A Tauopathy model of Alzheimer's disease in *Drosophila melanogaster*

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Statement of Sources

I declare that this report is my own original work and that contributions of others have been duly acknowledged.

Sarah F. Van Drunen

October, 2019.

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Abstract

Alzheimer's disease is the most prevalent cause of dementia, decreasing a person's functionality in memory, personality and eventually motor domains, leading to the need for full time care. Tau is a protein in the brain which becomes dysfunctional in Alzheimer's and the localisation of tau pathology is associated with the deficits people experience, starting in learning and memory areas such as the hippocampus. Drosophila melanogaster provide a useful model for studying Alzheimer's disease. In the current study I verified a model of tau dysfunction induced in the whole brain (pan-neuronally) and investigated a novel model specific to the learning and memory area of the brain in Drosophila, the mushroom body.

Consistent with the literature, the pan-neuronal tau flies had deficits in motor performance and a decreased lifespan compared to controls. Flies with mushroom body specific tauopathy had a deficit in learning and memory, decreased lifespan and abnormal morphology of the affected brain region compared to controls. The next step of this research is to study gene expression in the learning and memory neurons. This will allow us to understand the epigenetic changes induced by tauopathy, paving the way for the development of targeted screening tools and treatments for Alzheimer's disease.

Dementia is a syndrome which interferes with everyday functioning and is marked by changes in memory, personality and behaviour. As of 2015, 47 million people were affected by dementia (costing US $818 billion globally) and it is estimated that by 2050 this number will triple (El-Hayek et al., 2019; Livingston et al., 2017). Alzheimer's disease (AD) is the most prevalent cause of primary dementia accounting for approximately seventy percent of cases (Kametani & Hasegawa, 2018; Salardini, 2019). AD is a terminal condition which begins with deficits in memory, then progressively affects other areas such as personality and motor functioning. AD accounts for a large proportion of the global disease burden and will only increase with our aging populations (Shah et al., 2016). In the later stages of AD people require full time care, and the negative impacts of the disease are felt by the individual and their family, friends and carers. Currently there is no cure, and prevention strategies rely on decreasing modifiable risk factors. Understanding the mechanisms of the disease could allow targeted early detection, treatment and prevention strategies to be developed.

Causes of Alzheimer's disease

To develop a disease treatment, it is important to understand and target the cause of the disease. The majority of AD cases are sporadic (90%), meaning that there is no known set of causative genes or risk factors (Piaceri, Nacmias, & Sorbi, 2013). Population based studies have been used to identify risk factors that correlate with the development of AD (Livingston et al., 2017); due to the correlative nature of that research however, conclusions cannot be drawn about the cause. Hypotheses about the cause of AD therefore focus on the two main pathological changes that are found within the brain: amyloid beta plaques and tau neurofibrillary tangles. It is likely that the dysfunction of both amyloid beta and tau are important to understanding the cause and progression of the AD. A representation of amyloid beta and tau pathology in an AD context can be seen in Figure 1.

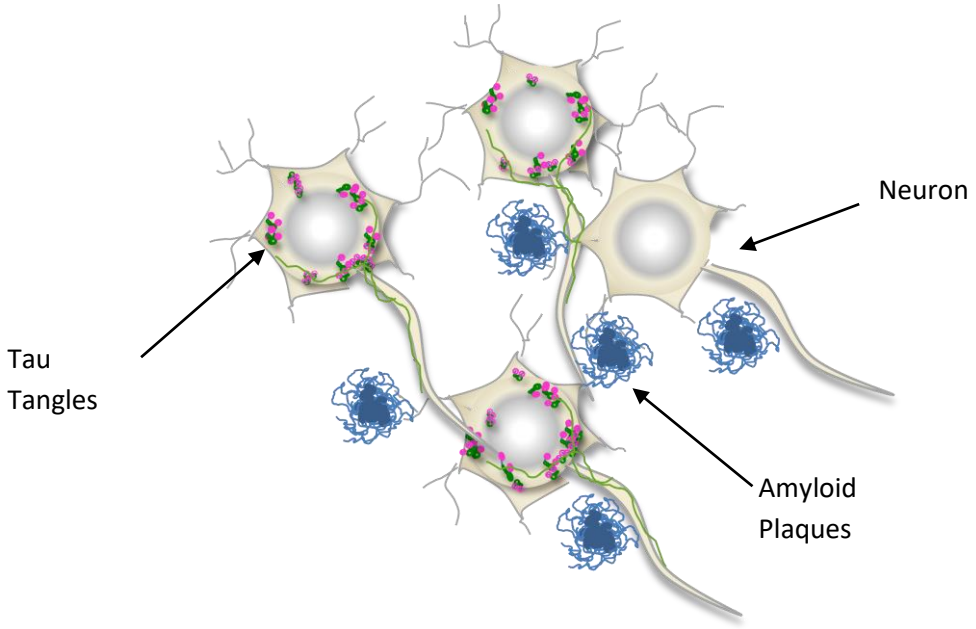

Figure 1. The pathological features of Alzheimer's disease.
Depicted are a set of neurons with Amyloid beta plaques outside the cells (blue) and tau tangles inside the cells (green and pink).

Amyloid beta pathology

The majority of the literature so far has focused on amyloid beta plaques as the main cause of AD (Selkoe & Hardy, 2016). Amyloid beta is a by-product arising from the modification of a larger protein, the amyloid precursor protein (Pearson & Peers, 2006). In healthy brains amyloid beta is thought to have a functional role, including involvement in signal transmission between neurons (Pearson & Peers, 2006). In AD amyloid beta forms aggregates (clumps) and as a result, insoluble plaques form outside cells in the brain (extracellularly), disrupting the communication between neurons, and causing death to neuronal networks (the systems of cells which allow internal and external stimuli to be perceived, interpreted and acted upon). This has been termed the amyloid or amyloid beta hypothesis (Selkoe & Hardy, 2016). However, there is debate about whether amyloid is the main or only cause of neurodegenerative symptoms in AD (Kametani & Hasegawa, 2018). Amyloid pathology load in the brain is not proportional to cognitive deficits experienced by patients which suggests that there are other mechanisms involved (Selkoe & Hardy, 2016).

Tau pathology

Tau is a protein which is found in normally functioning neurons, where its main role is to bind to and stabilise microtubules which form part of the scaffolding of the cell (Iqbal, Liu, & Gong, 2016). For neurons to function effectively they need to be structurally sound, but also be dynamic in order to change shape. This is important, as tau is mainly localised in the long cellular processes of neurons called axons, which allow signal transmission to occur through vast networks. In AD, tau becomes hyper-phosphorylated (adding of extra phosphate groups to the protein) which means that it can no longer bind to microtubules (Gistelinck, Lambert, Callaerts, Dermaut, & Dourlen, 2012). As a result, microtubule structures are destabilised and large tangles of tau form inside neurons (intracellularly) in the cell bodies and dendrites, disrupting cellular functioning (Iqbal et al., 2016). There has been relatively little research into the effect of tau pathology as a cause of AD in comparison to amyloid. Tau pathology is referred to as tauopathy in the literature, as it exists in several neurodegenerative diseases.

Tauopathy and AD progression

In human AD there is a predictable disease progression in terms of the symptoms an individual experiences. First, people experience deficits in learning and memory, then executive functioning, personality changes and eventually motor dysfunction. Interestingly, research suggests that tau pathology correlates better with the progressive cognitive decline experienced by people with AD compared to amyloid pathology (Pooler et al., 2013). It is first found in areas important for learning and memory such as the hippocampus, and in the late stages eventually affecting the neocortex (Pooler et al., 2013). As such, studying tau pathology is important for understanding disease progression and the resulting cognitive symptoms. The scope of this thesis focuses specifically on the effect of tau pathology in learning and memory structures of the brain.

Chromatin states: Epigenetic changes in neurodegenerative disease

In AD some neural dysfunction is caused by physical aggregates of protein; however research indicates that there are also changes in gene expression in affected neuron populations (Klein et al., 2018). Although all cells in the body contain the same genetic code, there is large diversity in their anatomy and physiology. This is achieved by the packaging of DNA in each cell (Ernst & Kellis, 2010). As seen in Figure 2, some parts of the genome are open and allow genes to be copied and therefore their subsequent proteins to be made easily. Other genes are tightly packed thereby repressing gene expression. Active and repressed parts of the genome can be further divided according to the genetic environment. The different types of DNA packaging are referred to as chromatin states and are defined by histone modifications and proteins (proteins that tag different areas of the genome) binding to these histones modifications (structural proteins around which DNA is wrapped), as seen in Figure 2 (Delandre & Marshall, 2019). It has been suggested that there are five distinct chromatin states which correlated with different gene roles (Delandre & Marshall, 2019).

Diseases such as AD can cause abnormal changes to chromatin states, which subsequently can cause gene and therefore protein deregulation resulting in changes in cell functioning and cell death. (Frost, Hemberg, Lewis, & Feany, 2014). Preliminary research in tauopathy models of AD, as well as human cases indicates that changes in histone modifications occur as part of the disease (Berson, Nativio, Berger, & Bonini, 2018; Klein et al., 2018). These are classified as epigenetic changes and are likely to contribute to neurodegeneration in AD.

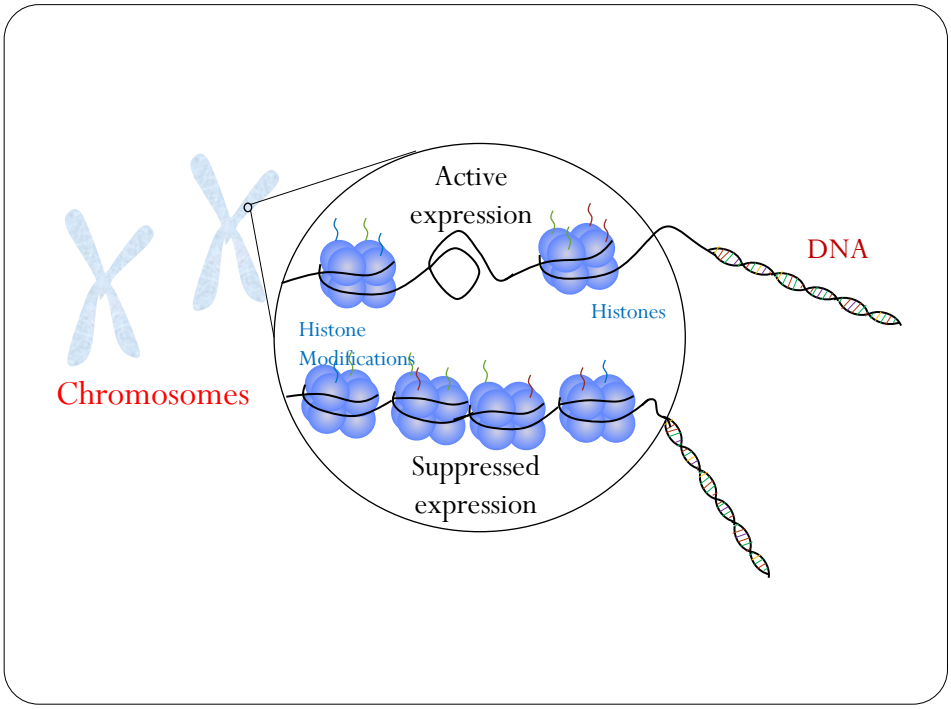

Figure 2. Packaging of DNA resulting in active or suppressed expression of genes. The groupings of blues spheres represent the histones around which DNA is wrapped. Various coloured lines represent the histone modifications which can be measured to determine chromatin states.

Using *Drosophila* to model Alzheimer's disease

To study the underlying cause of AD it is important to study the initial mechanisms that cause neurodegeneration. Changes in protein function and cell death in AD occurs before any noticeable cognitive deficits which means that the disease process is occurring long before diagnosis, and therefore it is not possible to identify and observe changes in human brains in the early stages of AD development (Sperling et al., 2011). Use of human data also constrains the investigations, as there are many contextual factors that cannot be controlled for.

Drosophila melanogaster (the vinegar fly) provides a useful model for studying human diseases. Approximately 75% of disease-related genes are conserved between *Drosophila* and humans (Bier, 2005). The *Drosophila* nervous system has also been shown to be similar to human nervous system, providing a useful model for brain related disorders (Venken, Simpson, & Bellen, 2011). They also have the benefit of sophisticated genetic

techniques which have been developed over 100 years allowing for unique control and investigation of disease related processes (Bellen, Tong, & Tsuda, 2010). Other benefits of using *Drosophila* are that they have a short life cycle and large numbers can be produced for high powered experiments. As a model *Drosophila* allows us to thoroughly investigate the pathology and causes of AD at different stages of disease progression.

Creating an Alzheimer's disease model in Drosophila

Manipulation of the gene that codes for tau has been established as a useful way to create animal models of AD, induced with certainty. In these models a copy of the wild type human tau gene (an unmodified version of the one found in healthy humans) is inserted into the *Drosophila* genome, labelled human tau fly line in Figure 3. On its own, the human tau gene is not active. To create an active copy, the human tau fly line can be crossed with a driver line, as seen in Figure 3 (Venken et al., 2011). The first part of the driver line is a promoter, a regulatory element that induces gene transcription in a cell specific manner. Different promoters are used to target cell populations specifically, in this case the promoters used target were in all neurons (pan-neuronal) and a specific sub-population of neurons (in the mushroom body) which allowed for specific control of gene expression. In cells where the promoter is recognised, the gene part of the driver line, LexA, is also transcribed which causes the creation of LexA protein. This protein acts on the LexA operator in the human tau fly line to the drive expression of the human tau protein.

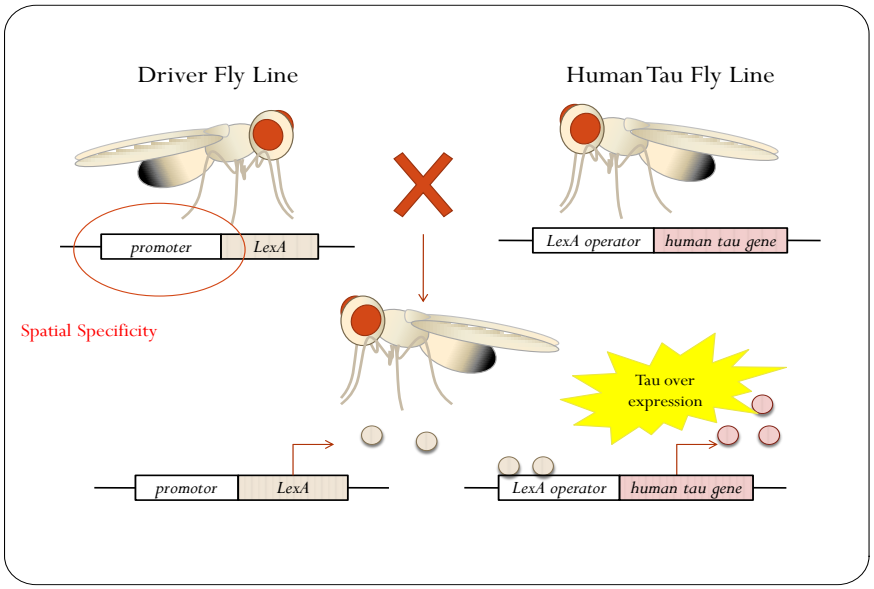

Figure 3. Two part expression system in *Drosophila.*
When the driver fly line and human tau fly line are crossed, the resulting fly possesses both constructs. The driver is then able to drive expression of the human tau gene in a cell specific manner.

To change the severity of tauopathy, flies with different mutations in the tau gene are used (Gistelinck et al., 2012). Some common mutations are associated with early-onset front-temporal dementia. In the mutant form of the human tau gene I used (R406W), one base pair in the genetic code is substituted out which results in incorrect functioning of the protein (Iqbal et al., 2016). Flies expressing this mutant human tau have a further reduced life span and motor deficits (Wittmann et al., 2001). Therefore, using a mutant tau gene allows a more severe tauopathy phenotype to be studied compared to a wild type human tau model. These different models increase the scope of studying tauopathy in the fly.

Tauopathy in *Drosophila*

Pan-neuronal tauopathy models

The majority of the literature has focussed on pan-neuronal expression of tauopathy, i.e., where tau is expressed in all neurons. In pan-neuronal *Drosophila* tauopathy models there is age-related neurodegeneration of the cortex and associated motor deficits, consistent with human disease (Tan & Azzam, 2017; Wittmann et al., 2001). The main neurological

features of *Drosophila* tauopathies are neurodegeneration, as seen by increases in vacuoles (holes in the neuronal tissue), changes in axonal morphology and axonal blebbing (enlargement, or swelling of the axon) and cell death (Gistelinck et al., 2012). These cellular changes result in several behavioural changes.

In a test of motor performance (where flies are tapped down a vial and subsequently climb back up as a reflex), *Drosophila* with tauopathy do not climb as high as controls in the given time frame (Ali, Ruan, & Zhai, 2012). This is similar to the motor deficits experienced by patients with late stage AD when the whole brain affect. Disease-affected flies also have a decrease in performance in learning and memory tasks (such as an associative shock–stimuli paired learning task) and a shortened life span (Gistelinck et al., 2012; Mershin et al., 2004). Neurodegeneration, memory decline, locomotor decline and a decrease in lifespan are all common features of human AD.

One of the main limitations of pan-neuronal studies of tauopathy is that tau pathology is induced in all areas of the brain at the same time, which does not reflect the progressive nature of human disease. As discussed above, AD in humans begins in areas of the brain important for learning and memory (the hippocampus), and then progresses throughout the brain. An interesting area of research, therefore, would be to look at how the hippocampus is specifically affected in early stages of disease.

The mushroom body: A learning and memory structure of the fly brain

In a *Drosophila* model, tau pathology can be induced specifically in the analogous structure to the hippocampus, the mushroom body. The mushroom body is made up of two groups of cell bodies, which extend processes to form two dendrites bundles called the calyces (one on either side), and a series of axon bundles making up the lobes (Ito, Awano, Suzuki, Hiromi, & Yamamoto, 1997). These lobes are split into three groups, the α/β, α’/β’

and γ lobes, as seen in Figure 4 below. Similar to the human hippocampus, the mushroom body is vital for learning and memory in *Drosophila.*

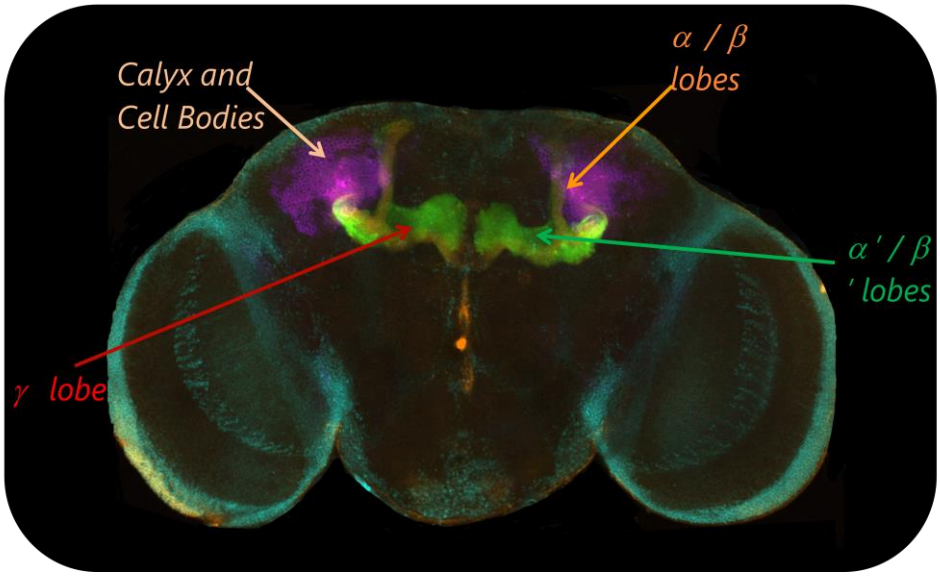

Figure 4. The whole fly brain with the mushroom body highlighted
Drosophila brain outlined in aqua with mushroom body lobes in green and cell bodies in purple.

The mushroom body is an important structure for learning and memory, therefore the main functional test to determine if it has been disrupted is a learning assay. One *Drosophila* learning and memory assay is the T maze; in this test flies learn to associate an odorant with a shock and therefore avoid that odorant. To date there has been one mushroom body model of tauopathy in *Drosophila,* which found that there was a decrease in learning performance of approximately 20% in tauopathy flies compared to control flies (Mershin et al., 2004).This functional deficit developed even when tauopathy was induced after nervous system development (Mershin et al., 2004).

In pan-neuronal tauopathy models, imaging studies indicate that there are large scale changes to the morphology of the mushroom body, such as enlargement of the axon bundles and in some cases only partial development of the structure (Beharry, Alaniz, & Alonso Adel, 2013; Kosmidis, Grammenoudi, Papanikolopoulou, & Skoulakis, 2010).

Other common measures of tauopathy phenotype, as mentioned above, are climbing performance and survival. These features have not been fully tested in a mushroom body specific model of tauopathy. Research in a *Drosophila* model of Parkinson's disease suggests that there is important connectivity between the mushroom body and areas of motor planning (Riemensperger et al., 2013). In this research they found that the inhibiting different mushroom body lobes had opposing effects on motor performance.

In the literature it has been commented that the mushroom body is not vital for survival (Mershin et al., 2004). One study using mushroom body specific tauopathy flies found no difference in survival; however flies were only tracked to 30 days, which is approximately half their normal lifespan (Mershin et al., 2004). Studying the effect of tauopathy in the mushroom body allows us to characterise how these neurons, which seem to be most vulnerable in AD, are specifically affected.

Chromatin state changes in Drosophila tauopathy models

Beyond the phenotypic changes it is important to investigate how gene regulation changes after disease onset. Human studies indicate that there are changes in gene regulation in AD (Klein et al., 2018). Preliminary studies in *Drosophila* have found that there are also changes in gene regulation in tauopathy models. One study by Frost et al. (2014) indicated that there is a general relaxation of chromatin, which caused an increase in gene expression. Their research observed chromatin relaxation in *Drosophila* and mouse models, as well as human cases; however only one chromatin marker was investigated (Frost et al., 2014). In profiling only one marker, the broad picture of changes in the genome can be missed.

To accurately identify changes in chromatin state, several markers must be analysed across the genome (Delandre & Marshall, 2019; Ernst & Kellis, 2010). Probability models can then be used to predict the chromatin states across the entire genome according to the combined presence of several markers (Marshall & Brand, 2017). *Drosophila* provide a

unique system for chromatin profiling in a holistic manner, which allows predictions to be made states transitions. Studying how chromatin states differ in a tauopathy model compared to healthy brains, specifically looking at the neurons involved in learning and memory, is important for understanding the mechanisms of neurodegeneration, and therefore developing early diagnosis tests and interventions (Qureshi & Mehler, 2015).

The current project

Aim 1: Verifying the behavioural features of a pan-neuronal tauopathy model

Based on previous literature it was expected that tauopathy flies would have a decrease in motor performance and lifespan in comparison to control flies.

Aim 2: Investigating the behavioural and morphological features of two (wild type and mutant human tau) mushroom body specific tauopathy models

Based on pan-neuronal models of tauopathy, it was hypothesised that there would be a stronger disease phenotype for the mutant human tau model than the wild type human tau model. In light of limited evidence it was expected that there would be no effect of mushroom body tauopathy on survival and a small decrease in motor performance of tauopathy flies. Based on mushroom body specific and pan-neuronal tauopathy models it was hypothesised that tauopathy flies would perform worse in a learning and memory task and have abnormal mushroom body morphology compared to controls

Aim 3: Investigating epigenetic changes in a mushroom body tauopathy model

Based on previous literature it was hypothesised that there would be a change in chromatin states of some of the genome. Due to time constraints and unexpected genetic issues with the flies, this aim was not fully completed.

Methods

Drosophila Populations

Fly populations were created from crosses of existing stocks with a standard background (w^{1118}). This means that the variability seen in the models can be solely attributed

to the genes of interest and not a systematic variation due to random mutations in other genes. *Drosophila* were raised on standard food at 25^{o}C, the optimal growing temperature for flies. Detailed crossing schemes can be seen in Appendix A.

Design

Aim 1: Verifying a pan-neuronal tauopathy model

The first aim was to confirm the characteristics of a pan-neuronal tauopathy model. It used a one-way independent samples design with genotype (control or tauopathy) as the independent variable expressed in all neurons. Tauopathy phenotype was measured by two dependent variables: lifespan survival probability and climbing performance.

Aim 2: Characterising a mushroom body specific tauopathy model

The second aim was to investigate and characterise a novel mushroom body specific tauopathy model. For this aim the factor genotype had three levels: control, wild type human tau and mutant human tau (R406W). I used a mushroom body specific driver which meant that each of these genotypes was expressed only in the mushroom body neurons. There were four dependent measures of phenotype: lifespan survival probability, climbing performance, learning and memory performance, and brain morphology.

Aim: Investigating epigenetic changes in a mushroom body tauopathy model

The third aim was to investigate the epigenetic changes that occur in mushroom body neurons during tauopathy. For this aim, genotype had two main levels: control and wild type human tau. Once again a mushroom body specific driver was used to limit the expression of each genotype to mushroom body neurons only. For each level, genotype was further split into eight levels, corresponding to the eight chromatin markers to be profiled. The dependent variable was the binding profile of the chromatin markers across the genome. These chromatin marker binding profiles could then be combined for each of the main genotypes to create a model of the overall chromatin states.

Procedures

Climbing Assays

Climbing assays are used to determine the motor ability of flies and are conducted with adult flies across the lifespan. When flies are tapped to the bottom of a vial, as an innate startle response they climb to the top. The height they are able to climbing in 10 seconds is used to measure motor ability of flies. For the current experiments a cohort of flies were collected for each genotype over a 24 hour period to ensure a similar age. Flies were housed in vials with approximately ten flies per vial. For the experiments with a pan-neuronal tauopathy model, there were 30 control and 21 tauopathy flies. The lower number of tauopathy flies was due to the low number of flies that reached adulthood within the 24 hour period, likely due to the pan-neuronal tau expression. For the mushroom body specific experiment there were 40 flies for each genotype.

Climbing assays were performed with modifications to established procedure (Mudher et al., 2004). Flies were transferred into empty vials thirty minutes before the experiment to allow for environmental habituation. During the procedure a set of five or six vials were tested at the same time, as seen in Figure 5. Flies were tapped to the bottom of the vials and allowed to climb up for one minute. This was repeated three times for each set of flies at each time point. The climbing assays were recorded on video, and flies were scored as being in the bottom, middle, or top third of the vial, as seen by lines behind the vials at ten seconds after being tapped down.

Figure 5. Scoring for climbing performance index.

Drosophila climbing performance was manually scored as per the sections indicated above (top, middle and bottom). Fly position was counted as the height climbed in the first ten seconds.

Due to the effect of time of day on *Drosophila* behaviour, all experiments were started between 8:05 and 8:25am. Motor assays were conducted twice a week in the first few weeks, and then once a week after climbing performance decreased. This was done to ensure sensitivity to changes at the start of the lifespan. Experiments were conducted until climbing performance reached approximately zero. All vials were given a random identification number until raw scoring was complete to ensure no experimenter bias was introduced.

After scoring, the counts were converted into a climbing performance index with weightings as indicated:

$$Performance\ Index = \frac{0.5 \times (no.\ in\ middle\ section) + 1 \times (no.\ in\ top\ section)}{no.\ of\ flies\ in\ vial\ \ at\ time\ 0} \times 100$$

The number of flies originally in the vial was used as the denominator to control for differential attrition. The average climbing performance index was taken for each vial at each time point. Data were analysed using a general linear model with genotype as a between

subjects factor and time as a covariate. In the literature it is common to use a two-way ANOVA with time as a factor (Ali et al., 2012). The use of time as a covariate rather than a discrete factor increases the power of the analysis and acknowledges that I sampled different time points across a continuous scale.

Lifespan analysis

Lifespan assays can be used to determine whether an experimental manipulation changes the median lifespan, or distribution of deaths in a population. The same cohort of flies was used for climbing and lifespan experiments. Fly deaths were recorded one to three times a week. Survival data were collected until the death of all flies.

The Kaplan-Meier survival analysis was conducted using the statistical package R (R Development Core Team, 2011) to determine the probability of *Drosophila* survival over time. Kaplan-Meier survival analysis is well established in the field, and it is used to statistically account for the dropouts in an experiment. In this context, several flies were lost during experimental procedure. By using this analysis, the length of lifespan for these flies can contribute useful information to the analysis without knowing the exact date of death.

Learning and Memory Assay

An associative learning task, as shown in Figure 6, was used to determine if there were specific deficits in mushroom body functioning. Flies were collected over two consecutive 24 hour periods and tested at three to five days. I used young flies as the preliminary imaging results indicated changes to mushroom body morphology by three days. T maze learning and memory assays were conducted according to a standard protocol (Tully & Quinn, 1985). Flies were sorted prior to the experimental day to prevent CO_2 exposure on the day of testing, as this has been shown to affect fly behaviour (Bartholomew, Burdett, VandenBrooks, Quinlan, & Call, 2015). For the training, flies were first transferred into the T maze and given 90 seconds to habituate to the environment. They were then were exposed to

an odorant through a vacuum at the same time as brief shocks were administered at consistent intervals over one minute. After a thirty second rest period, they were exposed to a second odorant without shock. Flies were then transferred into the testing part of the T maze, where they chose between the two odorants, one on either side of the maze. After two minutes flies were blocked from being able to move between the two vials. The numbers of flies in each side were then counted. Consistent with the literature, I defined flies to have learnt if they avoided the shock associated odorant. During the procedure some flies were lost. Each time a group of flies did not make a choice and were left in the middle of the maze. These numbers were recorded and checked for consistency.

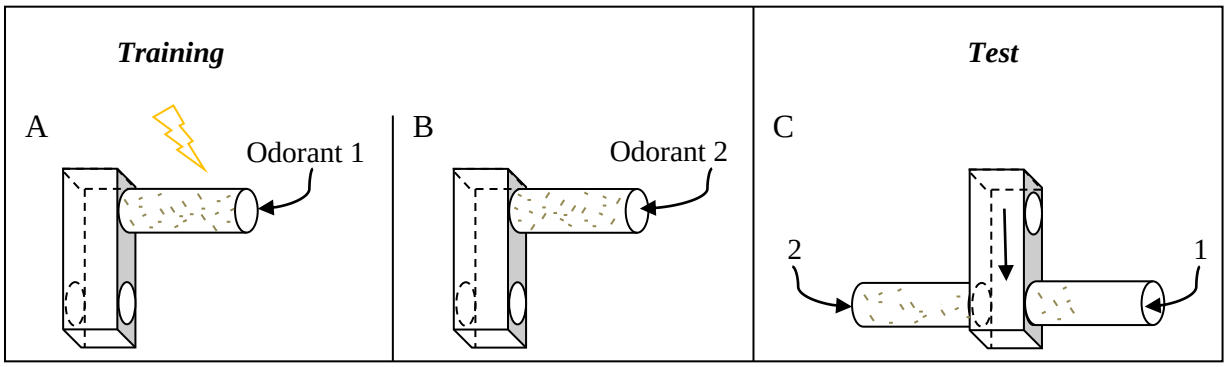

Figure 6. Phases of the T maze procedure.
In the training phase, flies were first exposed to an odorant paired with a shock (A). Second they were exposed to a different odorant without a shock (B). Flies were then transported to a choice point, where they could fly towards either of the odorants they were originally exposed to. Learning was defined as a fly avoiding the shock-paired odorant.

For each data point the experiment was completed with two cohorts of 100 flies of the same genotype. Alternate odorants were paired with the shock for the two cohorts to eliminate a potential confound of fly preference for one odorant. Two aversive odorants were used, octanol (10 µl to 10ml of mineral oil) and methylcyclohexanol (MCH, 13 µl to 10ml of mineral oil). To decrease the effect of room cues on behaviour the training was completed under red light (not visible to *Drosophila*) and the test was completed in the dark.

The learning index of each group of flies was calculated using the following formula:

$$Learning\ Index\ =$$

$$\frac{(no.\ flies\ avoiding\ shock\ pair\ odorant) - (no.\ flies\ on\ the\ side\ of\ the\ shock\ paired\ odorant)}{Total\ number\ of\ flies\ that\ made\ a\ choice.}$$

This resulted in a learning index for each genotype, for each odorant shock pairing at all time points. The two corresponding learning indices for an octanol and MCH shock pairing respectively were then averaged, creating an overall learning index for that session. A one-way, between subjects ANOVA was used to determine whether the learning indices differed between the three genotypes. Using effect sizes derived from existing literature, a power analysis indicated that 12 observations per group were required to achieve a power of .8. Unfortunately, insufficient numbers of flies eclosed to meet this target and two data points were excluded from the analysis due to less than 50 flies making a choice in at least one of the corresponding trials. This resulted in 10 learning indices for both the control and wild type tauopathy flies and 8 for the mutant tauopathy flies and a power of approximately 0.73.

Neuroimaging

Fly brains were examined to determine whether there was an overall change to mushroom body morphology in response to tauopathy. I was able to visualise the mushroom body using a fly line that expressed membrane-bound GFP specifically in the mushroom body neurons. As the GFP was targeted to the cell membrane the dendrites, axons and membrane around the cell bodies could be visualised.

Brains of both experimental and control flies were dissected and fixed as per previously described protocol (Wu & Luo, 2006). The α and β lobes of the mushroom body were stained using an antibody associated with the membrane protein Fasciclin 2. This allowed us to determine whether any absence of GFP was a result of there being no mushroom body axons, or a lack of gene expression. Imaging was conducted using an Olympus confocal FV3000 inverted microscope with a 60x lens under oil. Images of the

calyx were taken at a z stack slice size of 0.5 µm and images of the lobes were taken at a z stack slice size of 2.0 µm.

Three to eight brains per genotype were dissected approximately weekly from three to 62 days resulting in eight time points. Images from five time points were visually inspected for changes in cell body and lobe morphology. A quantitative analysis was done on the cell bodies of three day old fly brains to determine changes in the amount of membrane in these areas by analysing fluorescence intensity. Both cell body areas were examined for each brain resulting in 12, 8 and 6 data points for controls, wild type human tau or mutant human tau respectively. It should be noted that 3 wild type human tau brains had no GFP expression.

In the sample, fluorescence intensity is dependent on both the amount of membrane present and the overall expression of GFP (lower expression will result in a lower fluorescence). For this analysis I was interested in the amount of membrane only, therefore to control for the effects of GFP expression, an automatic threshold (with levels allocated using the kmeans algorithm in icy) was used and example scaled images are presented below in Figure 7. A region of interest that contained cell bodies was selected for each image and measured for fluorescence intensity. Fluorescence intensity values were compared using a one-way ANOVA.

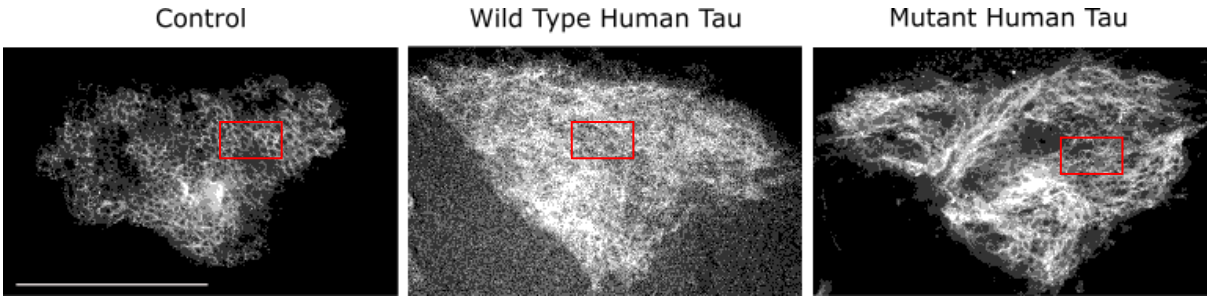

Figure 7. Example images with thresholding of fluorescence intensity.
Scale bar: 100µm.

Chromatin Profiling

I used a chromatin profile to determine whether the deficits in cell functioning of tauopathy flies (seen in aim one and two) were partially due to epigenetic changes in the cells. To determine where chromatin markers were in the genome I used the chromatin TaDa system (Marshall & Brand, 2017). The data from these markers could then be modelled to determine the likely chromatin states across the genome. In this technique it was important to be able to collect data from the specific cells of interest during a single temporal window.

To create spatial specificity, I used a two-part expression system (as described in the introduction). As chromatin states change over the lifespan, it is important to obtain the profile after development for a discrete amount of time. To create temporal specificity, I used a system that is temperature sensitive. As seen in Figure 8 (A), at 18˚C, the system was active, which suppressed the creation of tagged chromatin proteins. Ideally parents would have been crossed at this temperature and progeny kept at 18˚C for one day after eclosion. At 29˚C (Figure 8. B) the system was inactivated and therefore tagged chromatin proteins were created and bound across the genome. After 24 hours at 29˚C flies were snap frozen ready for processing. I was not able to complete the final aim, and therefore the processing and data analysis procedures have not been described.

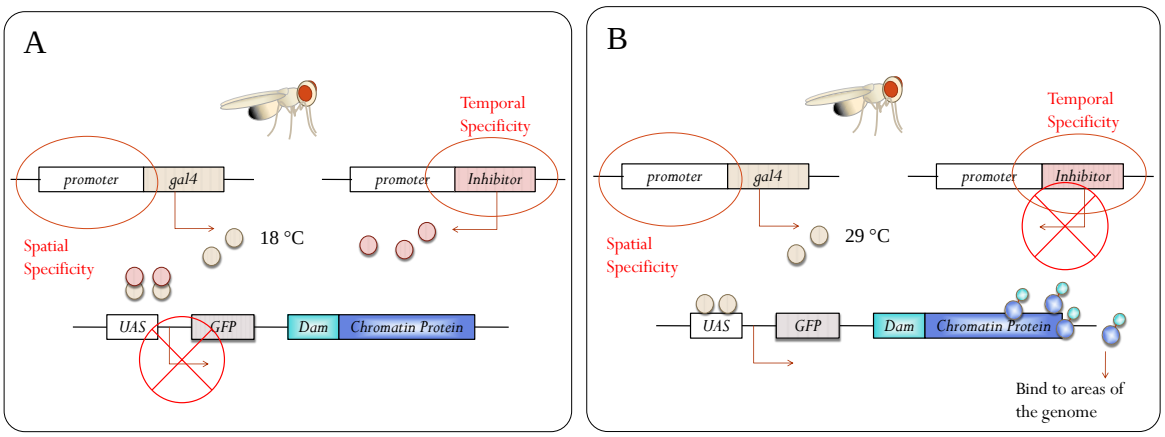

Figure 8. The genetic system to profile chromatin states in *Drosophila*.
At 18°C an inhibitor is active which stops chromatin binding proteins from being transcribed (A). At 29°C the inhibitor is no longer active and the genome can be profiled for a discrete amount of time (B).

Results

Aim 1: Verifying a Pan-neuronal Tauopathy Model

Aim one was to verify the phenotypic characteristics of a pan-neuronal tauopathy model in *Drosophila.* As described above I tested the tauopathy phenotype of these flies using reflexive climbing assays and tracked their survival across the entire lifespan.

Lifespan Analysis

A Kaplan-Meier Survival analysis with a log-rank comparison test indicated that the lifespan of Drosophila (in days) was significantly shorter for flies expressing wild type human tau (M =51.7 days, SE = 5.1), compared to controls (M =71.0 days, SE = 1.1), χ^2 (1) = 4.5, p = .033. As shown in Figure 9, there was a large difference in the median lifespan of tauopathy flies compared to controls. There was also higher variability in the 95% confidence intervals around the median lifespan for tauopathy flies at 59 days, 95%CI [35, 71], compared to 71 days, 95%CI [71, 74], for the controls. This provides support for my hypothesis that pan-neuronal tauopathy decreases lifespan.

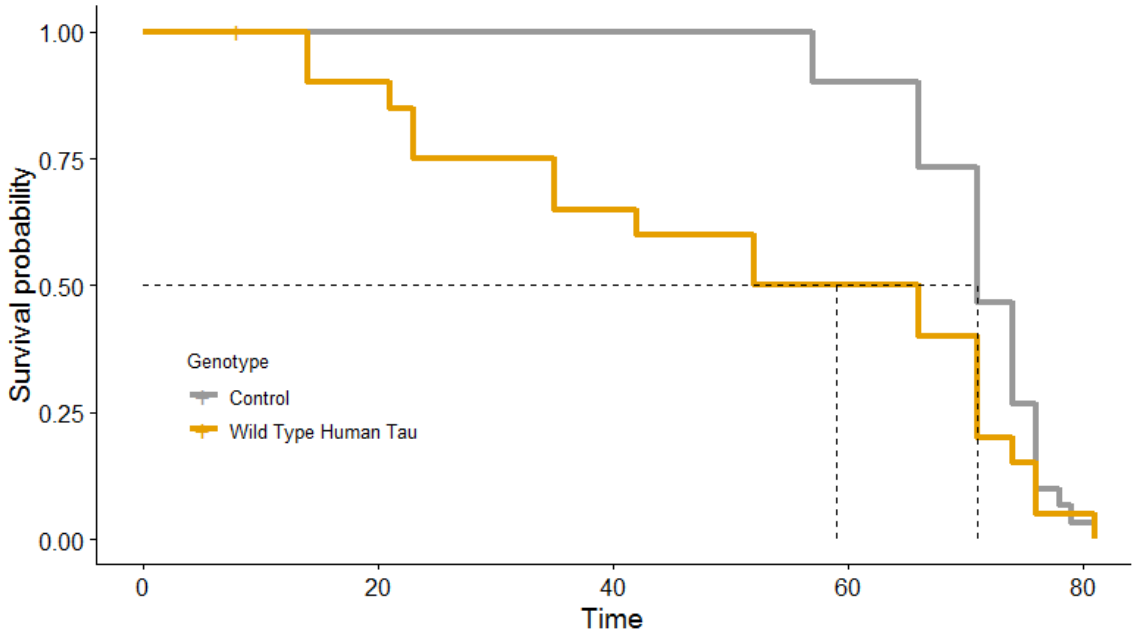

Figure 9. Survival of pan-neuronal tauopathy flies.
Flies with a tauopathy genotype (yellow) had a significantly shorter lifespan compared to controls (grey), p =.033. Median survival shown by dotted lines for tauopathy (59 days) and controls (71 days).

It was also noted that the majority of tauopathy flies eclosed later than the majority of control flies, suggesting a developmental delay. This finding however, was not directly measured.

Climbing Performance

A Shapiro-Wilk test of normality indicated that the climbing performance index scores significantly deviated from normal for both tauopathy and control samples, *skew* = 1.19, *kurtosis* = 0.97, p = .005 and *skew* = -0.25, *kurtosis* = -1.55, p = .003, respectively. This was expected as proportion data does not fit a normal distribution. Several data transformations were tried but did not improve data normality (p< .05 for control genotype for all transformations). In light of the low magnitude of normality deviations and logic of interpretation, raw data was analysed without transformation. A general linear model was used to analyse the data as these models are robust to normality violations; however due to the non-normal data, results should be interpreted with caution.

The general linear model was conducted with a factor of genotype (tauopathy or control) and continuous covariate of time as presented in Figure 10. A Levene's test of homogeneity of residuals was non-significant (p = .956) and inspection of a Q-Q plot and statistical tests indicated that there was normality of residuals (p > .05). The overall model accounted for 80% of the variance in climbing performance index, $F(3, 51) = 68.07$, $p <$ .001, R^2 = .80. As described in the methods, means represent a climbing index from zero to 100, where high scores indicate a stronger climbing reflex and a score of 100 means that all flies climbed to the top third of the vial.

There was a main effect of genotype such that climbing performance was poorer in tauopathy flies (M = 22.3, SD = 22.8) compared to controls (M = 46.9, SD = 30.2), $t(51) = 6.50$, β = .82, $p <$ .001. As part of the overall model, genotype accounted for a large proportion of the variance, η_p^2= .453. This supports my hypothesis that *Drosophila* climbing ability would be negatively affected by tauopathy.

Drosophila climbing performance also decreased over time, $t(51) = -11.72$, β = -.75, $p <$ .001. This accounted for a large amount of the variance, η_p^2= .729. There was an interaction effect such that the difference in climbing performance between each genotype decreased over time, $t(51) = -2.52$, β = -.32, p = .015, which accounted for a small amount of the variance, η_p^2= .11. A test of simple effects indicated that there was a significantly larger difference in performance index between the genotypes at earlier time points compared to later time points. Differences in fly climbing performance were 34.2 at nine days and 15.0 at 34 days, with control flies consistently higher than tauopathy flies. It should be noted that the tauopathy flies reached minimum performance by approximately 25 days.

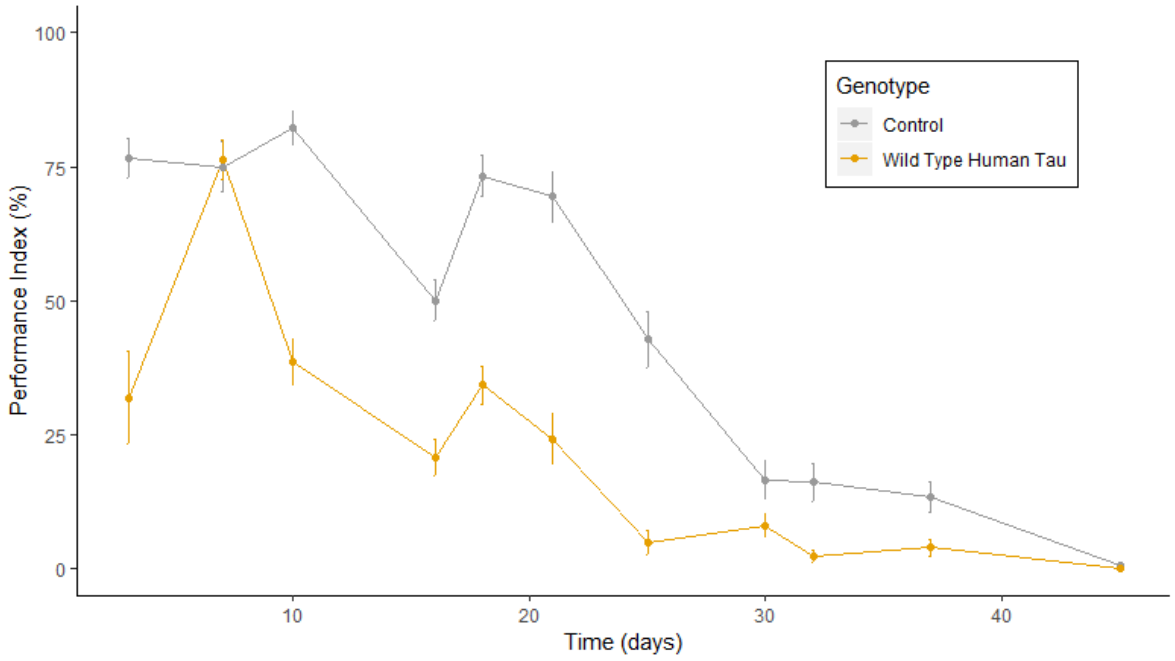

Figure 10. Climbing performance of pan-neuronal tauopathy flies
Tauopathy flies (yellow) had a decreased motor ability from three days old compared to controls (grey), $p < .001$. Error bars indicate standard error of the mean for each time point. A score of 100 indicates that all flies reached the top third of the vial for all trials at that time point.

Aim 2: Characterising a Mushroom Body Specific Tauopathy Model

In aim 2 I characterised a novel mushroom body specific model using both a wild type human tau and a mutant human tau expressing fly population. Flies from these two experimental populations, along with controls, were tested for average lifespan, climbing ability, learning and memory performance and mushroom body brain morphology.

Lifespan analysis

A Kaplan-Meier survival analysis indicated that there was a significant difference in the lifespan of flies (in days) depending on the genotype, $\chi^2(2) = 19.1$, $p < .001$. A set of pair-wise comparisons indicated that the lifespan was significantly shorter for both wild type human tau (*M* =59.5, *SE* = 1.8, *Median* = 64, 95% CI [59, 64]) and mutant human tau (*M* = 61.9, *SE* = 2.2, *Median* = 64, 95% CI [59, 64]) flies compared to controls (*M* =66.7, *SD* = 1.7, *Median* = 73, 95% CI[73, 73]), $p < .001$ and $p = .022$ respectively. The lifespan of flies

in the two tauopathy conditions was also significantly different, such that wild type human tau flies had the worst survival probability, $p = .044$. This was surprising given that I hypothesised there would be no difference in the survival of tauopathy compared to control flies. This is represented in Figure 11 below.

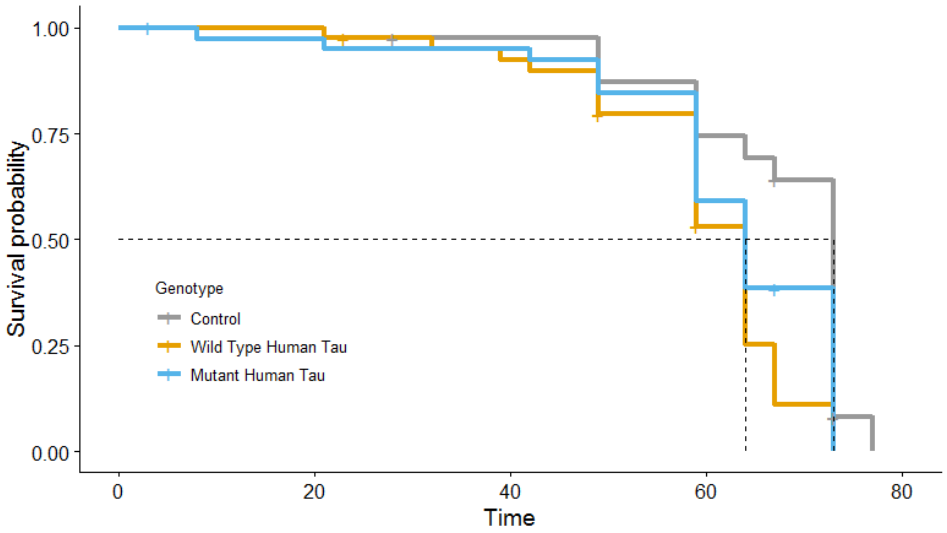

Figure 11. Lifespan of mushroom body specific tauopathy flies
Flies with a tauopathy genotype (yellow and blue) had a significantly shorter lifespan compared to controls (grey), $p < .05$ for wild type and $p < .001$ for mutant human tau respectively. There was also a significant difference in lifespan depending on the type of tauopathy, with wild type human tau flies having a lower average lifespan than mutant human tau flies, $p < .05$. Dotted lines represent median lifespan.

Climbing performance

A general linear model was conducted with genotype (wild type tauopathy, mutant tauopathy or control) as a factor and time as a covariate, as seen in Figure 12. The performance index data for the control group was significantly different from normal, $p = .004$ and kurtosis was around 1 for all genotypes. As previously discussed, this analysis is robust to normality violations, however results should be interpreted with caution. A test for normality residuals and inspection of a Q-Q plot were checked ($p > .05$). There was a violation of homogeneity of residual variances ($p < .001$) however, the ratio of the smallest

to largest variance did not exceed 4, therefore the effect size was small (Tabachnick & Fidell, 2013).

The overall model accounted for a large proportion of the overall variance, $F(5, 150) = 198.9$, $p < .001$, $R^2 = .864$. There was a moderate effect of genotype on climbing performance, $F(2, 150) = 5.25$, $p = .006$, $\eta_p^2 = .065$. Surprisingly post hoc tests indicated that climbing performance was significantly better for flies with a mutant human tau ($M = 47.0$, $SD = 26.5$) and wild type human tau ($M = 45.0$, $SD = 28.1$) compared to controls ($M = 40.4$, $SD = 30.4$), $t(150) = -3.17$, $p = .002$, $d = 0.23$, and $t(150) = -2.18$, $p = .032$, $d = 0.16$, respectively. There was no difference between the two tauopathy genotypes. No adjustment was done for the three way comparison as it is not considered necessary when the overall model is significant (Howell, 2013).

There was a significant effect of time, such that fly performance decreased over time, $t(150) = -30.62$, $\beta = -.92$, $p < .001$. There was no interaction between genotype and time.

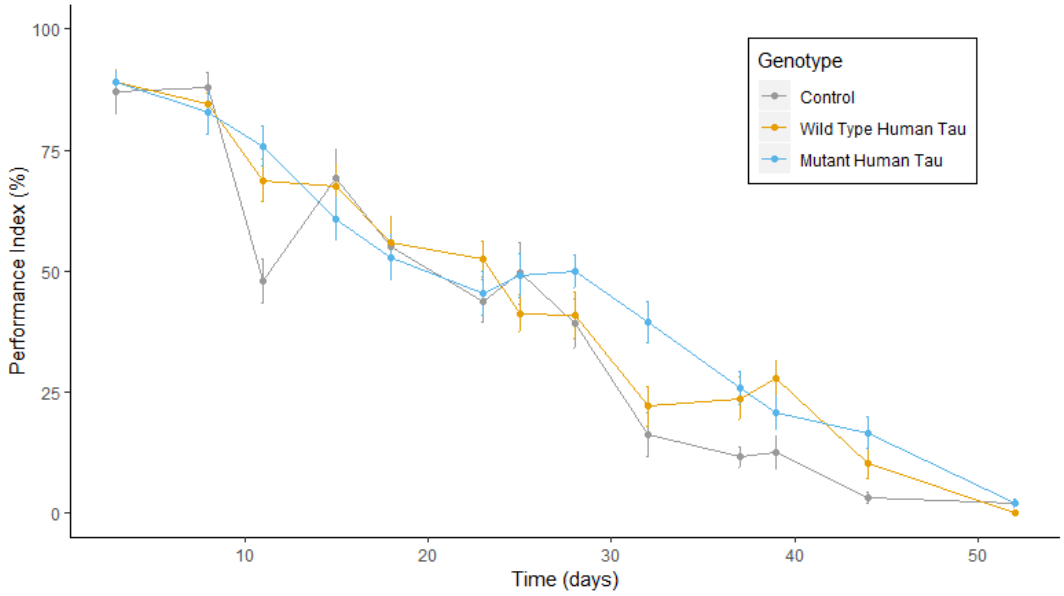

Figure 12. Climbing performance of mushroom body tauopathy flies.
Tauopathy flies (yellow and blue) had an increase in motor ability from three days old compared to controls (grey), p <.05 for both. Error bars indicate standard error for each time point. A score of 100 indicates that all flies reached the top third of the vial for all repeats.

Learning performance

Flies were tested on a shock-odorant associative learning assay. A Shapiro-Wilk test of normality indicated that the data was normal for all genotypes, all p > .05. There were minor deviations of normality for skew and kurtosis of both wild type (*skew* = 0.97, *kurtosis* = 0.57) and mutant (*skew* = - 0.83, *kurtosis* = - 0. 23) tauopathies. However, due to the small number of data points (n= 10, 10 and 8), this is not unexpected. Histograms are provided in Appendix B. Learning index is a value between -1 and 1. A value of 1 indicates that all flies avoided the shock-paired odorant and a value of -1 indicates that all flies went towards the shock paired odorant. A value of 0 indicates that there were equal numbers of flies for each odorant.

A one-way, between subjects ANOVA indicated that there were significant differences in learning index depending on the genotype, $F(2, 25) = 3.95$, $p = .032$, $\eta^2 = .24$. A Levene's test was non-significant indicating there was homogeneity of variance. Pair-wise

comparison tests indicated that memory performance was lower for both wild type (M = 0.11, SD = 0.07) and mutant (M = 0.11, SD = 0.10) tauopathies compared to controls (M = 0.20, SD = 0.09), $t(25) = -2.44$, $p = .022$, $d = 1.12$ and, $t(25) = -2.37$, $p = .026$, $d = 0.95$ respectively. There was no difference between the two tauopathy genotypes. This is represented in Figure 13 below.

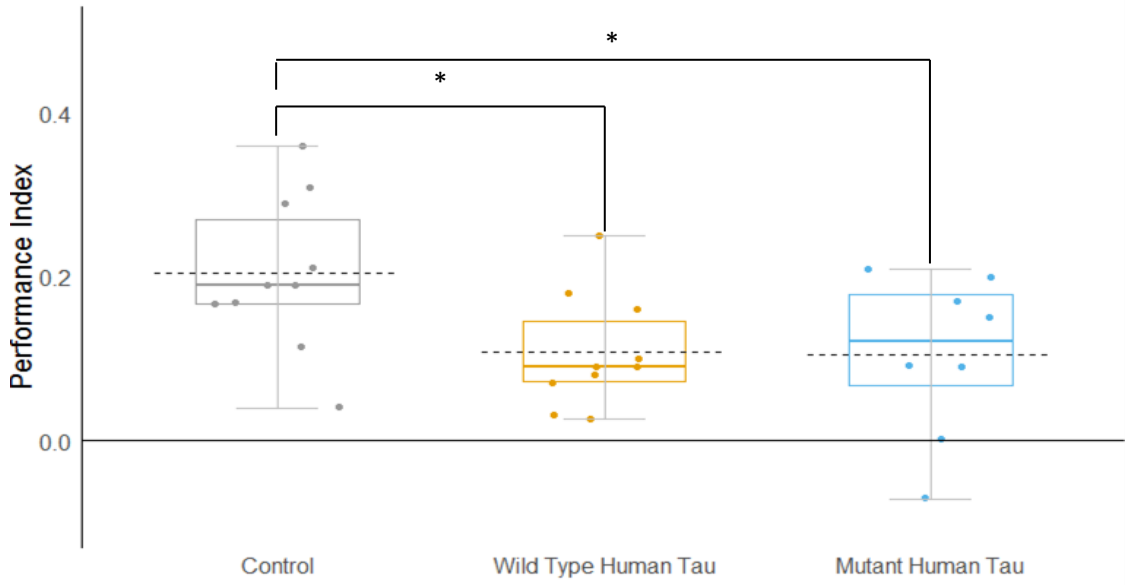

Figure 13. Learning and memory performance of tauopathy flies.
Drosophila with a tauopathy genotype had impaired learning and memory on an associative learning task when compared to controls, indicated by a one-way ANOVA. Dashed lines represent means, *$p < .05$.

Brain Imaging

Brain imaging was done to determine whether there was physical disruption to mushroom body cells in light of the phenotypic differences. The mushroom body cell bodies and axon bundles were examined for differences. For a reference to the different images presented, please see Figure 14 below.

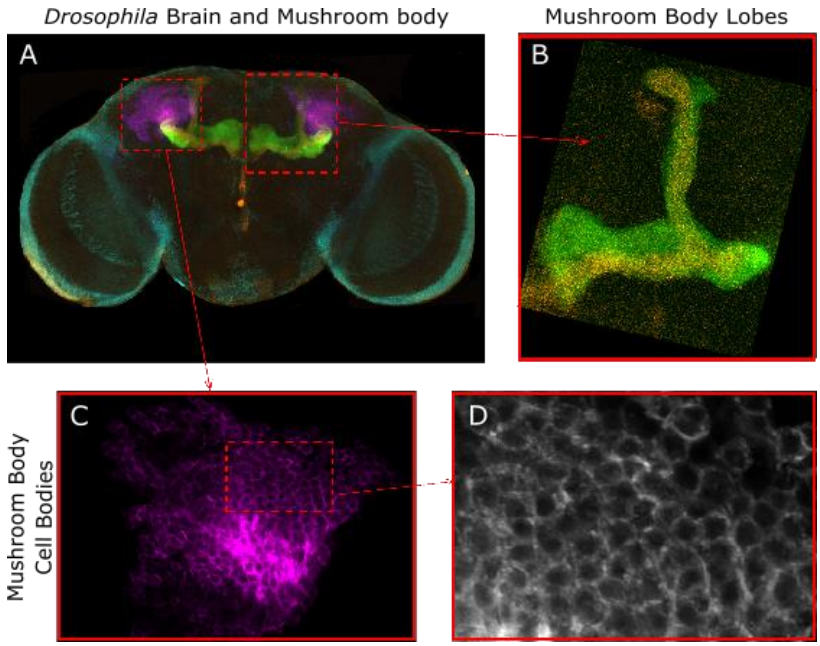

Figure 14. Drosophila brain and mushroom body.
Panel A is an image of the entire fly brain with the mushroom body in green and pink. Panel B is a zoomed in picture of the mushroom body lobes, representative of the images in Figure 15. Panel C is a larger picture of the cell body area of the mushroom body, with a further zoomed in picture in panel D, representative of the images in Figure 17.

Visual inspection of the mushroom body lobes indicated that there were some differences in GFP and Fasciclin 2 expression (representative images in Figure 15 below). The structure of control brain lobes is very uniform, which is not as apparent in tauopathy lobes. Due to time constraints these differences were not quantified, however this analysis can be conducted in the future.

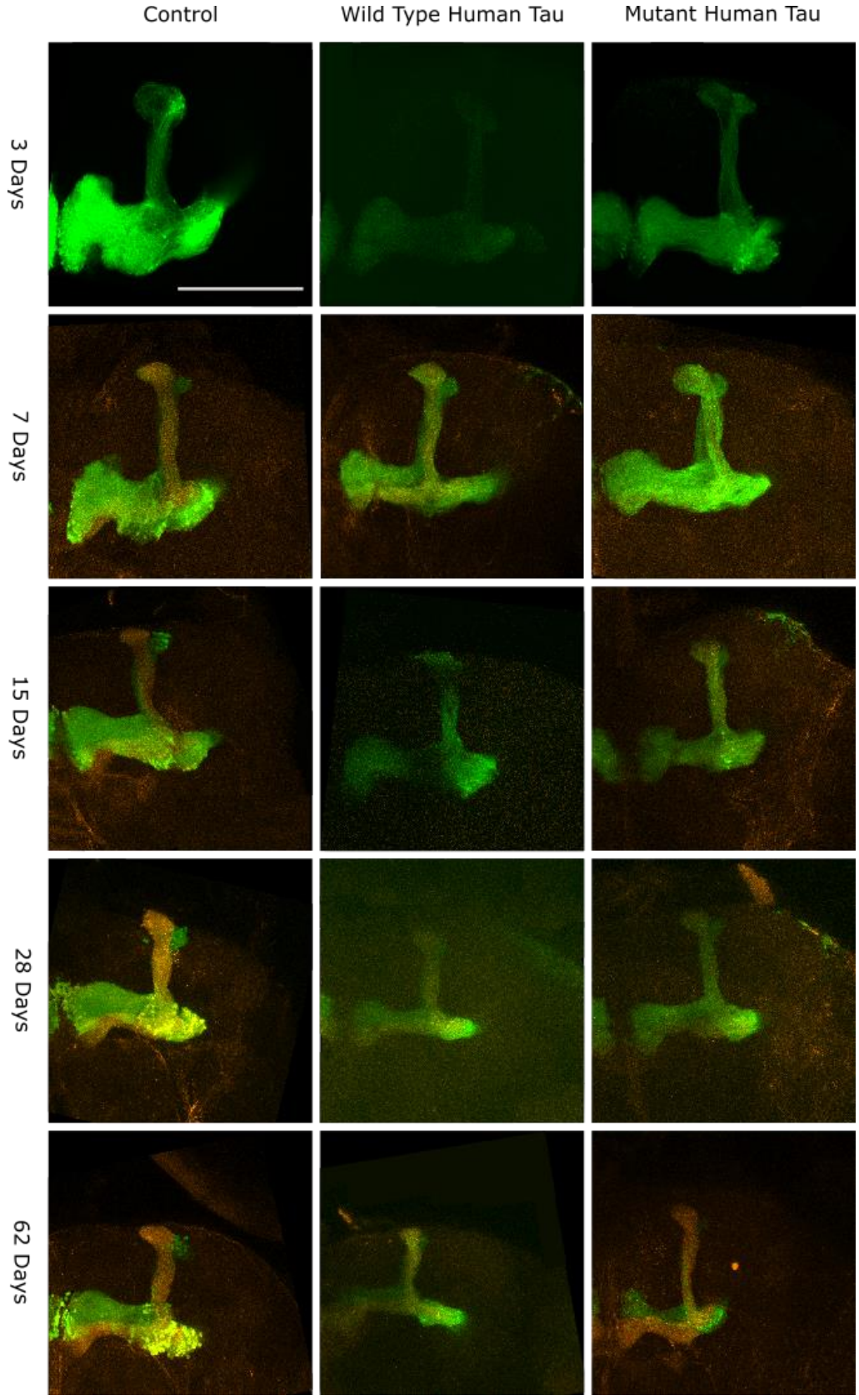

Figure 15. Mushroom body lobes of tauopathy flies compared to controls

There were minimal disruptions to the lobes structure of the mushroom body in tauopathy flies. The expression of GFP however, is generally lower in tauopathy genotypes. Scale bar: 80 µm.

Visual inspection of the membrane around the cell bodies of the mushroom body indicated that there were changes to the overall morphology of this area. From three days old, the cell bodies of the tauopathy models exhibited different morphology to controls. As seen in Figure 17 below, instead of an organised honeycomb type structure, the arrangement of cell bodies was disorganised, and in some cases there was a greater amount of membrane in the cell body areas (shown by thicker areas of GFP). Three dimensional reconstructions of the cell body area (Figure 16) indicated that there were abnormal projections which resembled dendrites surround the cell body area of tauopathy brains. These projections were not present in control brains. A common feature of neuronal degeneration is blebbing (swelling) of the membrane (Ghavami et al., 2014). As represented in Figure 17, approximately half of the mushroom body cell body areas exhibited blebbing by 15 days for the mutant human tau flies and 28 days for the wild type human tau flies which was earlier than the controls.

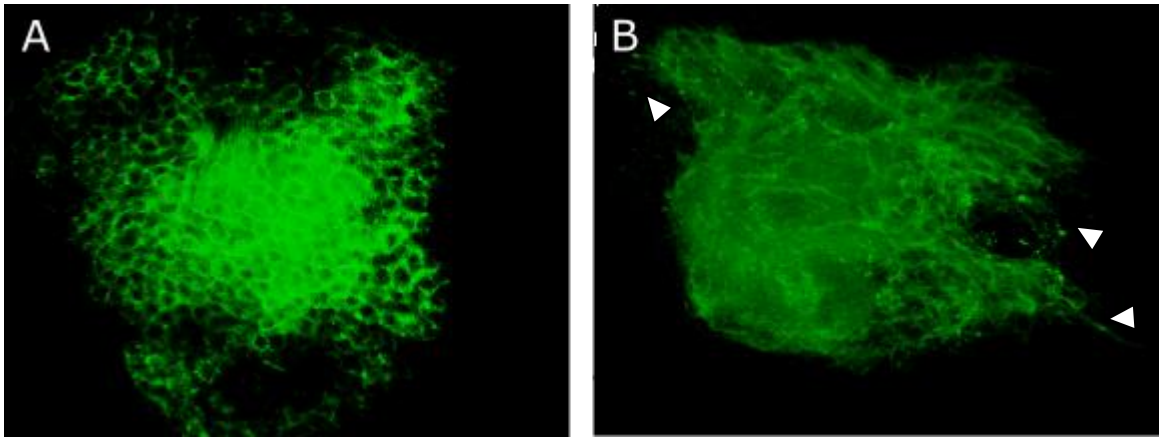

Figure 16. Three dimensional construction of the cell body area of the mushroom bodies The edge of the cell body area was well defined in control fly brains (A), however there were abnormal projections around the cell body area of tauopathy brains (white arrows in B).

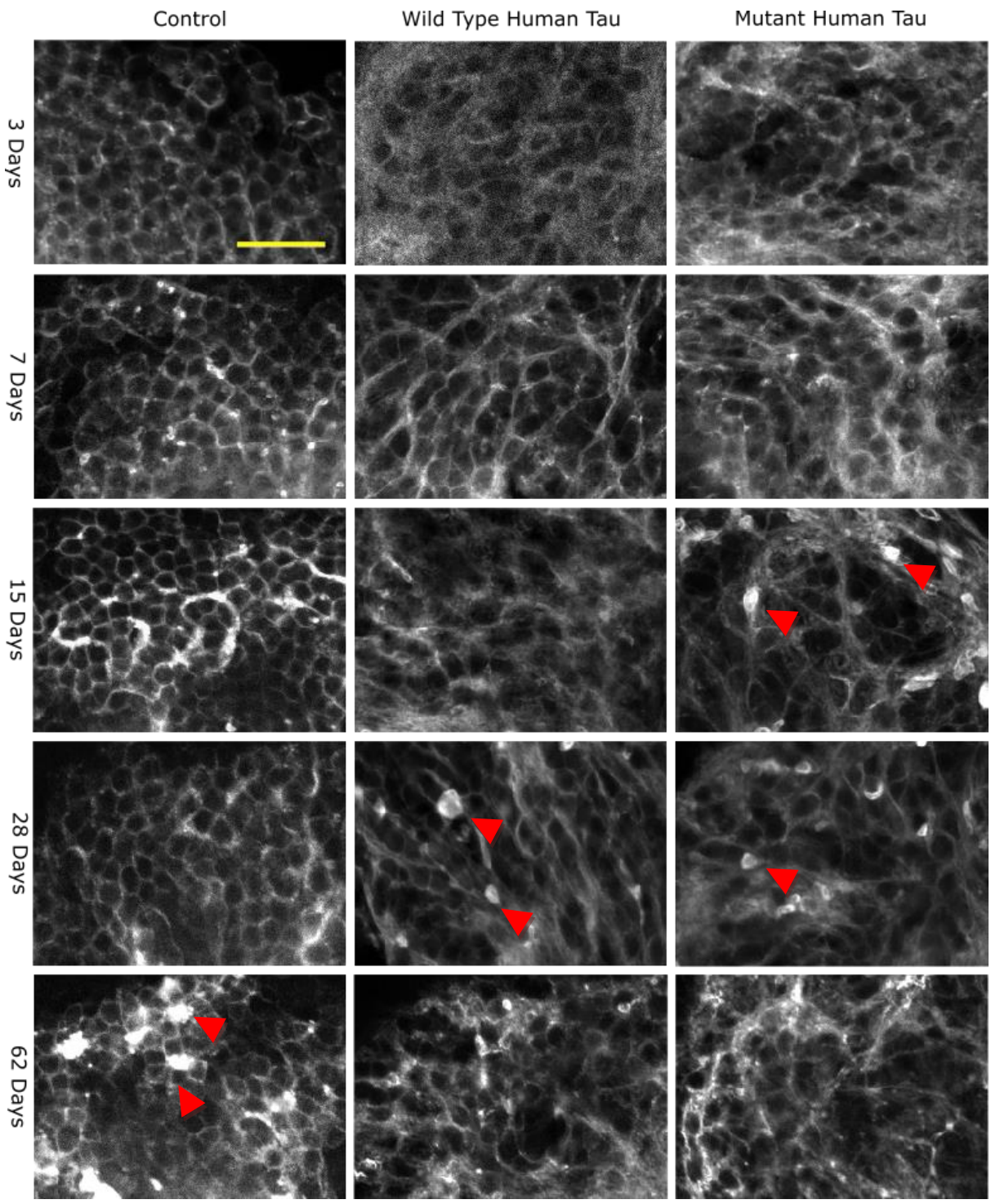

Figure 17. Cell body area of the mushroom body in tauopathy flies compared to controls. The highly organised structure of the cell body neurons in the mushroom body was disrupted in tauopathy. This was seen across the lifespan with irregular cell membranes, an increase in the amount of cell membrane and the appearance of blebbing (red arrows) earlier than in control *Drosophila* brains. The scale bar in the first picture is representative of 15 µm.

A quantitative analysis of amount of cell membrane at three days indicated that there were significant differences between the genotypes, F (2, 23) = 31.8, $p < .001$, $\eta_p^2 = .734$. A Levene's test and inspection of Q-Q plot indicated that there was homogeneity of variance ($p = .180$) and a normal distribution of residuals. As seen in Figure 18, pair-wise comparison tests indicated that there was less membrane present in the images of controls ($M = 2.06$, $SD = 0.22$) compared to wild type and mutant human tau genotypes, t (23) = 7.70, $p < .001$, $d = 3.41$ and t (23) = 4.71, $p < .001$, $d = 2.56$ respectively. The wild type human tau ($M = 3.20$, $SD = 0.42$) had significantly more membrane than the mutant human tau ($M = 2.82$, $SD = 0.36$) in the cell body area, t (23) = 2.15, $p = .043$, $d = 0.98$.

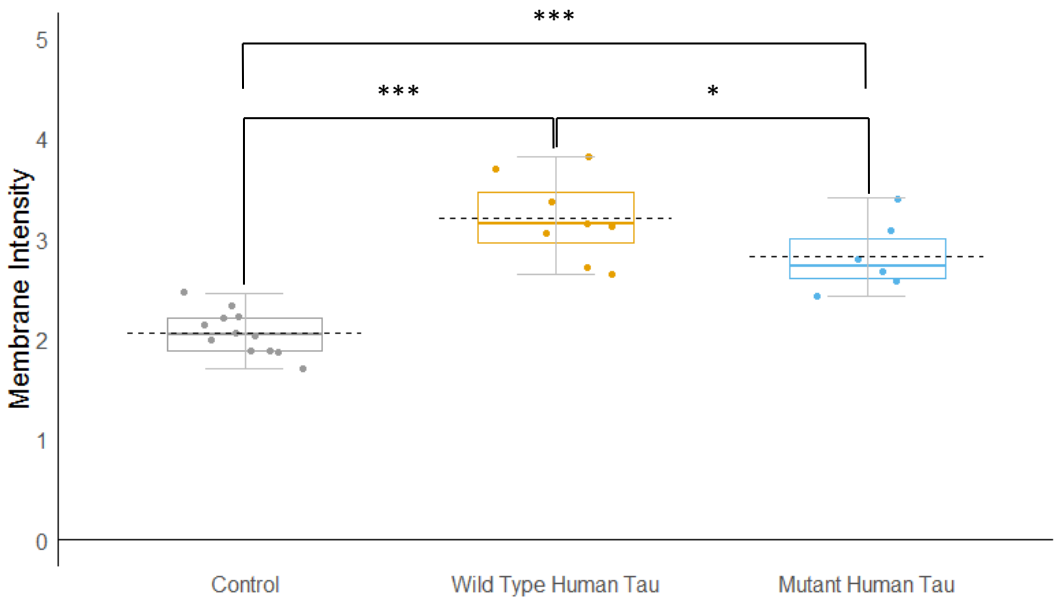

Figure 18. Membrane in the cell bodies of the mushroom body.
The mean light intensity (proportional to amount of membrane) was greater in the cell body regions of the tauopathy flies than controls. Wild type human tau flies had the greatest amount of membrane in the cell body area, * $p < .05$, *** $p < .001$. Means are shown as dotted lines and each dot represents one data point.

As well as the changes in membrane structure, there were differences in GFP intensity. In wild type human tau samples there was less fluorescent intensity. In 30% of these samples no GFP was present. This may indicate that there are changes to gene expression of GFP in the mushroom body of tauopathy models.

Aim 3: Creating a Chromatin Profile of Mushroom Body Tauopathy Flies

Due to time constraint I was not able to collect the data for the final aim. Crosses were schemes were completed and therefore generation of a data set can be easily done in future.

Discussion

The current project verified an established *Drosophila* model of tauopathy and characterised a novel mushroom body specific model. For the pan-neuronal model of tauopathy, as hypothesised there were significant decreases in motor performance and lifespan of tauopathy flies compared to controls. For the mushroom body specific model there were some novel findings. Contrary to my hypothesis, there was a decrease in average lifespan for mushroom body tauopathy flies compared to controls. There were also changes in motor performance; however they were in the opposite direction than expected, such that tauopathy flies had a slightly better climbing performance than controls. My other hypotheses for this aim were supported: tauopathy flies had a decrease in learning performance and abnormal mushroom body morphology. I was not able to test the hypothesis of the third aim, that a mushroom body tauopathy model would have changes in epigenetic expression.

Aim 1: Verifying a Pan-neuronal Tauopathy Model

I was able to verify that pan-neuronal tauopathy in *Drosophila* results in a decrease in climbing ability and survival which supports the consensus in the literature (Gistelinck et al., 2012). Median survival decreased by approximately ten days which is similar to observations of other studies (Wittmann et al., 2001). One observation which has not been reported on in the literature was a delay in eclosion of tauopathy flies compared to controls. In the current experiment the delay was noted but not directly measured, therefore it would be interesting to investigate further.

Tauopathy flies had a decrease in climbing performance compared to controls by three days post-eclosion which suggests that a phenotype develops quickly in the model. This is supported by the literature as decreases in motor ability have been observed in both larvae

and adult flies (Ali et al., 2012; Mudher et al., 2004). An anomaly in my results is that at seven days tauopathy flies climbing performance seemed to return to the same as control flies which had not been previously commented on in the literature. *Drosophila* are unable to regulate their body temperature intrinsically and therefore their motor behaviour is very sensitive to temperature and humidity (Dillon, Wang, Garrity, & Huey, 2009). The climbing assays are also performed manually. It is entirely possible that a random deviation in environment conditions or conducting of the assay caused a specific change in behaviour at that time point.

A more speculative explanation, however, is that the data point represents a real change. In the literature, few time points for tauopathy climbing performance are measured or in some cases reported, which means that I am unable to determine whether other studies have had a similar finding (Ali et al., 2012; Wittmann et al., 2001). The data point could represent a fluctuation in tauopathy symptoms. Interestingly, symptom fluctuation have been observed in people with various neurodegenerative conditions, including AD and other tauopathies (Palop, Chin, & Mucke, 2006; Walker et al., 2000). The high variability over short periods of time suggests that the changes are due to dysfunction of networks, not cell death (Palop et al., 2006). If the fluctuation in my results represents a real fluctuation in cognitive behavioural symptoms, this provides further evidence that tauopathy models in *Drosophila* effectively represent human disease and would allow subsequent investigation of the causes behind these fluctuations in a fly model. Though, it is important to note that further replicate experiments are required to confirm this result.

Aim 2: Characterising a Mushroom Body Specific Tauopathy Model

Wild type compared to mutant human tau

Unexpectedly, the wild type human tau flies had a shorter lifespan and greater deviations in mushroom body morphology than mutant human tau flies. This was surprising

given that in pan-neuronal studies, mutant human tau produces a stronger phenotype when measured by climbing assays and survival (Gistelinck et al., 2012; Wittmann et al., 2001). Interestingly, it has been suggested that wild type and mutant forms of tauopathy have different effects on mushroom body neurons (Kosmidis et al., 2010). Different wild type human tau expressing flies have been found to have greater abnormalities in mushroom body morphology and learning and memory performance compared to controls (Beharry et al., 2013; Kosmidis et al., 2010). This reflects human disease, as in sporadic AD wild type human tau causes dysfunction in the learning and memory neurons first, whereas in familial forms of dementia (where there is a mutant form of human tau) other areas of the brain are affected first. This suggests that there are differential vulnerabilities of different neuronal subtypes in the brain.

Lifespan

This experiment was the first to track survival in mushroom body specific tauopathy across the entire lifespan. The consensus among researchers is that the mushroom body is not essential for survival in *Drosophila* (Mershin et al., 2004). In the primary reference for this, the mushroom body was ablated and flies were able to function normally and there were no effects on viability (i.e., whether they could survive to adulthood) (De Belle & Heisenberg, 1994). The average lifespan of the flies, however, was not tested. Subsequent research has used the primary paper as justification for following fly lifespan for only 30 days in a mushroom body tauopathy model (Mershin et al., 2004). In my experiment, the mushroom body specific tauopathy flies had a significantly shorter lifespan than controls flies.

Interestingly, the mushroom body in *Drosophila* has important roles in sleep and insulin pathways (Takahama et al., 2012). One study found that inhibition of the mushroom body using Shi^{ts} resulted in a decrease in lifespan of flies which was proportional to the decrease in sleep (Joiner, Crocker, White, & Sehgal, 2006).

This is of particular note because sleep disturbance is considered a risk factor for AD in humans (Benedict et al., 2015; Sherman, Mumford, & Schnyer, 2015). In a mouse model sleep disturbance resulted in the suppression of neurogenesis in the hippocampus, which in turn decreased memory formation and changed search strategies in a spatial location (water maze) task (Hairston et al., 2005). These results combined suggest that there is an important relationship between sleep and the functionality of learning and memory neurons. It also points to the plasticity in the learning and memory structures as a reason for the greater vulnerability to dysfunction (Bartsch & Wulff, 2015). To discover whether sleep is a mediator in the relationship between mushroom body disruption and a decrease in lifespan, mutant human tau flies and wild type human tau flies could be tracked for wakefulness at periods across the lifespan and a survival analysis conducted. As established in this study, wild type human tau has a stronger phenotype; therefore I would be able to track if there is a dose-dependent change in sleep in the two groups proportional to lifespan.

Climbing Performance

Surprisingly, given the deleterious effects on survival, morphology and learning and memory, both sets of tauopathy flies had significantly better climbing performance than controls. Mutant human tau *Drosophila* performed best in this test of reflexive motor ability (although not significantly better than wild type human tau flies). This is in contrast to the pan-neuronal tauopathy literature (Ali et al., 2012). Previously only one group has researched the role of the mushroom body in reflexive climbing assays (Riemensperger et al., 2013). When the β' lobe was inhibited, the result was a decrease in motor climbing performance, however when the α / β and γ lobes were inhibited there was a slight increase in climbing performance. My results suggest that disruption to activity in the whole mushroom body results in an increase in climbing performance and adds to the literature suggesting that the mushroom body plays a role in reflexive motor function.

Learning and Memory Performance

As expected, memory performance for tauopathy flies was significantly worse than controls. In the literature one previous study tested learning and memory in a mushroom body model and found that there were significant decreases in learning index (Mershin et al., 2004). It is worth noting that although the driver used in this experiment has the greatest expression in the mushroom body, it is also expressed at low levels throughout the brain which could have confounding effects (Waddell, Armstrong, Kitamoto, Kaiser, & Quinn, 2000). My model is therefore the first to express human tau with high specificity to the mushroom body and demonstrate that learning deficits occur. Inconsistent with the literature, there was no difference between wild type and mutant human tau genotypes in memory performance. In my experiments, although many flies were used to create each performance index, there were a maximum of 10 data points for each condition in the statistical analysis. Due to the genetic background, the learning index of control flies was very small in comparison to the literature, possibly resulting in a floor effect (De Belle & Heisenberg, 1994).For these reasons my experiment might not have had the power to detect a small difference, and in future higher powered experiments using flies with a background for learning and memory assays (canton-s) should be conducted to confirm my results.

Imaging: Mushroom body morphology

Detailed mushroom body imaging had not previously been completed in a mushroom body tauopathy model. In the mushroom body images there was evidence of neurodegeneration earlier in the tauopathy flies than controls. Blebbing (swelling or bursting of the membrane seen as bright spots on images) was seen in the cell body area of the mushroom body in tauopathy flies by 15 to 28 days and is a marker of cell death in neurodegenerative disease (Ghavami et al., 2014). Quantitative markers of neurodegeneration were not measured.

More interestingly, my imaging results suggest that there was a change in the overall structure of the mushroom body by three days post-eclosion. Previous literature has looked at overall morphology and the presence of the mushroom body lobes however, none of these experiments looked at morphology of the cell body area (Beharry et al., 2013; Kosmidis et al., 2010; Mershin et al., 2004). Inspection of the images suggests there is an increase in the amount of cell membrane, and there are also abnormal projections that resemble dendrites surrounding the cell bodies, in tauopathy but not control brains.

If the increase in membrane is representative of abnormal dendrites around the cell bodies, a speculative explanation is that the formation or pruning of dendrites is dysfunctional in the tauopathy model. In the *Drosophila* mushroom body, during development neurons which make up the γ lobe send out an initial set of dendrites (Yu & Schuldiner, 2014). These dendrites are completely pruned before the cell develops into a mature neuron and sends out new processes. For dendrite pruning to occur the microtubules forming the structural basis of a dendrite must be destabilised and de-assembled. In both mammals and *Drosophila* kinase PAR-1 promotes dendritic pruning (Herzmann, Krumkamp, Rode, Kintrup, & Rumpf, 2017). In *Drosophila,* PAR-1 acts to inhibit tau binding to microtubules by phosphorylation, which promotes the breaking down of dendrites. A model with decreased tau was also able to rescue some of the pruning deficits in a PAR-1 mutant (Herzmann et al., 2017). This suggests that in my model endogenous PAR-1 may have been insufficient to stop tau binding to microtubules in the dendrites, due to the increase in the amount of tau. As a result, there may have been a lack of dendrite pruning, decreasing the adaptability of the cell. Further research may be able to answer this question by measuring pruning factors present in the mushroom body when pruning should be occurring.

Imaging: GFP expression in the mushroom body

In the wild type human tauopathy flies approximately one third of the brains had no GFP expression. In these brains, Fasiciclin 2 staining (an antibody to visualise the α / β lobes of the mushroom body) confirmed that mushroom body structures were present. It is possible that these flies were lacking the gene for GFP expression in the first place, although flies were carefully crossed and sorted so this is unlikely. There was also a noticeable decrease in GFP expression in some tauopathy brains without complete absence. This suggests that there was a genuine difference in GFP expression of tauopathy flies brains with variable penetrance. Further analysis is required to quantify these changes, but it suggests that in the tauopathy conditions there was a change in gene expression of GFP. If there may have been a change in GFP expression, it is possible that in these models there are epigenetic differences compared to controls. Excitingly, this is evidence that there may be chromatin state changes in my model of tauopathy (even though I was unable to directly measure them), and warrants future chromatin state investigation.

Limitations

Due to time constraints I was not able to complete aim three and directly measure changes in gene expression. However, the change in GFP intensity with a tauopathy genotype that I observed further strengthens the rationale behind completing a chromatin profile of the mushroom body of tauopathy flies. The lab is currently working to create optimal fly stocks for these crosses. Once this is accomplished, I will be able to complete a chromatin profile of mushroom body tauopathy flies and compare that to controls.

An interesting consideration I did not test is that as *Drosophila* in the current experiment aged, human tau may not have been confined to the mushroom body. In human Alzheimer's disease, tau pathology is first found in the entorhinal cortex and the hippocampus, but then other more areas of the brain as person ages (Pooler et al., 2013). In a

mouse model, human tau expression which is localised in the entorhinal cortex results in pathological human tau protein appearing in connected areas of the brain such as the anterior cingulated cortex and limbic system, in an age dependant manner (de Calignon et al., 2012). In my model spreading of tau pathology may have further decreased lifespan. Future experiments could test this theory by staining the whole brain with and anti body specifically for human tau (and not *Drosophila* tau) in old flies. If the human tauopathy has spread, the stain will not be confined to the mushroom body. I also did not measure hyper phosphorylation of human tau or cell death directly. These are some important features of human AD. In future studies these features should be measured to verify the bounds and further areas of study in the model as a comparison to human disease.

Future Directions

The ultimate goal of disease-based research is to understand the mechanisms in order to identify novel therapeutic targets. As previously discussed, completing a full chromatin profile will help us to understand how tau affects gene expression. Therefore, the next stage of this research will be to complete the chromatin profile. A recent review, which further strengthens my rationale, indicated that tau may have an important role in the nucleus (Bukar Maina, Al-Hilaly, & Serpell, 2016). Tau is localised in nucleus during cell division, but also in mature neurons. Tau acts to stabilise DNA and promotes the formation of heterochromatin (tightly compact chromatin)(Bukar Maina et al., 2016). Tauopathy research has shown that at least one heterochromatin marker is altered, and there are signs of re-entry into the cell cycle (Frost et al., 2014). In tauopathy, tau forms aggregates which inhibits its ability to bind to DNA(Bukar Maina et al., 2016). It is therefore likely that pathological tau can no longer function properly in the nucleus, which would directly alter chromatin states and DNA stability. This indicates that chromatin state research is a topical and relevant step in the understanding and treatment of AD. Future research of chromatin states in the *Drosophila*

mushroom body will provide a full picture of what is going on the nucleus of cells with tauopathy.

Conclusion

Research in Alzheimer's disease has mainly focussed on the effect of amyloid pathology on the brain. Researchers in the field are increasingly becoming more aware of the strong links between tau pathology, gene expression and AD. My current study adds to the research in this area by characterising a novel, more specific model of AD in the learning and memory area of the *Drosophila* brain. My results indicate that there are systematic changes in the behaviour and brain morphology of the disease affected flies. Investigation of these cellular processes will help the identification of novel targets for potential therapies in the future. The characterisation of these phenotypic changes paves the way for further research into the epigenetic changes that occur in tauopathy and specific vulnerability of learning and memory structures.

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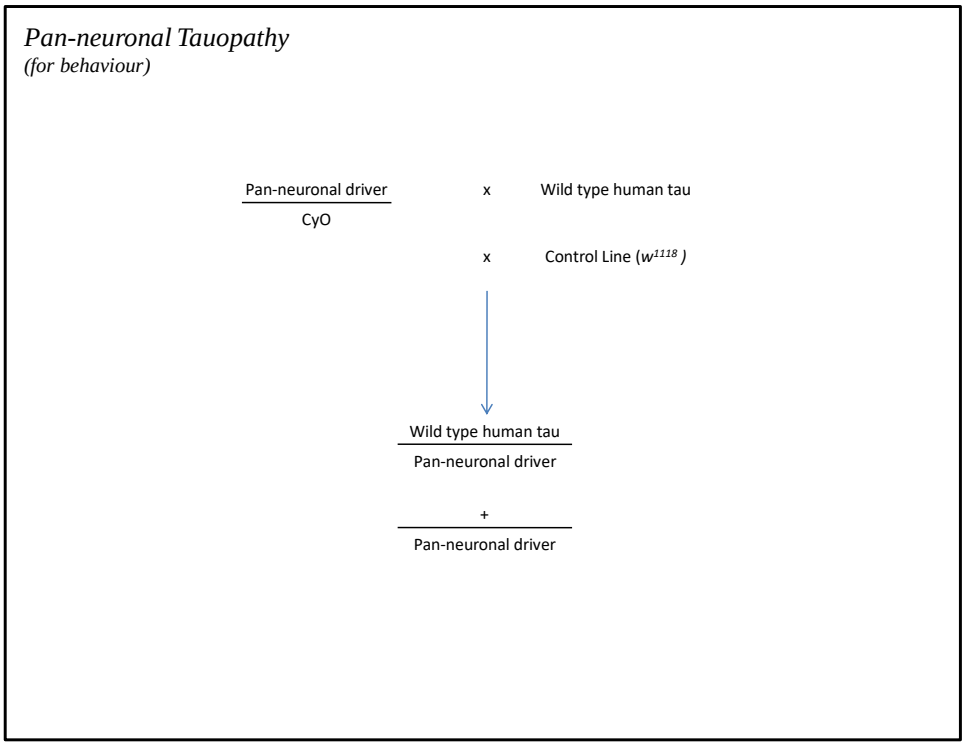
Appendix A

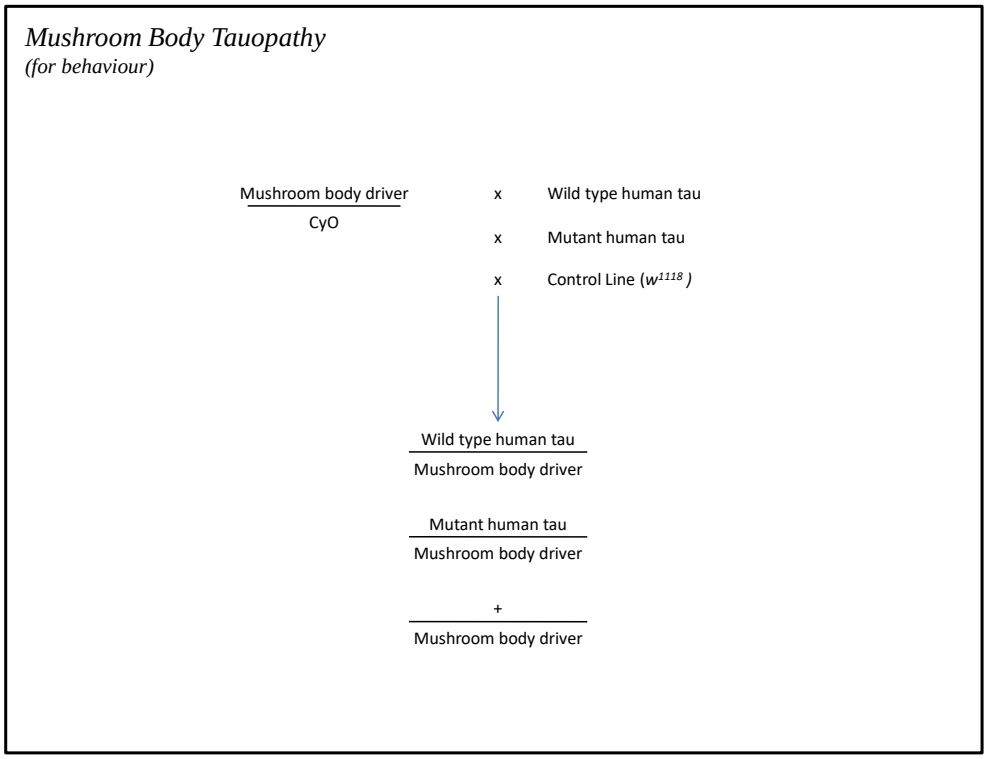
Detailed Fly Crossing Schemes for each Experiment

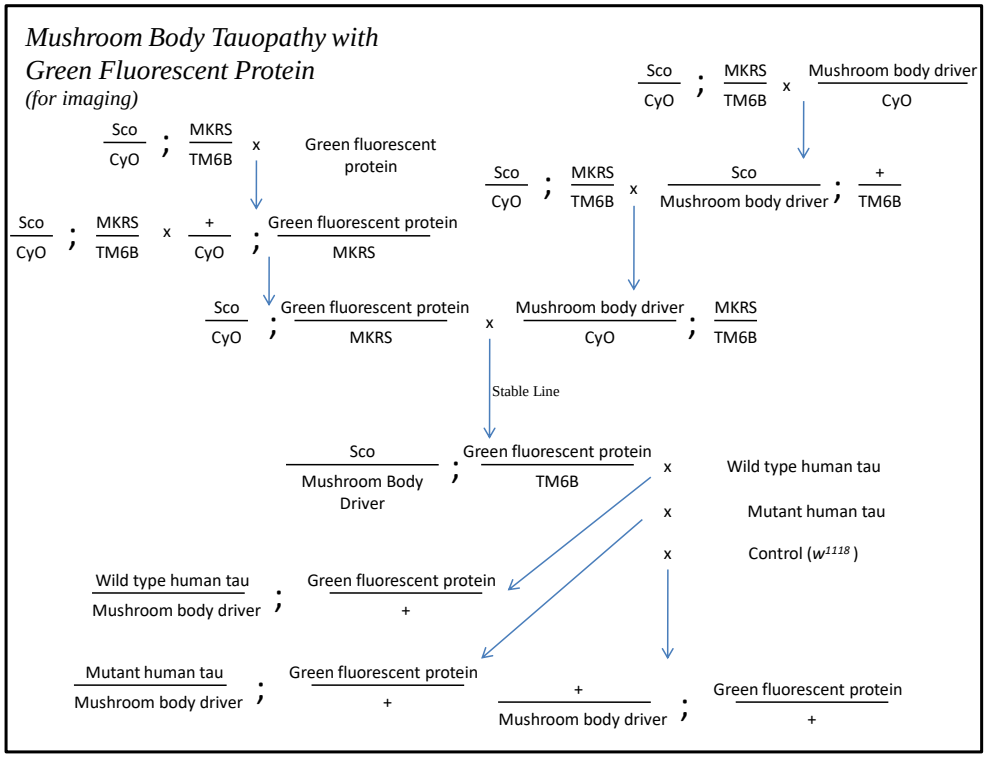
Below are the crossing schemes used to create the tauopathy fly models for each experiment. Artificially inserted genes are described by their function in the scheme. Different genes on chromosome pairs are separated by a horizontal line and different chromosomes are separated by a semicolon. CyO, Sco, MKRS and TM6B are all gene mutations that result in a change to a phenotypic characteristic in the flies (for example flies with CyO will have curly wings). This allowed me to screen out flies which did not possess the gene of interest.

Aim 1 Cross

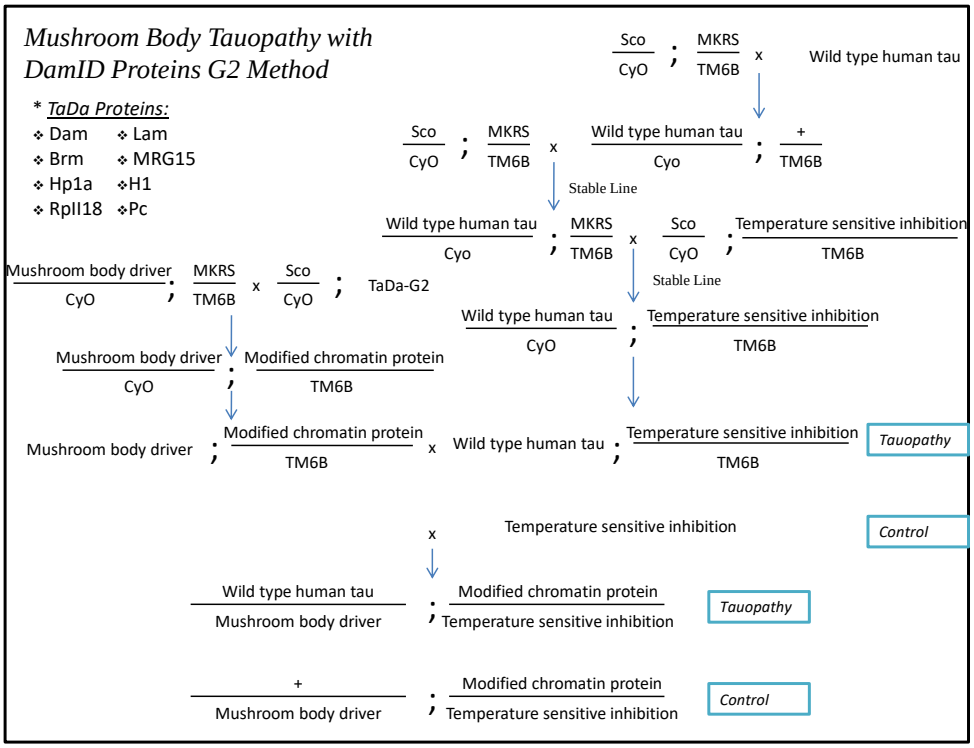


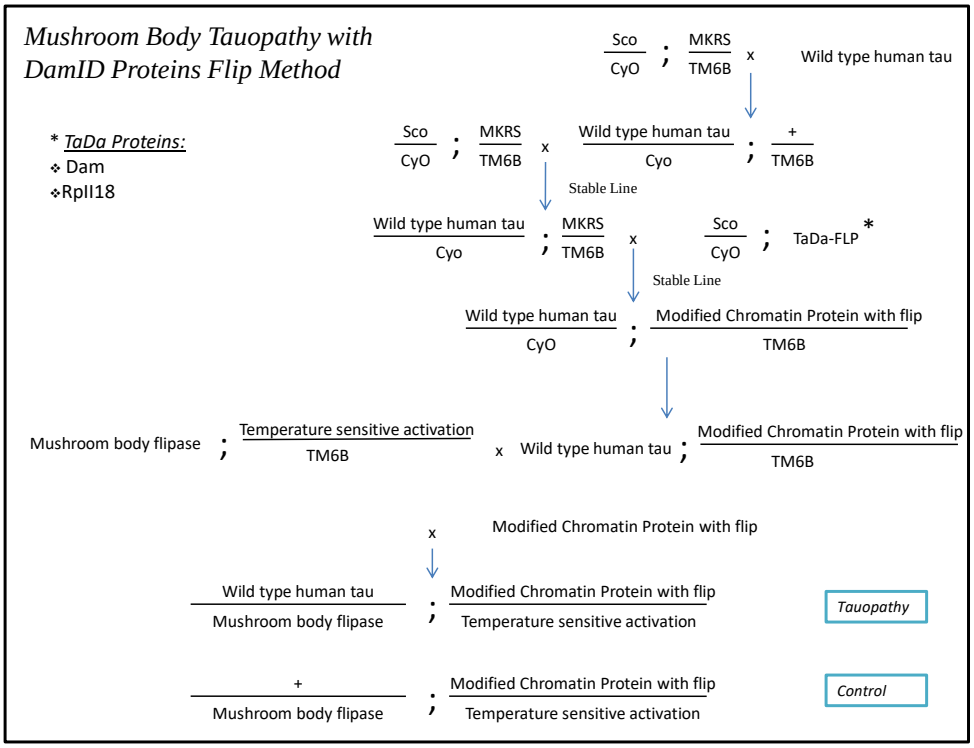
Aim 2 Crosses





Aim 3 Crosses

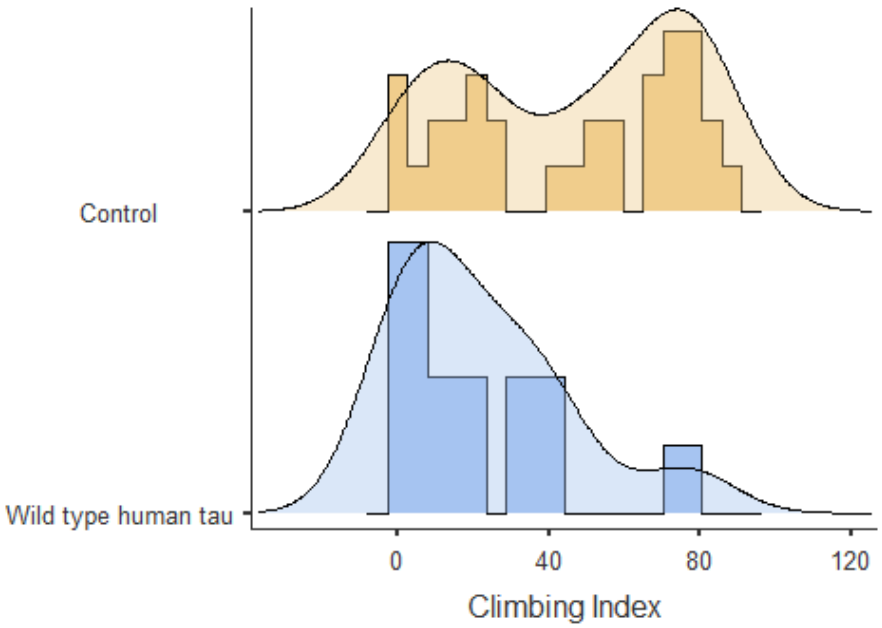




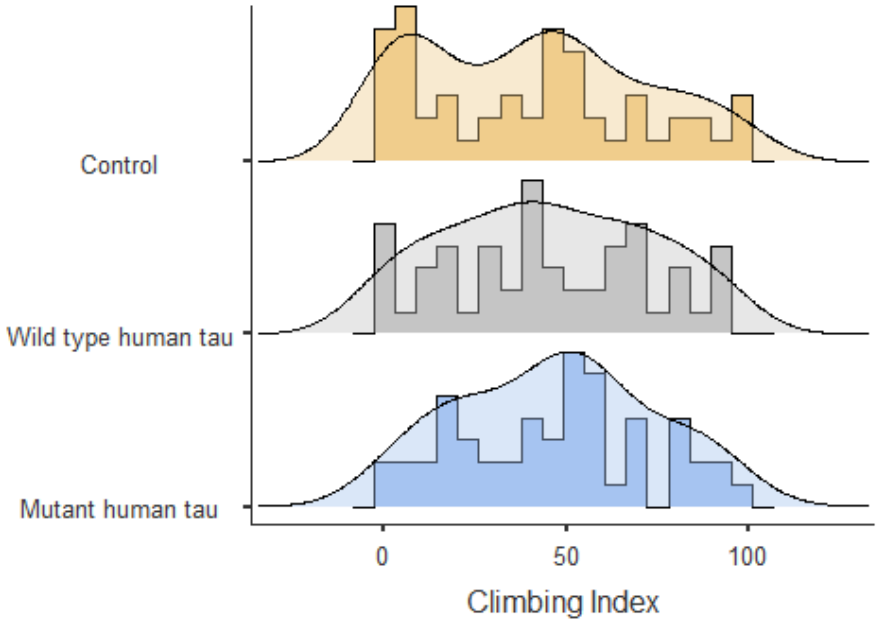
Appendix B

Histograms for Climbing and Learning and Memory Data.

Aim 1 Pan-neuronal climbing data (*n*= 33 for controls and *n* = 22 for tauopathy)



Aim 2 Mushroom body climbing data (*n* =52 per genotype)



Aim 2 Mushroom body learning index data (*n* = 10, 10 and 8 for controls, wild type human tau and mutant human tau respectively)