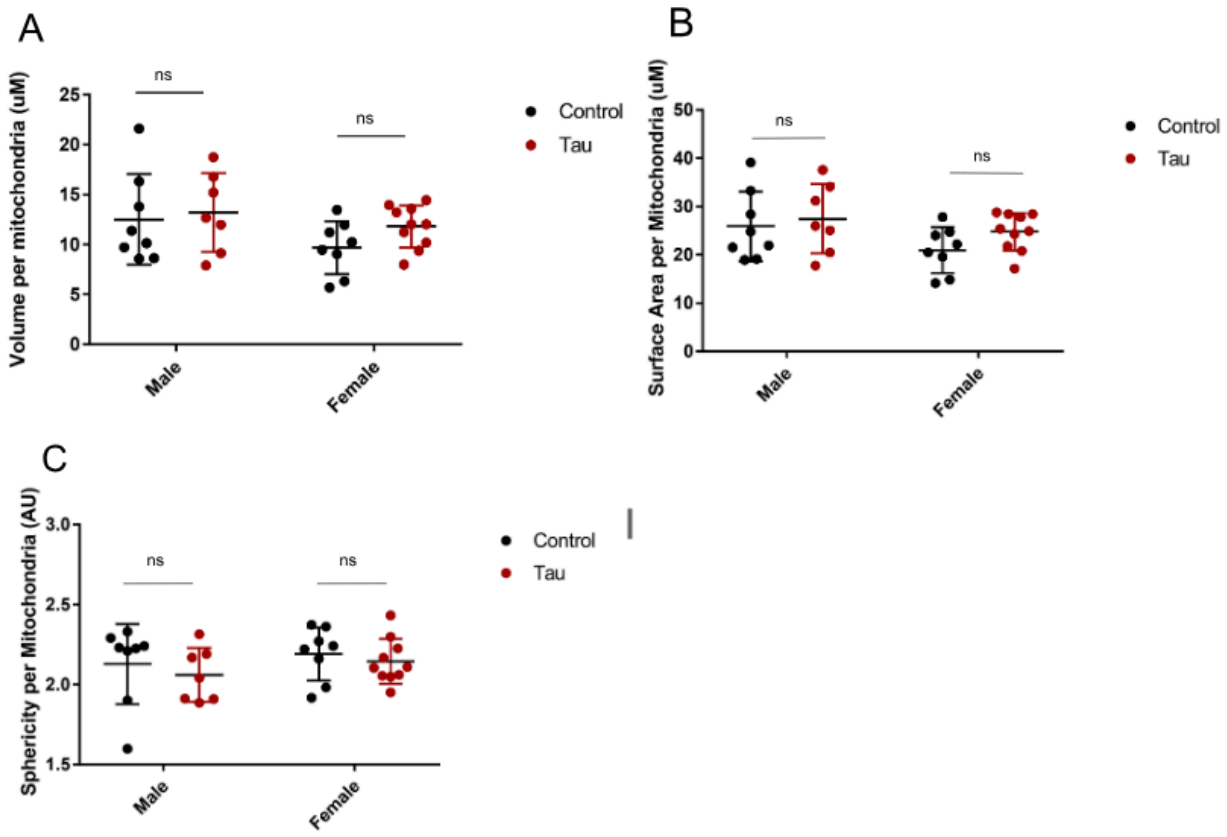


Figure 13. Volume, sphericity and surface area measurements are unaffected by panglial tau expression. Three two-way analyses of variance were conducted based on average volume (A), surface area (B), and sphericity (C) per mitochondria (n=10-12) brains per group. No morphological differences were found between sexes or in the presence of tau ($p > .05$).

Astrocytic mitochondria show age related, but not sex based differences in total puncta counts

To investigate whether astrocytic mitochondrial counts exhibited any age or sex differences, reported counts between sexes and across days 3, 10, and 30 were analyzed using a multifactorial ANOVA. Post-hoc Tukey HSD testing revealed that no significant differences were found between sexes ($p > 0.05$), however age comparison yielded significant results ($p < 0.001$). Male day 3 flies showed reduced puncta counts as compared with day 10 ($p=0.027$), however no differences were found between days 3 and 30, or between days 10 and 30. Among female flies, a significant difference was observed between days 3 and 30 ($p=0.0004$), but no differences were found between other ages (Figure 15). Day 3 flies showed visibly reduced GFP signal as compared with days 10 and 30 (Figure 14).

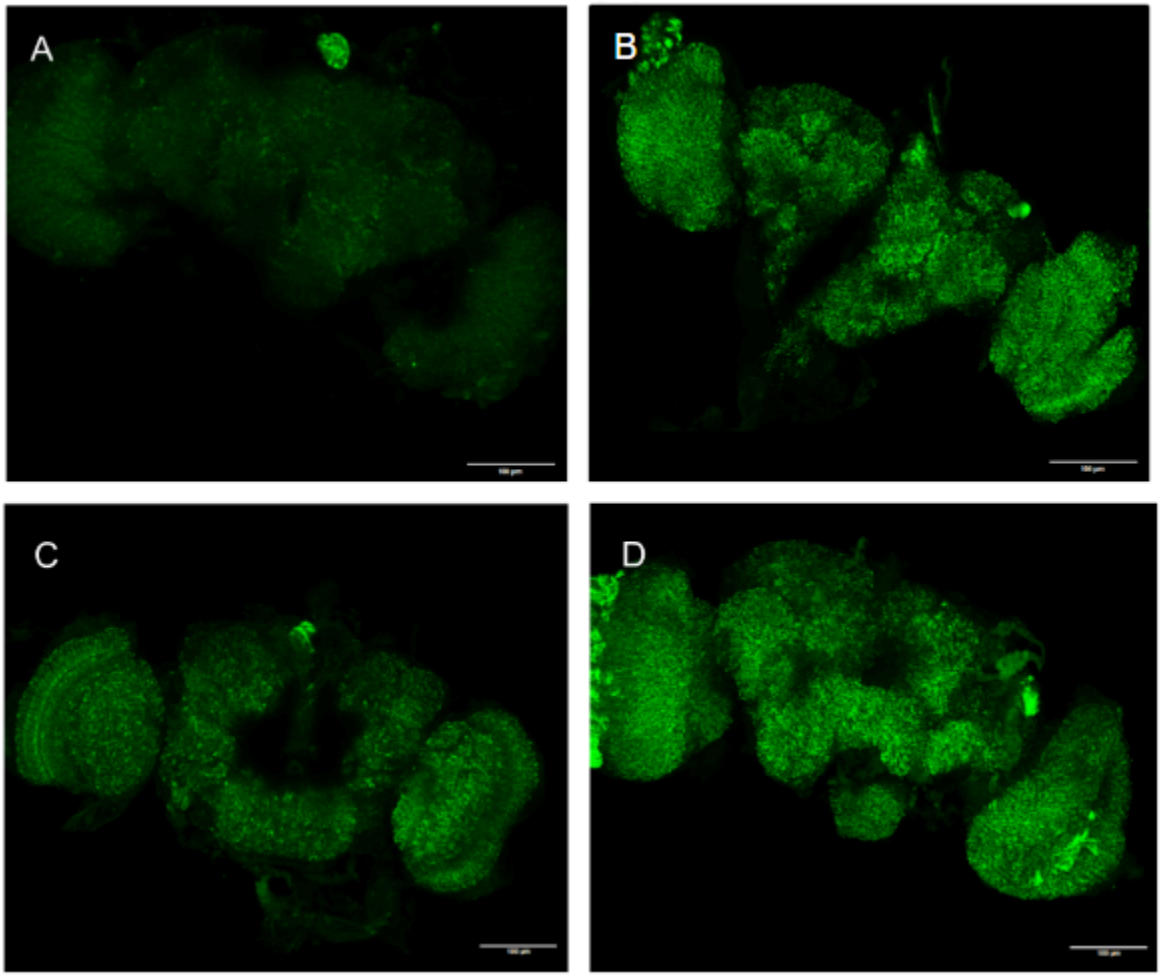


Figure 14. Astrocytic day 3 brains show visibly reduced fluorescent intensity and puncta as compared with older ages. Day 3 (A) and day 10 (B) female and day 3 (C) and day 10 (D) male *astroGAL4; UASmitoGFP* max intensity projection.

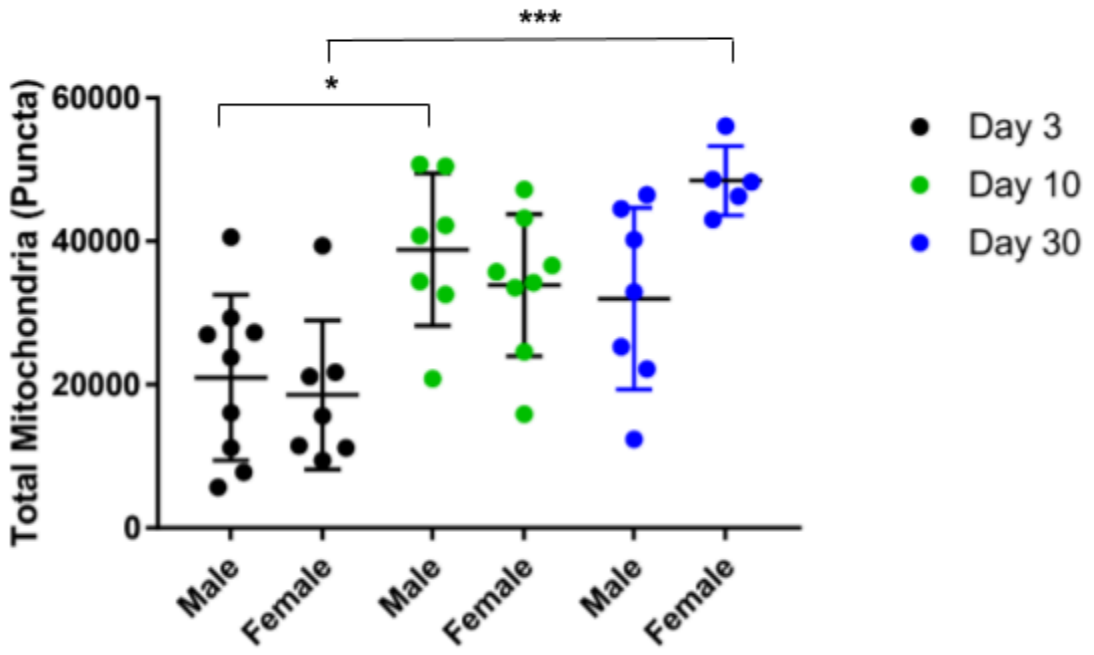


Figure 15. Age affects mitochondrial counts in a sex dependent manner. Total mitochondrial puncta were tallied among male and female day 3 (black), day 10 (green) and day 30 (blue) brains (n=5-9). A two-way ANOVA and Tukey's HSD testing showed that male day 3 brains showed a significant difference when compared with day 10 brains (* $p < .05$) , and female day 3 brains showed significant differences with day 30 brains (*** $p < .001$).

Astrocytic mitochondria do not display age or sex related differences in volume, sphericity, or surface area measurements

Multifactorial ANOVA analysis showed that the average volume, sphericity, and surface area measurements were not altered between male and female flies or across age ($p > .05$) (Figure 16). This indicates that all mitochondrial puncta are of a similar shape and size, despite changes in the number of puncta present according to age differences.

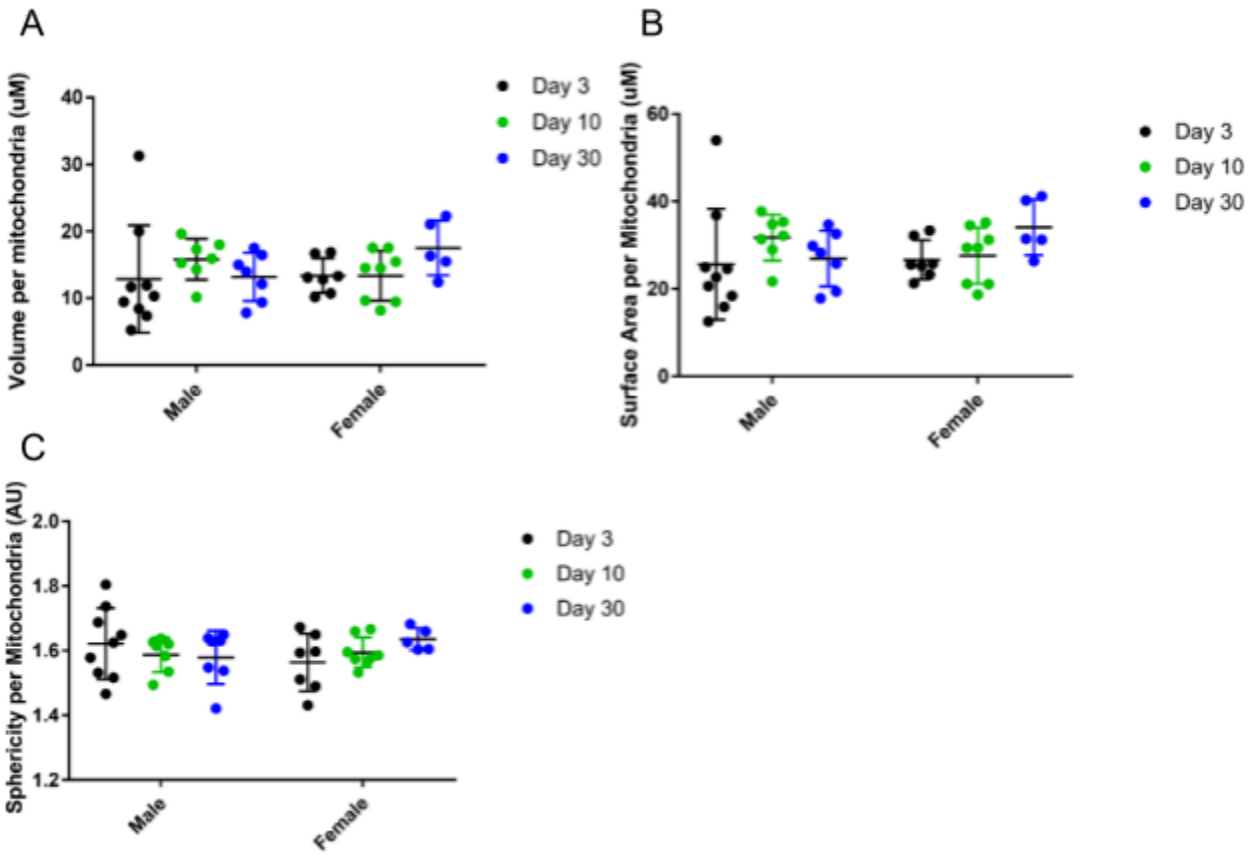


Figure 16. Average astrocytic mitochondrial volume, surface area, and sphericity measurements are not affected by sex or age. A two-way ANOVA revealed no significant differences in mitochondrial morphologies based on these criteria (n=5-9) ($p > 0.05$).

Glial mitochondria cluster in the ocellar lobe and this morphology is independent of age, sex, or the presence of tau

In many brains, a small circular cluster of intense mitoGFP signal located at the anterior portion of each brain was noted. (Figure 17) This locus of activity was visually identified in 52.63% of all brains and was localized to the ocellar ganglion region. The number of brains containing the ocellar ganglion were tallied for each condition across panglial and astrocyte specific experiments, and multiple 2-way ANOVAs showed that there were not significant differences in the presence of this morphology related to age, sex, or the presence of tau.

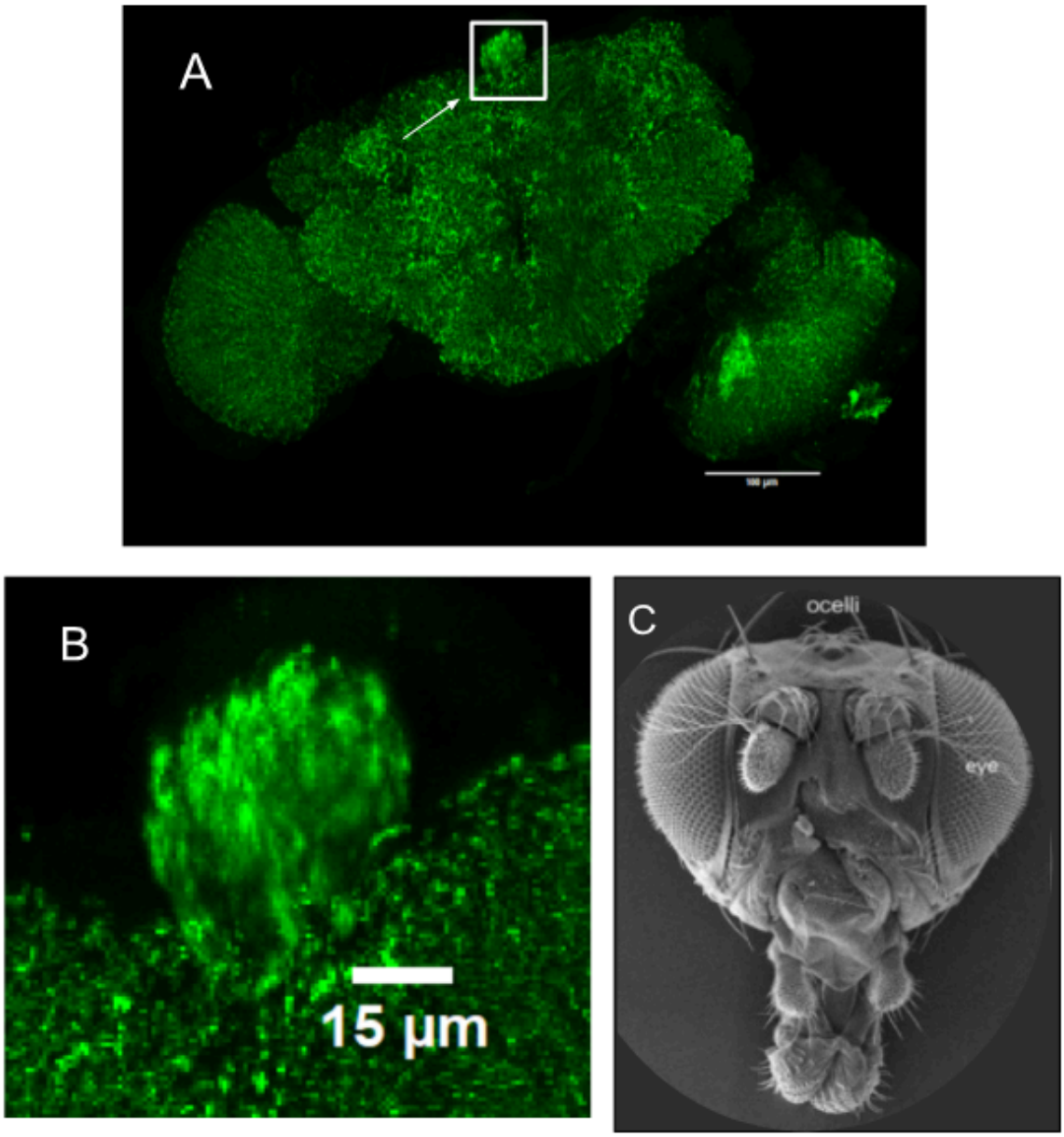


Figure 17. Glial mitochondria cluster in the ocellar ganglion. A. Day 3 male *astroGAL4;UASmitoGFP* brain. Arrow indicates locus of mitochondrial localization. B. 6.6x magnified ocellar region. C. Fruit fly eye and ocellar nerve exterior anatomy. Adapted from Garcia Alonso (1996).

Discussion

Summary of Findings

This study quantified and compared mitochondrial morphologies among glial tau expressing flies versus a panglial control group. We hypothesized that flies expressing tau would have a lower number of mitochondria due to the disruption of fission, and that they would exhibit abnormal, elongated morphology. Overall, this hypothesis was both supported and unsupported: expressing human tau led to a decrease in the number of mitochondria in both male and female flies at day 10, however no changes were found in the average volume, sphericity, and surface area of each mitochondria when tau was present. These findings suggest that tau may have a varied effect in the ways that it interacts with glial cells as compared with neurons, but that it still induces potentially mitotoxic effects irrespective of indicators of elongation.

Additionally, we performed an age and sex based analysis of astrocytic mitochondria at days 3, 10, and 30. We predicted an increase in the amount of glial mitochondria among female flies as compared with males due to their larger brain size, however this hypothesis was not supported by our results. We further predicted that day 10 and 30 flies would exhibit greater quantities of mitochondria than day 3 flies, and found that while we were correct that older flies possess more mitochondria, this depended on sex. Female flies showed increased puncta counts at day 30 as compared to day 3, while males displayed an increase at day 10. There were no significant differences between any other ages of interest. This study also sought to determine whether any morphological differences occurred in astrocytes, and found no effects based on age or sex.

Tau leads to glial mitochondrial loss

Consistent with prior research documenting disruptions to neuronal mitochondrial fission in the presence of tau (Perez et al, 2018; Szabo, 2020), our study found a marked decrease in the number of mitochondria present across brains from both sexes in tau expressing flies. For females, tau led to a 35.8% decrease in the total number of mitochondria as compared with control, and male flies showed an even further decrease with a 53.6% reduction in puncta. This substantial loss of mitochondria may indicate tau induced mitochondrial toxicity, where mitochondria begin to die as a result of the absorption of tau, as well as disruptions to their

ability to divide through fission (Szabo, 2020). These findings add to a growing body of evidence tying neurodegenerative disease directly to mitochondrial impairment, and indicate that glial cells, as well as neurons, are susceptible to mitochondrial toxicity.

Glial metabolic support is a crucial component of neuronal survival, and these results suggest that glial mitochondrial tauopathy is an important avenue for further research into neurodegenerative diseases. This loss of mitochondria may be linked to cellular impacts that have downstream effects on central nervous system health and the metabolic support of neurons. The loss of mitochondria is consistent with prior research that has found that tau inhibits mitochondrial fission and leads to substantial decreases in metabolic activity and respiration. In Alzheimer's disease, patients show 25% reduced cerebral glucose usage due to mitochondrial dysfunction and death (Szabo et al, 2020; Muddapu et al, 2020). Previously, this reduced metabolic capacity has been linked largely to mitochondrial dysfunction in neurons, however these results highlight the need for further study to determine if glial mitochondrial loss contributes meaningfully to the brain's reduced metabolic capacity in neurodegenerative disease.

While the loss of mitochondria in tau expressing flies is consistent with prior literature, it is important to note that these differences could be attributed to glial tau toxicity, rather than mitochondrial toxicity. Colodner and Feany (2010) found that in the presence of tau, *Drosophila* glial cells exhibit stress and undergo apoptosis, leading to neurodegeneration. Though glial mitochondria were visualized, in our study model it was impossible to identify or count individual glial cells themselves. This made it infeasible to confirm whether the loss of mitochondria was associated with overall cell death and reduced counts, rather than disruption to mitochondrial fission or direct mitochondrial damage/death. Based on findings by Rose et al, (2018) that glia rely predominantly on glycolysis for homeostatic ATP production, the loss of mitochondria in these cells may not lead directly to their death. In order to determine the underlying cause of mitochondrial loss in glial populations, it is crucial to repeat the experiment with glial specific nuclear markers so that cells can be visualized in tandem with their mitochondria.

Tau does not lead to glial mitochondrial morphological differences, including elongation

Ultimately, our hypothesis that mitochondria would exhibit reduced sphericity (indicating their elongation) in the presence of tau was not supported. Our results indicated that the

dimensions of volume, surface area, and sphericity were not significantly different in tau versus control flies. This is inconsistent with several prior studies documenting their elongation in neurons (Perez et al, 2018; Szabo et al, 2020; Varte et al, 2023). However, there may be multiple explanations for these findings. While the Mitochondrial Analyzer imageJ plugin has been well established in stem cells and other 3-D samples, it may lack the required resolution to properly measure the morphology of mitochondrial puncta in a *Drosophila* brain. Additionally, most studies identifying mitochondrial elongation utilized electron microscopy techniques, with substantially more powerful resolving techniques than confocal microscopy affords (Perez et al, 2018; Varte et al, 2023).

Despite some methodological limitations in this study, our results may also be indicative of the differential impacts of tau on mitochondria based on cell type differences. Neurons have been shown to possess more mitochondria and undergo oxidative phosphorylation more frequently than glia (Rose et al, 2018). Additionally, due to the lack of research on glial mitochondrial tauopathy, there is no established baseline for expected morphologies or fission dynamics among prior literature. While the fission protein DRP-1 is widely utilized in neurons, glial mitochondria may undergo different mechanisms to regulate their fission/fusion dynamics.

Another possible explanation for our results being inconsistent with prior findings is through the tau isoform we used in this study. Most studies identifying neuronal mitochondria elongation in the presence of tau utilize either human wild type tau or pathologically toxic isoforms such as 2N4R tau (Szabo, 2020; Varte et al, 2023). However, this study utilized 0N4R tau, an isoform shown to be toxic and cause functional deficits in *Drosophila* (Shahpasand-Kroner et al, 2022). This form of tau may interact differently with mitochondria than previously utilized forms, potentially inducing toxicity without directly affecting their morphology. Rather than reducing the number of mitochondria through inhibiting fission, glial 0N4R tau may lead to these results by inducing mitochondrial death.

Day 3 flies possess fewer astrocytic mitochondria than days 10 and 30, and this is associated with sex

Our hypothesis that age would have a role in mitochondrial quantity was overall supported, with day 3 flies possessing fewer mitochondria than their older counterparts. However, the prediction that female flies would have a greater number of mitochondria due to

possessing larger brains was not supported. This may indicate that males have a greater proportion of mitochondria to overall brain mass as compared with females, leading to similar puncta counts. Indeed, among both days 3 and 10 male flies trended towards having a greater number of mitochondria than females, though this was not found to be statistically significant and did not persist at day 30.

While *Drosophila* do not appear to have any sex differences in their mitochondrial count when compared within each age group, age- based differences were differentially expressed between sexes. Male day 3 flies showed a significant reduction in mitochondrial puncta when compared with day 10, but this difference did not persist among day 30 flies. However, this result was flipped among female flies-- females showed a difference between days 3 and 30, but not at day 10. This could indicate sex dependent aging dynamics, with female flies exhibiting a greater quantity of mitochondria as they age beyond 10 days, and male flies showing a loss of mitochondria as they get older. These sex-age interactions raise further questions and indicate that additional study into sex related mitochondrial dynamics and protein expression is needed to determine the ways that male and female individuals may be differentially susceptible to tauopathy.

Astrocytic mitochondrial morphology is not influenced by age or sex

Consistent with our findings that tau does not influence mitochondrial morphology, age and sex were not found to have a role in mitochondrial volume, sphericity, and surface area. This indicates that the normal aging process does not lead to mitochondrial elongation in astrocytes. However, this result may be influenced by the limitations of confocal microscopy and the Mitochondrial Analyzer software. We conclude that these morphological differences are not easily observed using confocal microscopy techniques and that electron microscopy would more accurately image mitochondrial puncta smaller than 1uM.

Glial mitochondria cluster in the ocellar ganglion in both astrocyte specific and panglial brains, irrespective of tau

Over the course of image analysis, a frequently occuring cluster of glial mitochondria was observed on the anterior portion of the brain. This cluster appears to correspond with the location of the ocellar ganglion, a nerve cord connecting *Drosophila* visual sensory organs

known as ocelli to the brain. Coined the “little eyes,” many fly species possess ocelli. *Drosophila melanogaster* possesses three ocelli which are responsible for detecting light/dark visual stimuli (Krapp et al, 2009). Additionally, it is believed that the ocelli function in part as a faster mode of visual processing than the compound eye due to their relatively primitive structure and short nerve connection to the brain (Mizanami, 1995). The ocellar ganglion connects directly with the lateral protocerebrum, a region responsible for coordinating movement and stabilizing flight reflexes (Krapp et al, 2009). The ocelli may thus allow flies to detect changes in light based on direction and coordinate their flight movements accordingly.

Despite their documented functions among invertebrates, no prior research has identified the presence of glial mitochondria clustered within the ocellar ganglion. While this research did not seek to characterize the localization of tau within the ocelli, over 50% of brains across ages, sexes, glial subtypes, and conditions possessed this morphology. Among the brains that do not possess this locus of mitochondria, we believe that the ocellar ganglion was removed during the dissection process. Additionally, for a small portion of brains possessing mitochondrial activity in the ocellar region, the ocelli appears to have been dislodged in the dissection process, leading to its appearance in other unrelated brain regions. Given that this region is present in so many brains irrespective of the presence of tau, age, or sex differences, we hypothesize that this is a fixed feature of the *Drosophila* nervous system. Despite this, our research must be repeated with careful dissection techniques to confirm our findings.

The high concentration of mitochondria within the ocellar ganglion is consistent with the current scientific understanding of fruit fly visual processing. Raji and Potter (2021) determined that each of the fruit fly’s optic lobes possess over 100,000 nerve cells, together accounting for more than half of an adult fly’s total neurons. While many of these cells innervate the optic lobes via the lamina, rather than the ocelli, it is clear that *Drosophila* have evolved to prioritize visual processing systems. Given the massive amount of the *Drosophila* brain that is dedicated to sight, it stands to reason that the ocelli may experience a high metabolic demand, and may require a greater number of mitochondria than other systems (Raji and Potter, 2021). Particularly, wrapping, ensheathing, and astrocytic mitochondria may metabolically support this visual signaling at a high rate, necessitating a large number of glial mitochondria.

Study Limitations

Among astrocyte specific experiments, several limitations may have impacted experimental results. Due to software and mechanical errors, the confocal microscope underwent several weeks of malfunction. During this time period, several dissected brains appeared to lose fluorescent signals. After three weeks without the ability to image, several brains displayed dim signals and showed heavily reduced mitochondrial puncta counts (fewer than 2000). These brains were omitted from data, meaning that among day 30 female flies, the sample size was limited to five brains, and many dissections had to be replicated.

This loss of signal within a 3 week time window leads to the conclusion that the number of days between dissection and imaging heavily impacts experimental results. After the loss of several brains (and with the eventual restoration of the confocal microscope) we established a protocol of imaging brains within seven days of initial dissection to minimize loss of signal. All panglial tau versus control brains were imaged in accordance with this time table, however much of the previously collected astrocytic data was affected by variation in the time taken between dissection and imaging. Because of this variability, these results may not represent the true number of mitochondria, and are likely skewed by a tendency to undercount.

In addition to the difficulties associated with confocal microscopy technology, the dissection process itself may produce some degree of natural variation in the overall quality of results. In many brains, dissection artifacts such as trachea, ocelli, and lamina remained partially attached and influenced the number of mitochondria counted. Trachea innervates the brain to provide oxygen for respiration, however when present it can block certain sections of the CNS from being properly visualized. These trachea appear to cover small sections of the brain and prevent lasers from passing through, leading to reduced mitochondrial puncta counts. The lamina, on the other hand, appears to have led to increased mitochondrial puncta counts. Lamina, like ocelli, relates to *Drosophila* visual processing, connecting to the compound eyes and propagating visual signals to the rest of the CNS (Raji and Potter, 2021). The presence of these dissection artifacts may have subtly influenced the recorded number of mitochondria counted through mitochondrial analyzer software, however they were present in brains across different conditions leading to overall minor effects to average mitochondrial counts.

While dissection artifacts remained the primary source of variation in mitochondrial counts, damage to brains during the dissection process may account for additional variability.

Brains were considered intact if both optic lobes and the protocerebrum were present, however often these sections were partially detached from one another or had been nicked during the dissection process. In these cases, brains were utilized for data analysis but small, missing sections may lead to the underreporting of mitochondrial puncta. Despite these limitations inherent to the dissection of the *Drosophila* central nervous system, brains were overall in complete condition and alterations to the findings based on dissection appear negligible.

Future Directions

Ultimately, our findings raise many questions about the role of glial cells in tauopathies, and the ways that they may be uniquely impacted by neurodegenerative disease: are there fewer mitochondria due to the way that tau interacts with glial cells, leading mitochondria to die off? Alternatively, is tau so lethal to glial cells that neurodegeneration itself results in a decrease in mitochondria? Would glial mitochondria exhibit elongation under electron microscopy, or do glial mitochondria differ from neuronal ones and exhibit normal morphologies in the presence of tau In order to test these questions, the use of a panglial nuclear or membrane bound fluorescent protein, such as histone RFP is essential (Beliharz et al, 2015). These constructs allow for the identification and count of each individual glial cell, which in turn can be used to identify whether cells have died or remain intact. Further studies involving electron microscopy could similarly serve to confirm whether the use of the Mitochondrial Analyzer plugin is appropriate for determining mitochondrial morphology.

While several future directions relate to questions and next steps raised by our findings, the most pressing follow-up studies relate to the confirmation of our findings through additional genetic manipulations. Importantly, we hope to induce random protein expression to ensure our results were not caused by a dilution effect of GAL4.The GAL4-UAS system is highly effective at creating proteins of interest in specific cell types, however when multiple proteins are being created, a limited amount of GAL4 must be used to drive multiple UAS sequences (Johnston and Hopper, 1982). This results in a slight reduction in the available GAL4, leading to possibly lower UAS signaling for each transgene. In this case, flies possessing *UASmitoGFP* alone would have a greater available amount of GAL4 to drive its activity than flies possessing both *UASmitoGFP* and *UAStau* constructs. In order to establish that there is no dilution effect, a non-active protein such as lacZ is frequently used as a control in *Drosophila* research (Small, 2000). If flies

possessing lacZ and mitoGFP exhibit normal, high mitochondrial signal, it can be assumed that the reduction in mitochondrial signal in the presence of tau is not caused by GAL4 dilution.

In addition to the confirmation of results, our panglial experiments provided a basis to further interrogate the effects of tau on mitochondria among different glial subtypes, such as wrapping glia, ensheathing glia, cortical glia, and astrocyte-like glia. Each of these glial subtypes possesses unique features and properties that may be influenced by tau in a myriad of ways. However, wrapping and astrocyte-like glia are of the greatest interest for immediate future research due to their roles in neuronal metabolism (Cezeriat et al, 2024; Phillips and Rothstein, 2017). Astrocyte like-glia are particularly susceptible to mitochondrial tau pathology due to the astrocyte's roles in respiration, cellular metabolism, and gas exchange. Future studies will aim to observe astrocyte specific mitochondrial counts and morphologies in the presence of tau. Due to their potential implication in the spread of tauopathy through pyruvate transporters, the study of astrocytic mitochondria is highly salient to understanding neurodegeneration (Cezeriat et al, 2024).

While glial subtypes remain one of the most pressing followup studies, future directions of interest include observing the direct interactions between tau and mitochondria. Prior research has identified that misfolded proteins including tau may become localized to the mitochondria, indicating a possible mode of proteasomal degradation (Spinelli and Haigas, 2018). In order to examine this relationship, the use of immunohistochemistry to stain for hyperphosphorylated tau could be used to observe co-localization in glial mitochondria (Yoshida et al, 2006). If tau co-localizes to mitochondria, this would lend credibility to the theory that tau constitutes a direct burden to mitochondria, rather than acting on other upstream pathways or proteins that produces mitotoxic effects.

Moreover, to further interrogate the effects of tau on overall mitochondrial functioning and metabolic output, future studies could utilize fluorescent markers of oxidative activity. In addition to the mitoGFP protein that our study incorporated, several fluorescent proteins can be used to visualize metabolically active mitochondria. One of these such constructs in *Drosophila* research is known as *UAS-mito-roGFP2-Grx1*, a protein that fluoresces anywhere a redox reaction is occuring (Varte et al, 2023). Cellular respiration is highly dependent on redox reactions, thus metabolically active mitochondria are easily visualized using this fly line. Given that tau has been linked to reduced mitochondrial ATP production, it stands to reason that tau

expressing flies would display reduced redox reaction capacity, indicating a possible loss of mitochondrial activity in addition to reduced puncta counts (Szabo, 2020).

Finally, our research on the age and sex differences associated with astrocytic mitochondria raised many questions about the sexually dimorphic nature of fly mitochondrial expression. Our findings that astrocytic mitochondria increase at ages 10 and 30 as compared with day 3 in a sex dependent manner indicates sex specific aging dynamics that must be investigated to better understand the role of sex in tauopathy. Future studies could involve observing and identifying different mitochondrial regulation mechanisms and proteins at various stages in development to elucidate these effects.

Conclusion

This study sought to examine the effects of tau expression on glial mitochondria to provide insights into these understudied cells in the context of neurodegenerative diseases. Consistent with prior research connecting tauopathy to mitochondrial metabolic dysfunction, elongation, and failure to undergo fission in neurons, we found a substantially reduced number of glial mitochondria in flies possessing tau. Despite these changes, we did not see any evidence of mitochondrial elongation, indicating that tau may affect glial mitochondria differently than neurons. Our findings have raised a number of questions about the mechanisms of glial tau interactions with mitochondria. In the future, we hope to determine some of the underlying causes of mitochondrial loss and investigate various glial subtypes. Ideally, through investigating these salient future directions we can highlight important features of glial tauopathy and the role of mitochondria in this process.

We hope that our findings will serve as a basis for future studies to both understand the mechanisms of neurodegeneration and identify possible treatment avenues. If glial and astrocytic mitochondria are being impacted uniquely, possible dementia treatments could revolve around finding ways to prevent their dysfunction or replicate their roles in the brain. This could include using medicines to provide extra metabolites for suffering neurons, replicating the role of DRP-1 to restore fission, preventing the spread of hyperphosphorylated tau, or developing drugs targeting ROS toxicity. Hopefully, this research will add to a body of research committed to providing hope to patients with neurodegenerative diseases, as researchers inch closer to finding a cure.

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