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Effect of temporal expression of Aβ1-42 in a *Drosophila* model of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) affects tens of millions of people all around the world. In 2018, more than 47 million people were affected, and it is estimated that more than 131 million people will suffer from the disease by 2050. AD is classified as a neurodegenerative disease and is caused by aggregated proteins due to misfolding, which eventually can cause fibrils that damages neurons and blocks blood vessels in the brain. One of those proteins is called Amyloid β (Aβ). An excellent model for Aβ research is *Drosophila melanogaster*, also known as the fruit fly. One of the advantages of this organism is the possibly for the transgenic insert of the UAS/Gal4 system together with a temperature sensitive Gal80 (Gal80^{ts}). These systems combined with a gene of interest have the ability of temporal expression, i.e. expression can be regulated by letting *Drosophila* flies live in different temperatures.
In this thesis, Aβ1-42 was used in this context to observe expression from flies from two different temperatures, 18°C and 29°C, and then test both their lifespan and behaviour. UAS/Gal4, Gal80^{ts} and Aβ1-42 was expressed in neurons of the flies. Three main assays were used: fluorescence microscopy, lifespan assay and an activity assay called iFly. The results showed that the temperature regulation was possible, where both 18°C and 29°C showing distinct differences in all assays. Moreover, flies born in either of the two temperatures and then kept there for 3, 5 and 10 days before switching to the other temperature, also showed clear differences. These results indicate that Gal80^{ts} effectively inhibits Gal4 from activating UAS and downstream expression of Aβ1-42, when transgenetically inserted in the model organism *Drosophila*.

Acronyms and abbreviations

A$\boldsymbol{\beta}$	Amyloid β
AD	Alzheimer's disease
AICD	Amyloid intracellular domain
APP	Amyloid precursor protein
APPL	Amyloid precursor protein-like
CTF	C-terminal fragment
GAL	Galactose-responsive transcription factor
mNG	monomeric Neon Green
ROI	Region(s) of interest
sAPP$\boldsymbol{\alpha}$	Secreted amyloid precursor protein
UAS	Upstream activation sequence

Contents

1 Introduction

1.1 Background

1.1.1 Alzheimer's disease

In 2018, more than 47 million people around the world were affected by Alzheimer's disease (AD) [1]. With age being the highest risk factor, and with the world population progressively growing older, so does the occurrence of AD. In fact, it is estimated that more than 131 million people will suffer from the disease by 2050 [1]. AD is a progressive neurodegenerative disease, which means that patients with AD show gradual loss of neurons in the central nervous system (CNS). Other examples of progressive neurodegenerative diseases are Parkinson´s disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS) and Huntington's disease [2]. Symptoms of AD is memory impairment and cognitive decline and can eventually cause issues with behaviour, speech, visuospatial orientation and movement. However, the most common associated symptom is probably dementia [3], with 60-80% of the dementia cases being due to AD [4]. For pathological diagnosis, amyloid plaques (clusters of amyloid fibrils) and neurofibrillary tangles are used. These consists of proteins called Amyloid β (Aβ) or tau, which are also associated with other diseases such as cerebrovascular disease and Lewy body dementia [3].

1.1.2 Protein folding

An important concept to lift before discussing Aβ any further is protein folding and misfolding. The two- and three-dimensional structure (secondary and tertiary structure) of a protein is determined based on how the amino acid sequence (primary structure) binds to itself and folds. Protein folding is a complex mechanism and several different factors contribute to protein folding. These includes [5] [6]:

1. Hydrogen bonds, which stabilize helices and sheets. These structures are what makes up the secondary structures. Absence of hydrogen bonds render the protein to form structures called random coil, which can for example form random structures between secondary structures.

2. van der Waals interactions, important for keeping the folded state together.

3. Backbone angle preferences. Not all three-dimensional structures are possible due to these angles, which exist in many polymers, including proteins.

4. Electrostatic interactions, like salt bridges, is frequently formed between amino acids in a folding protein.

5. Hydrophobic interactions. Proteins usually fold in a way so that the hydrophobic side chains of amino acids are pointed towards the core of the folded protein and hydrophilic side chains forms the proteins surface, which in many times is in solution with water.

6. Chain entropy. The major factor opposing protein folding. The entropy decreases when the protein gets more compact and increases in the denatured and unfolded states.

To illustrate the complexity even further, a protein does not just appear in its native state (i.e. the lowest energy-state). Many different conformations are often possible, even though the native state is usually the most favourable when in physiological conditions. The different states can be described as a funnel-shaped landscape (see figure 1), where there are few states with low energy and many possible states for high energy. Even though this landscape is many times illustrated as smooth, many cases it is very rough. Along the way down to the native state, many "traps" can appear, where it will take energy before moving down to a more energy-favourable state. To assist both the folding and unfolding processes, proteins called chaperones are present. Nevertheless, proteins can still be stuck in non-native states, a process called misfolding.

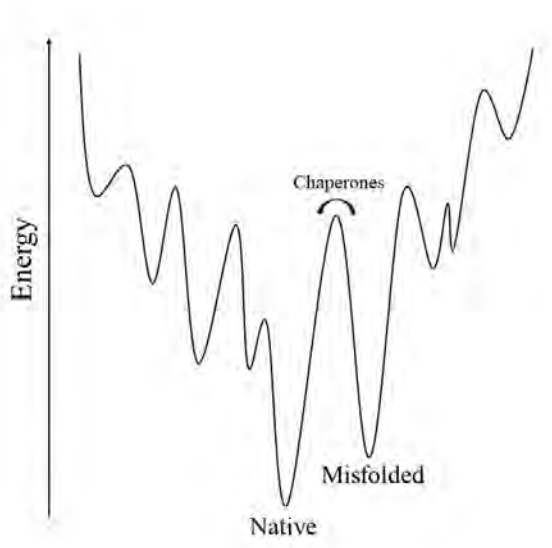

Figure 1: Simplified illustration of a funnel-shaped landscape. Lowest energy for a folded protein is the native state. The folded protein can get stuck in local minima, especially in the misfolded state. Chaperones is however present in many organism which can assist to properly fold the protein from misfolded to the native state. Figure created with Biorender.com.

1.1.3 Amyloid β

Aβ is a protein responsible for formation of amyloid plaques and is therefore highly associated with AD. Aβ is mainly found in extracellular environments around the neocortex, hippocampus and other regions involved with cognitive function [7]. The protein is also produced in humans without AD, even in the early stages of life. However, since Aβ is an aggregation prone protein, there is a risk for the protein to form aggregates that eventually can cause damage to the nearby cells and blocking cerebral blood vessels. This can lead to impaired function of the neurons, vasoconstriction and eventually AD [7]. Besides this, Aβ aggregates also have structure related toxicity and are thought to be involved in formation of intracellular neurofibrillary tangles via internalization (usually mediated by tau) [7]. Amyloid precursor protein (APP) is a single-pass membrane protein which has high level of expression in the brain. APP works as a receptor and is involved in several processes, including synapse formation and repair [8]. APP can be cleaved by α- and γ-secretase, which causes a nonamyloidogenic pathway. If the receptor instead is cleaved by β-and γ-secretases, this can cause several different Aβ peptides of various sizes, where mainly Aβ1-40 and Aβ1-42 leads to amyloidogenic aggregation (see figure 2).

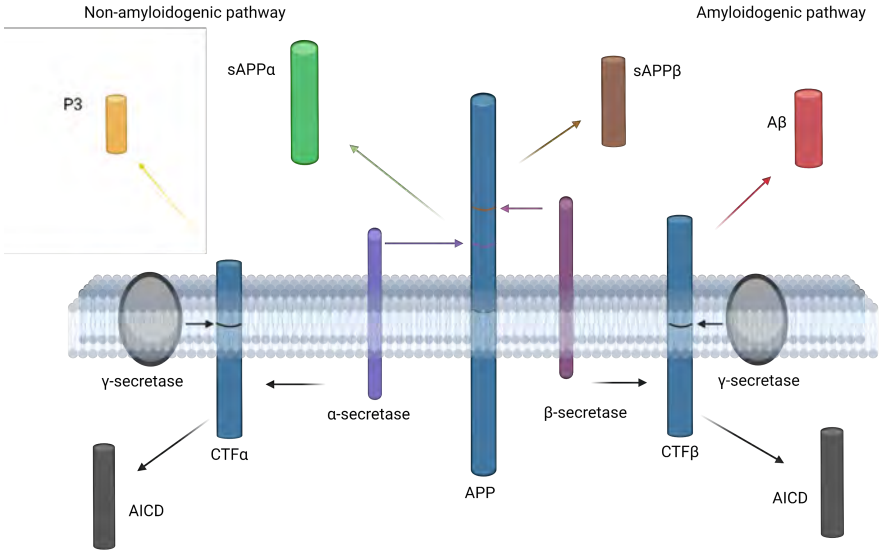

Figure 2: Illustration of non-amyloidogenic/amyloidogenic pathway. The non-amyloidogenic pathway starts when α-secretase cleaves APP, leading to sAPPα (extracellular) and CFTα (transmembranic). AICD (intracellular) CTFα is then cleaved by γ-secretase, forming AICD (intracellular) and P3 (extracellular), which is rapidly degraded. Amyloidogenic pathway is β-secretase mediated, leading to sAPPβ (extracellular), CTFβ (transmembranic), and AICD (intracellular) together with Aβ (extracellular) after CTFβ is cleaved by γ-secretase. Figure created with Biorender.com.

The aggregation is caused by protein misfolding of Aβ peptides and under normal conditions, there is a steady-state between the production and elimination of these misfolded peptides. However, in AD patients, this steady-state is disrupted and an over-production is induced. If amyloidogenic aggregation is not stopped, it goes through a number of phases (see figure 3). This starts with dimerization, called the lag phase because of its slower development. When the oligomers eventually starting to form, the process speeds up, leading to protofibrils and eventually mature fibrils is formed. The structure of these fibrils varies a lot, a concept known as fibril polymorphism.
There are today several different treatments of AD. Many of them target the amyloidogenic pathway, for example β-secretase and γ-secretase inhibitors [9] [10]. Other treatments instead target the Aβ aggregates directly with the use of antibodies. Examples are Aducanumab [11] and Lecanemab [12], both recently FDA approved drugs [13] [14].

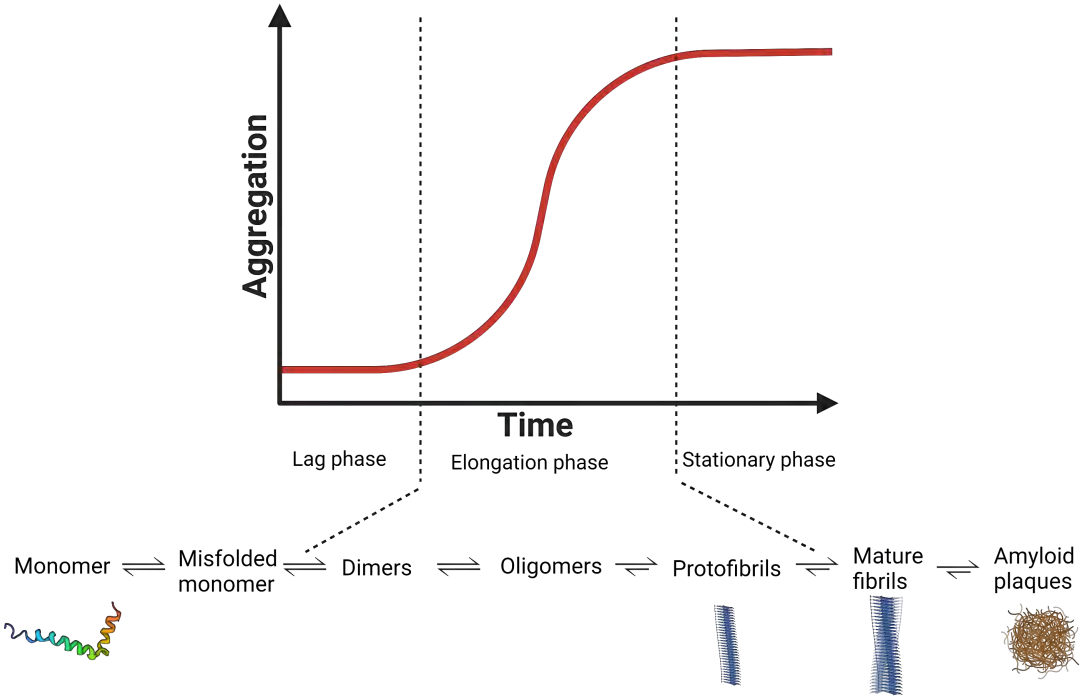

Figure 3: Plot illustrating the fibrillation from misfolded Aβ, with degree of aggregation on y-axis and time on x-axis. The process starts with a misfolded Aβ, which dimerize. The misfolding and dimerization occur in a slow manner, called the lag phase. When oligomers of higher degree form, the process speeds up in an elongation phase, eventually leading to mature fibrils and amyloid plaques. Figure created with Biorender.com.

1.1.4 *Drosophila melanogaster*

1.1.4.1 *Drosophila* as a model for Alzheimer's disease

Drosophila melanogaster, also known as the fruit fly, is a common small fly, about 2-3mm from head to tail. In the remainder of this report, *Drosophila melanogaster* will be referred to as "*Drosophila*" only or simply "fly". Male characteristics are a small body, sex combs, genital claspers and a dark and rounder abdomen. Females are usually larger, have a pointy tip and lacks the rest of the male characteristics. The fly is born from an egg that develop into an embryo, before a so called 1st instar larva is formed. As the larva develops, it becomes 2nd and 3rd instar, and eventually forms a pupa. When the pupa hatches, the fly is adult (see figure 4). Right after hatching, the fly goes through a virginity stage, where no mating is occurring, before the it reaches full development. The time of the virginity stage is, as the whole life cycle, temperature dependent, and continues for about 8 hours if in 25°C or 16 hours if in 18°C. Characteristic for the virgins is a swollen front head, folded wings, clear and swollen abdomen, and a black dot on the abdomen called meconium.

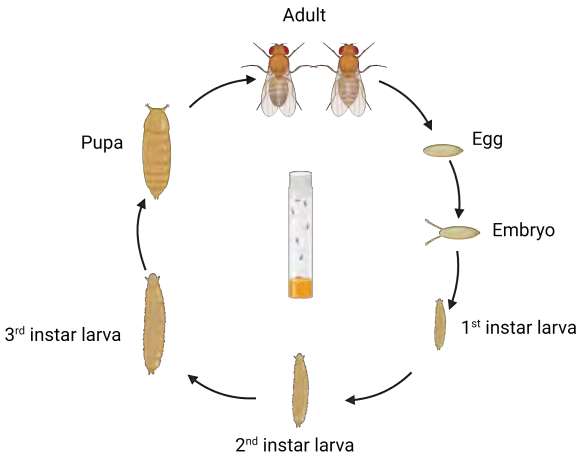

Figure 4: The life cycle of Drosophila melanogaster. The cycle starts with the females (right fly), laying eggs. The eggs develop into embryos, before going into a larva stage with 1^{st}, 2^{nd}, and 3^{rd} instar. The larva eventually forms a pupa, which after some time hatches and an adult fly is appearing. In the middle of the life cycle in this figure, a typical Drosophila vial is illustrated. Figure created with Biorender.com.

Drosophila is a model organism that has been involved in genetic studies for over 100 years [15] and since 2000, has a completely sequenced genome [16]. To add to the advantages, *Drosophila* experiments is relatively cheap and have high homology with many of the genes involved in human diseases, including many cancer-related and (as is important to this project) neurodegenerative-related genes [15]. As many as 75% of human disease related genes have orthologues or similar genes in *Drosophila*. One of those is the APP ortholog APPL (amyloid precursor protein-like), to which the corresponding gene has 25% identity and 39% similarity with APP. APPL does not however share high sequence similarity at the site of Aβ in human APP. Also, *Drosophila* lacks β-secretase. Because of these two differences, Aβ is not produced in endogenous wild type *Drosophila*. Even though there is evidence pointing towards the existence of an Aβ-like fragment when β-secretase is introduced [15], the simplest way of producing Aβ in the fly is instead to insert an Aβ transgene that directly leads to expression of Aβ peptides.
Drosophila have four pair of chromosomes, of which two is sex chromosomes and the rest is autosomes. One major advantage with *Drosophila* in a scientific context is the possibility to insert a so called balancer. A balancer is a chromosome that has been genetically modified so that each fly has a recessive lethal gene, i.e. homozygotes of that gene will not survive. Moreover, balancers can also include dominant genes for markers, giving the flies specific phenotypes. These markers include curly wings, white eyes, long bristles and many more [17]. These two properties of balancers can be very useful when conducting a *Drosophila* experiment since selective processes simplifies largely, especially when a combination of genes from parent flies is needed in the progeny.

1.1.4.2 The Gal4/UAS system and Gal80

Galactose-responsive transcription factor 4 (Gal4) is a transcription factor from yeast that do not exist in the endogenous genome of *Drosophila*. The Gal4 gene codes for the protein Gal4, which can bind to the enhancer upstream activation sequence (UAS), causing downstream upregulation of many different genes, depending on its isoform. This can be utilized by artificially inserting genes of interest downstream of UAS. Gal4 is constantly expressed in Gal4$^+$ organisms. However, Gal4 can be inhibited by the protein Gal80, and therefore, if Gal80 is expressed together with the Gal4/UAS system, expression of UAS enhanced genes will not occur. Furthermore, activation level of the variant

Gal80^{ts} (temperature sensitive Gal80) is temperature dependent, where lower temperatures, around 18°C, has been shown to inhibit binding of Gal4 to UAS, and higher temperatures, around 29°C, has been shown to not inhibit Gal4 [18][19] (see figure 5). Thus, a combination of Gal4/UAS and Gal80^{ts} have potential to regulate the expression of proteins of interest by regulating temperature of the model organism. The system has been tested with several genes successfully previously in *Drosophila*, though never before with Aβ.

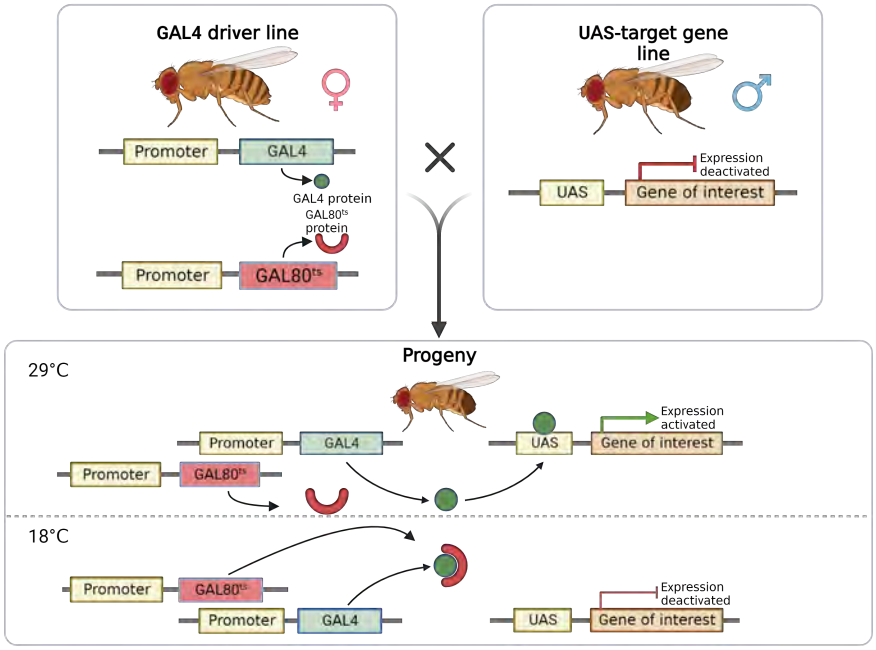

Figure 5: . Illustration of the properties of the Gal4/UAS system and the Gal4 inhibition caused by Gal80^{ts}. When a male carrying the genes of Gal4 and Gal80^{ts} mate with a female with a gene of interest coupled with an UAS enhancer, the progeny will contain a complete system of Gal4/UAS and Gal80^{ts}. This leads to the expression of the gene of interest being able to be regulated by temperature, where 29° C will turn on expression and 18° C will turn expression off. Figure created with Biorender.com.

1.2 Aim

The aim of this project was to observe how effective Gal80^{ts} blocks Gal4 from activating expression of Aβ in *Drosophila melanogaster*, when added to the Gal4/UAS system. The flies were transferred between different temperature and time combinations (between 18°C and 29°C), in order to observe the effectiveness at different life stages. If successful, this could lead to an easy and cheap method for not only analysing Aβ in *Drosophila* lines, but also in the same *Drosophila* individuals.

1.3 Ethical considerations

Since *Drosophila melanogaster* is an invertebrate, no ethical clearance was needed. The flies are nevertheless living organisms and was treated with care.
Regulations regarding Genetically modified organisms (GMO) from *Swedish board of agriculture* was applied. To prevent GMO release to the environment, the flies were kept in a laboratory with lock restricted air ventilation pipes and restricted access.
No other ethical considerations were needed.

2 Methodology

2.1 Fluorescence microscopy

Fluorescence is caused by emission of light that occur after a molecule have absorbed light and therefore been excited. This causes the electrons to momentarily jump to an excited state. When the electrons relax back to their initial state, they emit light of a certain wavelength. That difference of wavelength between the excitation and emission is called the Stokes shift. By blocking out the exciting light and still keep the emitted light, it is possible to see only the fluorescent molecules [20].
One problem with the emitted light is however that it can be "quenched". Meaning that the intensity of emitted light can decrease due to a number of reasons. One important factor is the light being absorbed by nearby molecules. For this reason, when designing fluorescent probes, the fluorescent region, called fluorophore, is usually shielded in a manner that let the emitted light stay for longer within the molecule. Many such probes are designed for attachment to organic molecules. Genetic engineering has however made it possible for a wide variety of protein-protein probes.
One such is called monomeric Neon Green (mNG) (see figure 6). As a mutant from the wild-type LanYFP, derived from *Branchiostoma lanceolatum* (a small fish-like animal also referred to as lancelets), the probe has an emission wavelength of around 517nm and a bright green-yellow colour. This probe has some advantages compared to some of the more traditionally used, like *Aequorea victoria* green fluorescent protein (avGFP) and its derivatives, with brightness being one of them [21].

Figure 6: Pymol illustration of the fluorescent probe monomeric Neon Green (mNG) (5LTR).

2.2 Lifespan assay

As mentioned in section 1.1.4, *Drosophila* has been used as a model organism in scientific experiments for over 100 years. One of the most simple studies, yet one very powerful, is measuring the lifespan of *Drosophila*. The method is also common in other organisms but is usually performed with organisms with shorter lifespan. Both effects of diet [22], environmental factors [23] and genetic modifications can be studied by this method, and within genetic modifications includes transgenic genes [24]. In section 4.2.2 a more in-depth explanation will be described on this project's specific set-up of lifespan assay. One disadvantage with a lifespan assay is that it is very binary, either the fly is dead or alive. It does not take gradual loss of neurological healthy behaviours and locomotion into account. For this, a complementary assay is needed.

2.3 iFly

A good complementary assay, especially when observing neurodegenerative behaviours, is an activity assay. These assays usually measure different factors as movement and behaviour over time. One activity assay is called iFly. This software uses a *Drosophila* vial combined with two mirrors and a video-recording camera to observe automated locomotion and behaviour of the flies by measuring several factors [25], including speed of climbing and angle of movement. A video is first recorded, and simultaneously the vial is dropped down in the iFly container. The idea for dropping the vial is to

cause the flies to fall to the bottom of the vial. The flies are negatively geotactic, this means that healthy flies will try to climb up as fast as possible (figure 7), in a straight angle. Un-healthy will do the opposite. Though in contrast to a lifespan assay, this is not binary, meaning the flies will not be climbing fast or slow. The change in climbing speed (for example) can be measured over several days, and a gradual change in the measured factors can be recorded. One disadvantage is however that the flies must have some movements and be able to climb, something which can cause insufficient data if the flies health decreases rapidly.

Figure 7: Snapshot images of one the iFly video recordings used for analysis. Figure is illustrating the negatively geotactic behaviour of Drosophila. In A, the vial has just been dropped down in the iFly container, causing the flies to fall to the bottom of the vial. In B, the flies start to climb up the vial. In C, the flies are at the top of the vial.

3 Process

In the following GANTT chart (see figure 8), an overview of the project plan is presented. The plan was made on the first week of the project. The planning is weekly and coloured dark green on the weeks were each of the activity/sub-activity is planned. The six milestones planned (M1-M6) is marked with white text on its corresponding weeks. As can be seen, the majority of the lab work was planned to be performed from week 40 to week 50. Because of this, more of the literature studies was planned to be before week 40, for preparation and compensation for the less time in lab in these first weeks. Exact dates of the final report submissions and presentation was set later and therefore those weeks are not here accurate. In table 1, a description of each milestone is presented.

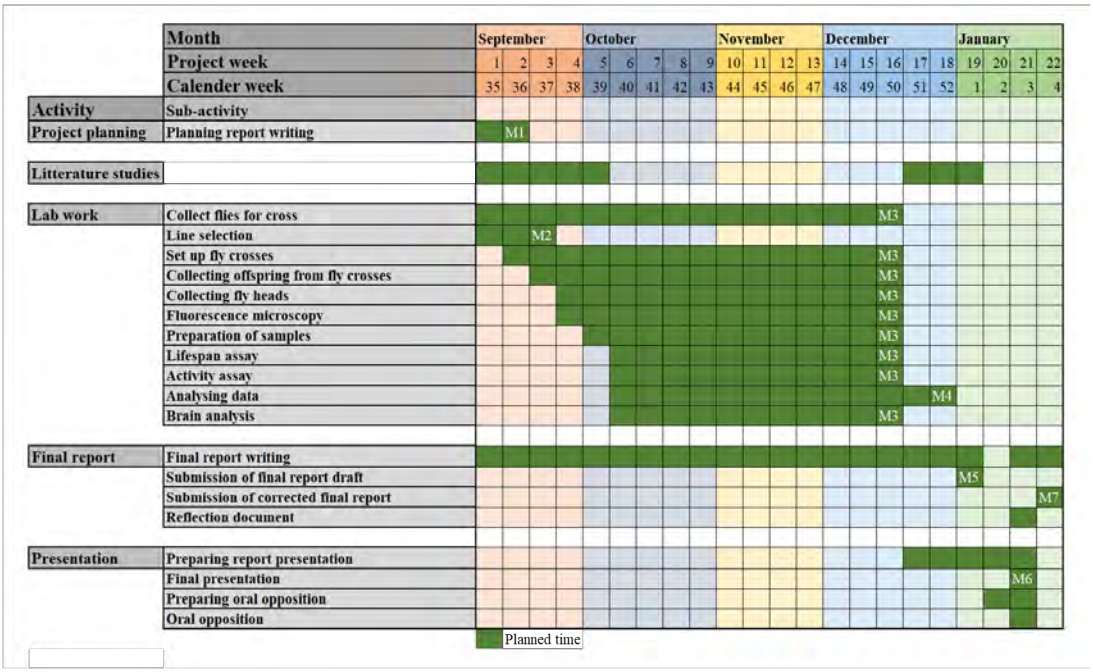

Figure 8: GANTT chart of the project, with milestones (M1-M6) marked.

Table 1: Table with description of the projects milestones with its planned calendar week.

Milestones	Description	Calendar week
M1	Submission of planning report	36
M2	Line selection finished	37
M3	Lab work finished	50
M4	Analysing data finished	52
M5	Submission of final report draft	1
M6	Final presentation	3
M7	Submission of corrected final report	4

4 Methods

4.1 Line selection

4.1.1 Drosophila lines

All together there were initially six different type of *Drosophila* lines and nine in total were used in this project (see table 2). All lines were provided by supervisor Ganesh Mohite and had been stored in room temperature.

Table 2: Table of all the lines and their corresponding genes and number of lines per genotype. All lines also included the balancer TM3-Sb. Gal4 and Gal80^{ts} were under nsyb promoter driving expression in neurons [26].

Gene	Description	Number of lines	Sex used for crosses
Gal80^{ts}+Gal4	Lines containing Gal80^{ts} and Gal4.	5	Females
Gal4	Line containing only Gal4	1	Females
Empty	Line without any transgenic genes.	1	Males
UAS+Aβ1-42	Line containing the UAS enhancer together with the gene for Aβ1-42.	1	Males
UAS+mNG-Aβ1-42	Line containing the UAS enhancer together with the gene for Aβ1-42 with a fused monomeric Neon Green probe.	1	Males

All lines also contained a balancer called TM3-Sb, where T is short for third chromosome, M for multiple inverted, and Sb for Short/Stubble bristles. The latter meant that flies not containing the correct genes after crossing would have short bristles. In figure 9, all different crosses are represented in typical crossing schemes. Only those flies without TM3-Sb had long bristles, which meant they contained the genes (or absence of genes) of interest.

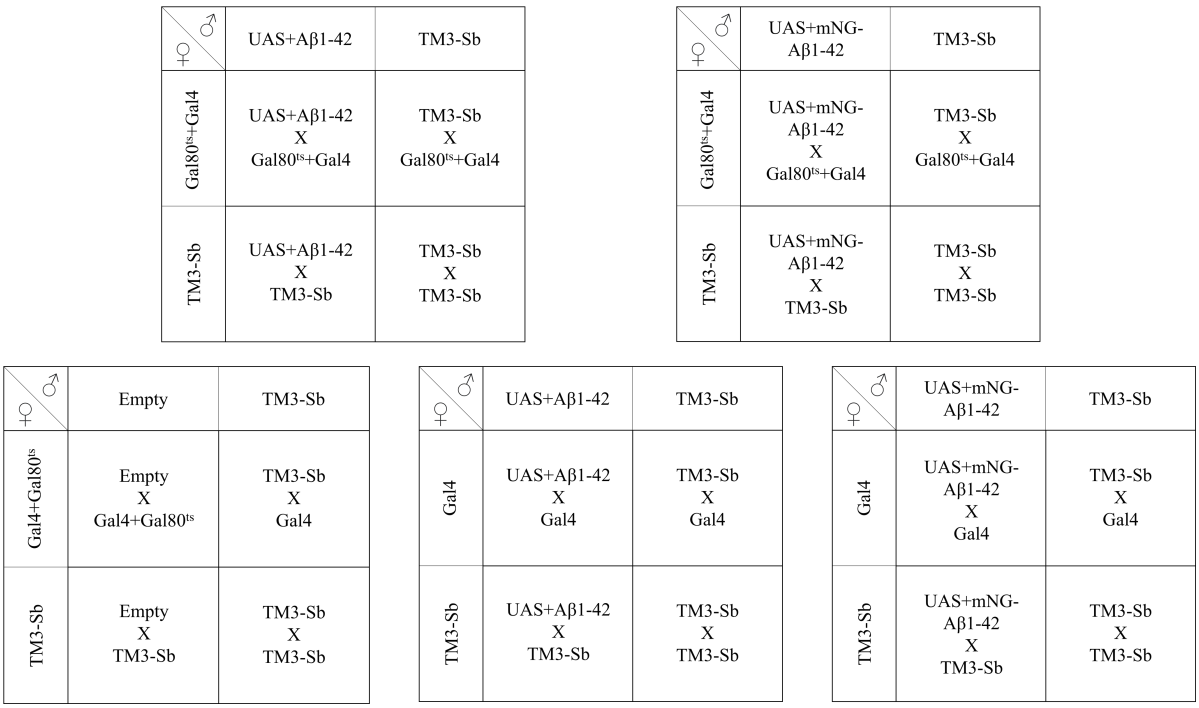

Figure 9: Crossing scheme of all 5 crosses made throughout the project. Upper two were the main crosses for this project, and the bottom three the controls. All progeny marked TM3-Sb x TM3-Sb (bottom right corner in each scheme) did not survive before selection, all progeny marked with only one TM3-Sb (upper right corner and bottom left corner) had short bristles, and those without TM3-Sb (upper left corner) had flies with long bristles.

All lines but Gal80^{ts}+Gal4 had been used and tested before. This transgenic procedure was therefore performed five times, i.e. on five different lines of flies. Only one of them needed to be selected and this needed to be picked before continuing the project. The selection process was performed by observing larvae and fly heads with fluorescence microscopy. Therefore, only the crosses with the fluorescent probe mNG were used in this step.

4.1.2 Setting up lines

The empty vials that were used contained food with a mixture of premade food and dry yeast. Before use, the vials were allowed to approach room temperature. Before collection and between each new line, the equipment was disinfected with ethanol. The flies were sedated with CO_2 and put under a light microscope for observation. About 6 males and 10 females from the same line were collected in each new vial. All new vials were stored in a 25°C incubator. The next week, all the vials were emptied of flies, so that only eggs, larvae and pupae were left. The flies were collected into new vials and stored as back-ups in 25°C. The new vials were about half as many as the previous vials and was also stored in 25°C.

4.1.3 Fly selection

About three days later, flies were detected in the vials previously only containing eggs, larvae and pupae. The flies from the vials containing the female lines (see table 2) were sedated with CO_2 and observed under microscope. If female virgins were detected, they were put into new vials. The female virgin vials were stored in 25°C. The vials containing pupae were stored in 25°C if next collection were within 8 hours and in 18°C if next collection were within 16 hours. The virgin collection was performed daytime at around 09.00, 14.00 and 17.30. Since the last collection of the day was made at 17.30, the vials were stored in 18°C overnight, and at 25°C in between 09.00-17.30. If these time-windows were certain, also the females without clear virgin characteristics could be considered as

virgins. This procedure was repeated until 50-90 virgin females were collected for each line. The males were collected only by observing the male characteristics.

4.1.4 Setting up crosses and progeny collection

The crossing vials were prepared by inserting a twisted paper tissue in the food (increases chances of mating). About 10-12 females from the female lines and 6-8 males from the male lines were collected in each vial. The empty female/male vials were then continued to be stored in 25°C. If larvae were spotted in one of the vials, this meant that it hadn't been exclusively males or virgin females in that particular vial, and the crossing vial was disregarded. In this way, higher assurance could be made that the crossing had been successively performed.
The progeny from this step were now UAS+mNG-Aβ1-42 X Gal80^{ts}+Gal4 (all five lines) and UAS+ mNG-Aβ1-42 X Gal4. For convenience, in the remaining text, UAS+mNG-Aβ1-42 X Gal80^{ts}+Gal4 will be called Gal80^{+}FL1-5 (Gal80 positive fluorescent line 1-5) and UAS+mNG-Aβ1-42 X Gal4 will be called Gal80^{-}FC (Gal80 negative fluorescent control). The vials were either transferred to 18°C or 29°C.

4.1.5 Fluorescence microscopy

4.1.5.1 Observing fluorescence in larvae

Drosophila larvae from the crosses were consequently born either in 29°C or 18°C. Because of difficulties with sedating larvae with CO_2, live larvae were used. To limit the movement, the larvae were put on ice for about 30-40 minutes. The microscope was set to 480nm of excitation wavelength.
3rd instar larvae were collected from the vials stored in the 29°C incubator. These were then observed with fluorescence microscopy. Images were taken mainly on the larvae that showed clear fluorescence at an emission wavelength of 520 nm. Focus of the images were also on the central brain, but other images were also taken if possible (depending on how mobile the individual larva were) and on i.e. if fibrils on other parts were seen.
2nd and 1st instar larvae from the same vials were also taken, observed and imaged in the same way. If clear fluorescence were shown at emission wavelength 520 nm, these larvae were transferred to new vials that was stored in 18°C. Observations of these were to be made again as soon as 3rd instar larvae could be seen.
The exact same procedure, but with reversed temperatures was also made. I.e. 3rd instar larvae from the 18°C incubator were observed, and 2nd and 1st instar larvae were observed, transferred to new vials, stored in 29°C and then observed as soon as 3rd instar larvae could be seen. Because of a varying quality of images, due to the larvae movement, only assessment of high/low fluorescence intensity at 520 nm were made, the images were not further analysed. Each image was analysed by choosing regions of interest (ROI) were the fluorescence seemed to peak at 520 nm. If no intensity at that wavelength, the ROI were picked were fluorescence of other intensities seemed to be high.

4.1.5.2 Observing fluorescence in flies

Preparations

Step 1 – Fly collection
The flies stored in 29°C were collected from their vials after three days and observed under microscope. Flies with long bristles was collected and stored in two new vials, which either was kept being stored in 29°C or transferred to an 18°C incubator.
The exact same procedure, but with flies from the 18°C incubator was also made.

Step 2 – Preparation of fly heads
Three days later, the 29°C->29°C and the 29°C->18°C flies were transferred to eppendorf tubes (10 flies in each) and was dropped in liquid nitrogen. After this step the tubes were vortexed, and their heads could then be collected. The fly heads were stored in -20°C before further steps. This step were also repeated for the reverse procedure, i.e. flies born in 18°C and kept there or transferred to 29°C.

Step 3 – Cryosectioning
Before observing the fly heads with fluorescence microscopy, the heads needed to be cut in thin slices. A cryostat is a machine which can cut ultrathin slices of tissues that has been frozen in a freezing medium called optimal cutting temperature compound (OCT).
OCT were first placed in a small plastic container, one for each line and temperature. The heads were then carefully placed in the bottom in their respective container by carefully pressing the head to let air out and minimize the risk of the heads to fall out of the sections later. Each container was dropped in liquid nitrogen and was kept frozen until the cryosectioning. The cryostat was set on around -20°C and cut thickness setting "FINE", which gave slices with 10 μm thickness. Each slice was put on a glass slide. Each glass slide was filled with head slices, and about three glass slides were used per line and temperature. The slides were kept frozen until next step.

Step 4 – Slide preparation
Each glass slide where prepared by washing away excess material. This was carried out by first put down the glass slides in 96% ethanol for 10 minutes, in 70% ethanol for 5 minutes, in water for 5 minutes and finally in PBS solution for 5 minutes. Mounting medium (Dako) was thereafter put on each slide before covering them with a thin cover glass.

Imaging and analysis

The microscope was first set on 480nm excitation wavelength. Each successfully sectioned slice was imaged by taking one overview image with 100 ms exposure on 5x zoom. Then six images were taken with 20 ms exposure and 20x zoom, three on the central region, one on the left and right lobe respectively and one additional on either left or right lobe were that particular head section was most intact. Each image was analysed by choosing ROI were the fluorescence seemed to peak at 520 nm. Based on this data, one of the Gal80^{+}FL1-5 lines could be selected for further experimentation.

4.2 Assays

4.2.1 Preparation

Based on results from the line selection process, Gal80^{+}FL4 was selected, and new crosses was prepared in the same manner as described in section 4.1.2-4.1.4. This time however, since only the toxicity of Aβ1-42 was of interest, a fluorescent probe was not used. For that reason, UAS+Aβ1-42 X Gal80^{ts}+Gal4 (Gal80^{+}) was used instead. As controls, both UAS+Aβ1-42 X Gal4 (Gal80^{-}) and Empty X Gal4 (Empty) was used. Each cross was either put in 18°C or 25°C. The latter was instead transferred to 29°C only when in pupae stage. The lower initial temperature increased the flies chances of survival to adult stage.
The fly food for the lifespan assay was prepared by mixing 1 litre of water, 20 g sugar and 20 g agar. This was then heated for about one hour in a water bath. 7 ml of this mix was added to each vial, that was slightly tilted. Within 2 hours, the mix had solidified in the vials. A small amount of yeast mix (1g of yeast for 2,166 ml water) was then added with a spatula spoon in each vial. The yeast mix was allowed to dry for about 1-2 days before use. This step was repeated whenever new vials were needed in the lifespan assay.

4.2.2 Lifespan assay

In this assay three lines was now used: Gal80^{+}, Gal80^{-}, and Empty. These were also divided into different sets, depending on when those particular progenies came from the crosses. In total, there were four sets, where two of them contained flies that was born in 29°C and later transferred to 18°C, with the exception of one group of vials that were kept in 29°C. These two sets were initially kept in 25°C until the Drosophila has developed pupae (as mentioned before), then transferred to 29°C (see figure 10A). Due to time limitations, these two sets were also the only ones that involved the Empty line. The two sets born in 29°C) each had one group that was transferred to 18°C after three days, one that was transferred after five days and one that was transferred after ten days. The other two sets were first kept in 25°C until late larva/early pupa stage, before transferred to 18°C. The flies were then born in 18°C and either kept in that temperature or transferred to 29°C after three, five or ten

days (see figure 10B). All sets and groups are summarized in figure 10. The vials were usually changed every second day and number of dead flies were noted every to every second day. Maximum 20 flies were kept in each vial at the start of the experiment.

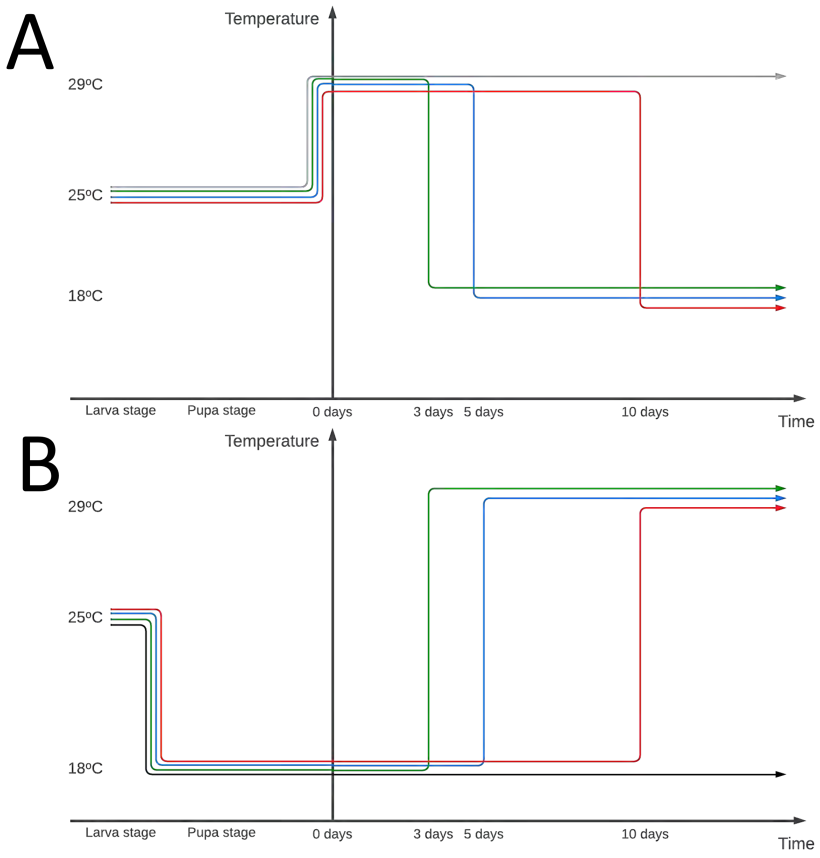

Figure 10: Set up of the project's lifespan assay. A includes set 1 and set 2, which had progeny kept in 25° C until late stage pupae and then transferred to 29° C. These flies were born in 29° C and then either kept in 29° C or transferred to 18° C after 3, 5 or 10 days. B includes set 3 and set 4, which had progeny kept in 25° C until late stage larvae/early stage pupae and then transferred to 18° C. These flies were born in 18° C and then either kept in 18° C or transferred to 29° C after 3, 5 or 10 days.

4.2.3 Activity assay (iFly)

The iFly assay began by using flies from the lifespan assay sets which was born in 18°C and transferred to 29°C. The videos were recorded after that the flies had been in 29°C for one day. In the first day of recording, three different vials were used which all contained ten flies each. The first group of flies had been three days in 18°C and one day in 29°C. This group contained only females due to insufficient number of males. All other groups started with 20 female flies and 10 males. Since the groups were taken from two sets, there were a total of 60 flies from each group and line on day one. Both $Gal80^{+}$ and $Gal80^{-}$ was used, but not the Empty line due to time limitation. Flies from each vial was sedated with CO_2, before transferred to the iFly-vial. To make sure that flies no longer was affected by the CO_2, 5 minutes passed before starting the recording. Right at the start of the recording, the iFly-vial was dropped from an approximate height of 1 decimetre into the container. This was repeated at 30 seconds and 60 seconds. The recording ended at 90 seconds. This was repeated for all vials from all groups and lines and continuously recorded each day until insufficient movement had been reached. The videos recorded were then analysed using the iFly software [25].

5 Results

5.1 Line selection

5.1.1 Fluorescence in larvae

In figure 11 and 12, images of larvae are shown for each line of Gal80^{+}FL1-5 and Gal80^{-}FC, both for 18°C and 29°C. Only one of the three larvae from each line imaged is shown here and only the image with ROI. In figure A1 – A2 all larvae with ROI are shown. All larvae that were born in 29°C showed clear fluorescence intensity at the mNG-specific wavelength of 520 nm. This can also clearly be seen in figure 11, with the light green parts being the central brain of the larvae. In Gal80^{+}FL1 and control (i.e. Gal80^{-}FC) a high degree of fluorescence intensity scattered along the body could also be seen. This was not detected in as high degree in the other lines.

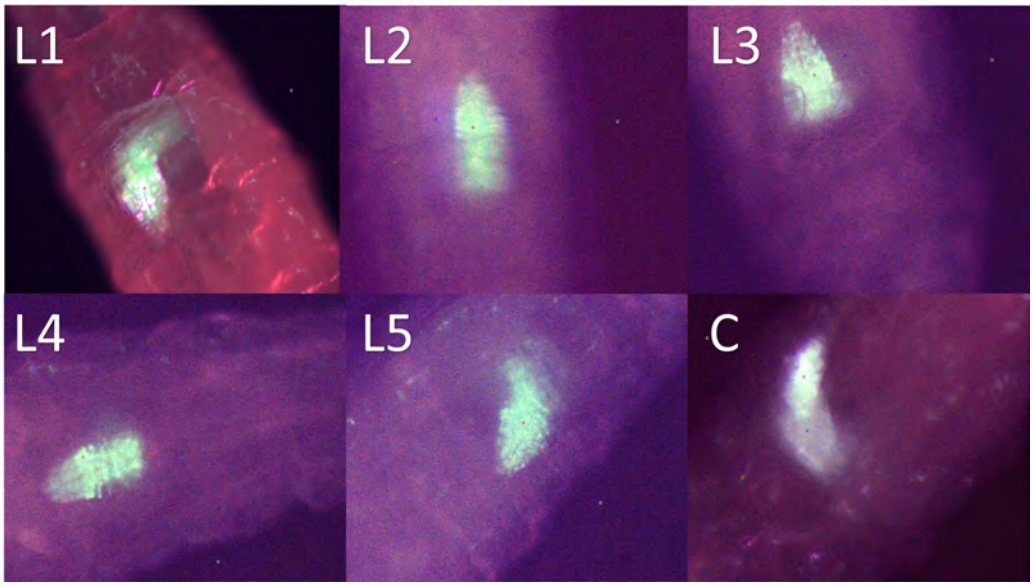

Figure 11: Images of live larvae born and kept in 29° C. L1 is Gal80^{+}FL1, L2 is Gal80^{+}FL2 and so on. C is Gal80^{-}FC.

For larvae kept in 18°C (see figure 12), only larvae from Gal80^{+}FL1 and Gal80^{-}FC had clear fluorescent intensity peaks at 520 nm, which can be seen in the light green regions of figure 12L1 and 12C. In figure 12L2-12L5, luminous regions can also be spotted. However, these regions did not have the characteristic light green colour, nor did they have any major fluorescent intensity peak at 520 nm.

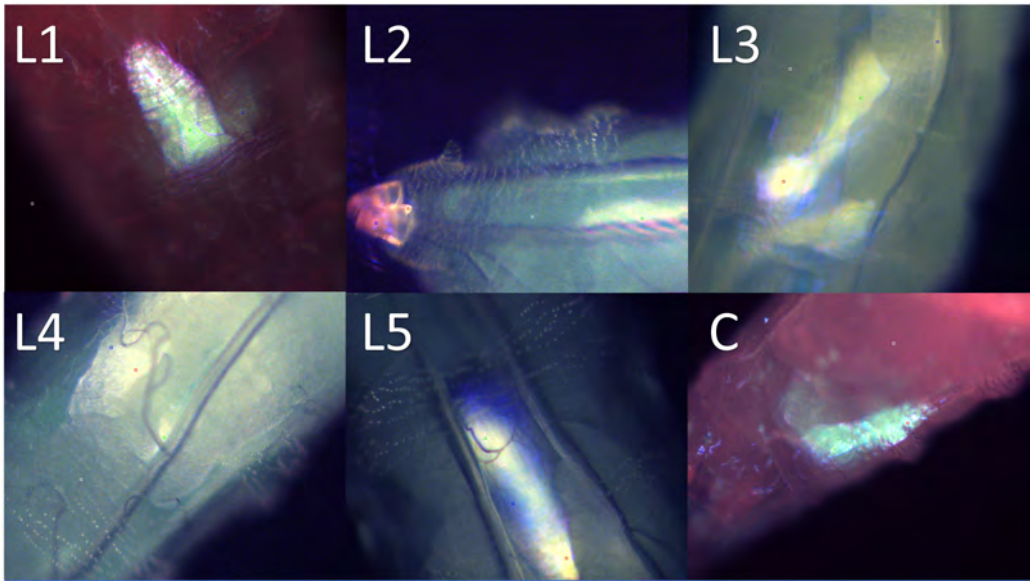

Figure 12: Images of live larvae born and kept in 18° C. L1 is Gal80$^+$FL1, L2 is Gal80$^+$FL2 and so on. C is Gal80$^-$FC.

Because of a very low survival rate of the 1st and 2nd instar larvae (after imaged the first time) that was supposed to be imaged when developed to 3rd instar, these were not used in any further experimentation and were not imaged.

5.1.2 Fluorescence in fly heads

5.1.2.1 Images

In figure 13-16, the overview images are shown of the sectioned fly heads from all lines from all combinations of temperature. As mentioned, seven images were taken for each head: one overview, three on the central region and three images spread out on left lobe and right lobe. In cases where one region/lobe were not intact, this was instead taken on another section of the same line and temperature combination. Therefore, not all heads from these overview images were analysed fully with left lobe, right lobe and central region. These overview images can however be seen as representations of that particular line and temperature combination. No significant difference was seen between sections of the line and temperature combination.
In figure 13, images of fly head sections from flies born in 29°C and kept there for six days, are shown. All flies showed clear fluorescence intensity at 520 nm, and no major differences was clear by observing the images.

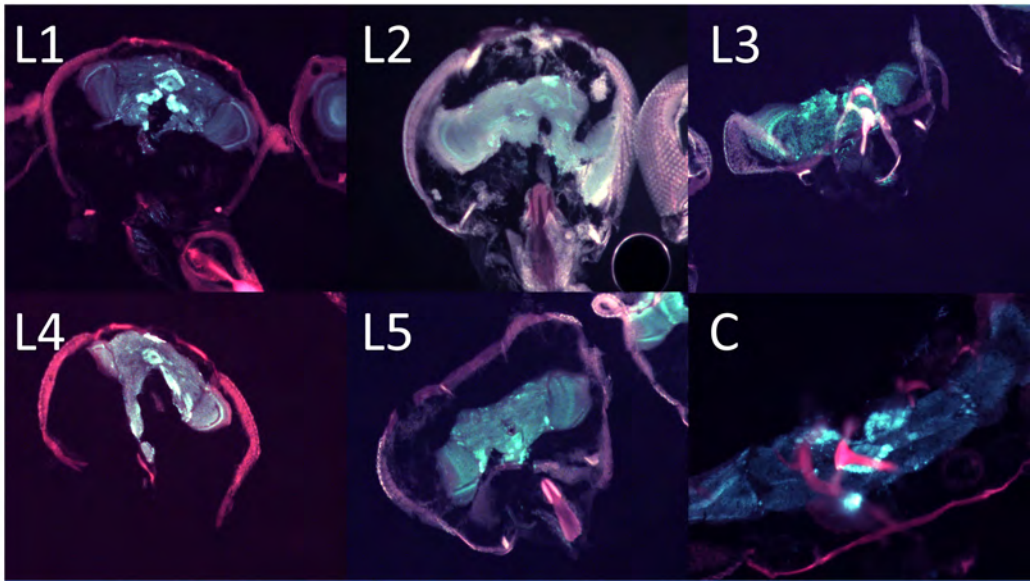

Figure 13: Images of fly head sections from flies born in 29° C and kept there for six days. L1 is Gal80$^+$FL1, L2 is Gal80$^+$FL2 and so on. C is Gal80$^-$FC.

In figure 14, images of fly head sections from flies born in 29°C which were later transferred to 18°C after three days and kept there for an additional 3 days, are shown. All flies showed clear fluorescence intensity at 520 nm, and only by observing these images, no clear difference could be seen, with the exception of Gal80$^+$FL5 (L5). However, mainly images of the central region were taken from this fly head section, and as will be described in section 5.1.3, the difference where not as major as one might believe when observing only this fly head section.

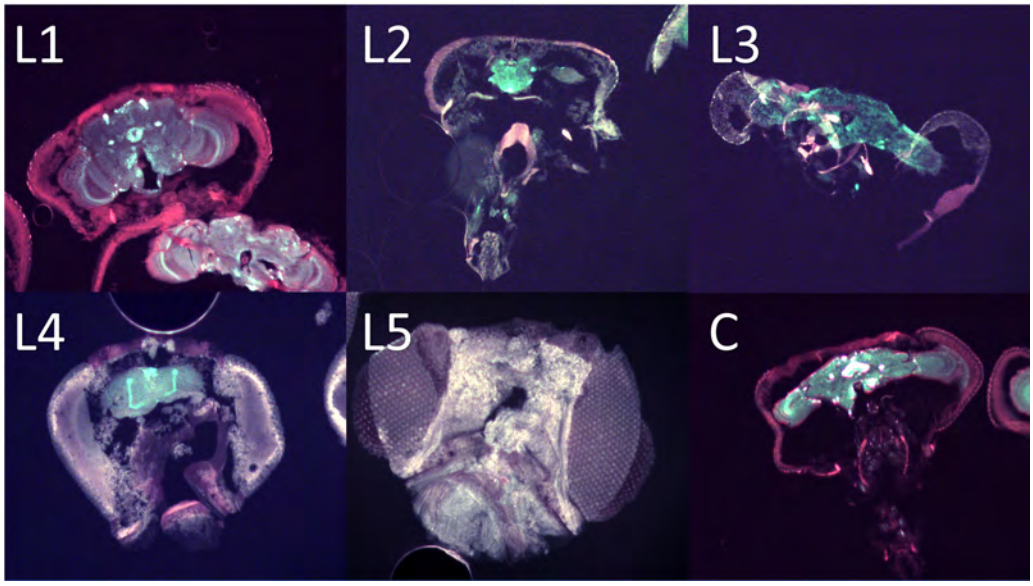

Figure 14: Images of fly head sections from flies born in 29° C, transferred to 18° C after three days and kept there for an additional three days. L1 is Gal80$^+$FL1, L2 is Gal80$^+$FL2 and so on. C is Gal80$^-$FC.

In figure 15, images of fly head sections from flies born in 18°C and kept there for six days, are shown. Notably, Gal80$^+$FL1 seemed to be having even more fluorescence intensity at 520 nm than Gal80$^-$FC. All other lines didn't seem to have any major fluorescence intensity at that wavelength.

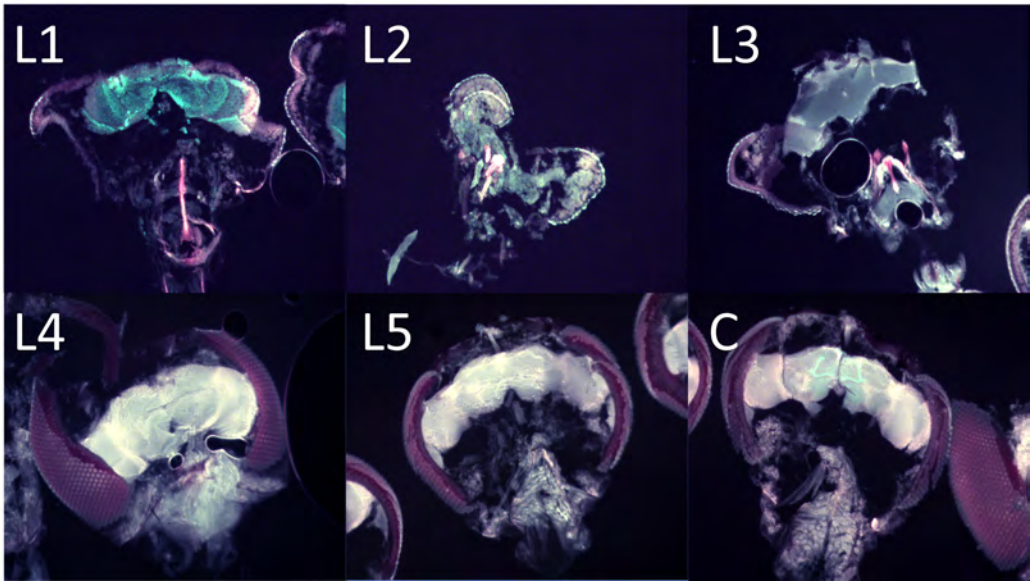

Figure 15: Images of fly head sections from flies born in 18° C and kept there for six days. L1 is Gal80$^+$FL1, L2 is Gal80$^+$FL2 and so on. C is Gal80$^-$FC.

In figure 16, images of fly head sections from flies born in 18°C which were later transferred to 29°C after three days and kept there for an additional 3 days, are shown. All heads analysed showed fluorescence intensity at 520 nm.

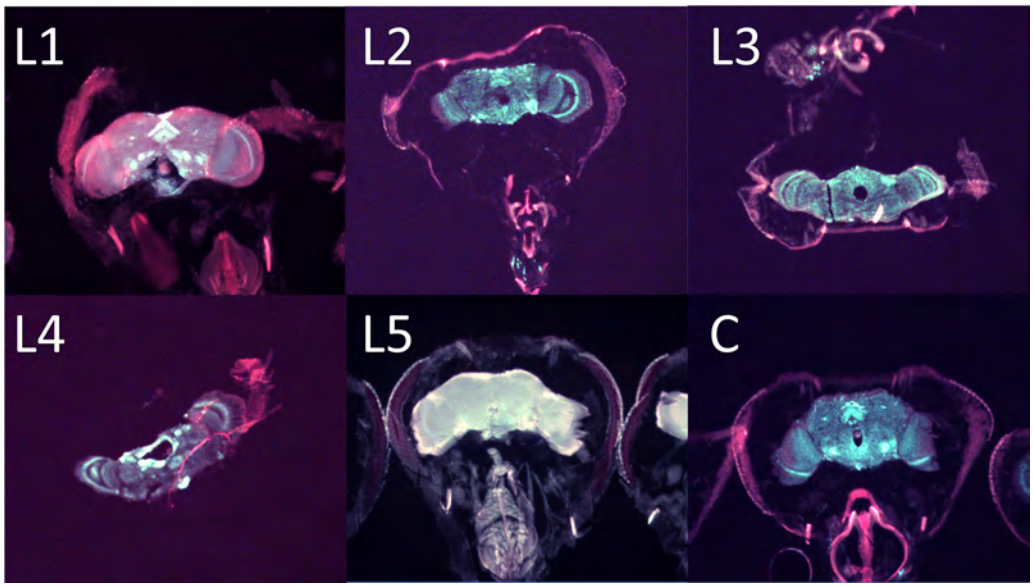

Figure 16: Images of fly head sections from flies born in 18° C, transferred to 29° C after three days and kept there for an additional three days. L1 is Gal80$^+$FL1, L2 is Gal80$^+$FL2 and so on. C is Gal80$^-$FC.

5.1.3 Fluorescence data from fly head images

The images were analysed by taking the intensity at 520 nm for each ROI on each image. The results are presented in figure 17. From these results two conclusions could be drawn: 1: Gal80$^+$FL1 did not have significant changes when changing the temperature. In fact, when changing the temperature from 29°C to 18°C it was performing even worse than the control. 2: Gal80$^+$FL4 and Gal80$^+$FL5 stood out from the rest when considering effectiveness. When taking the mean differences, Gal80$^+$FL4

were slightly more effective. However, to distinguish between them with higher degree of confidence, Gal80$^+$FL4 and Gal80$^+$FL5 were remade. Only flies born in 29°C and transferred to 18°C were used (collected the same time as the first set). When this data was added to the old data (see figure 18), it could be concluded that the two lines was once again very similar, but that Gal80$^+$FL4 had slightly higher mean difference. Because of this, Gal80$^+$L4 was selected for the rest of the project.

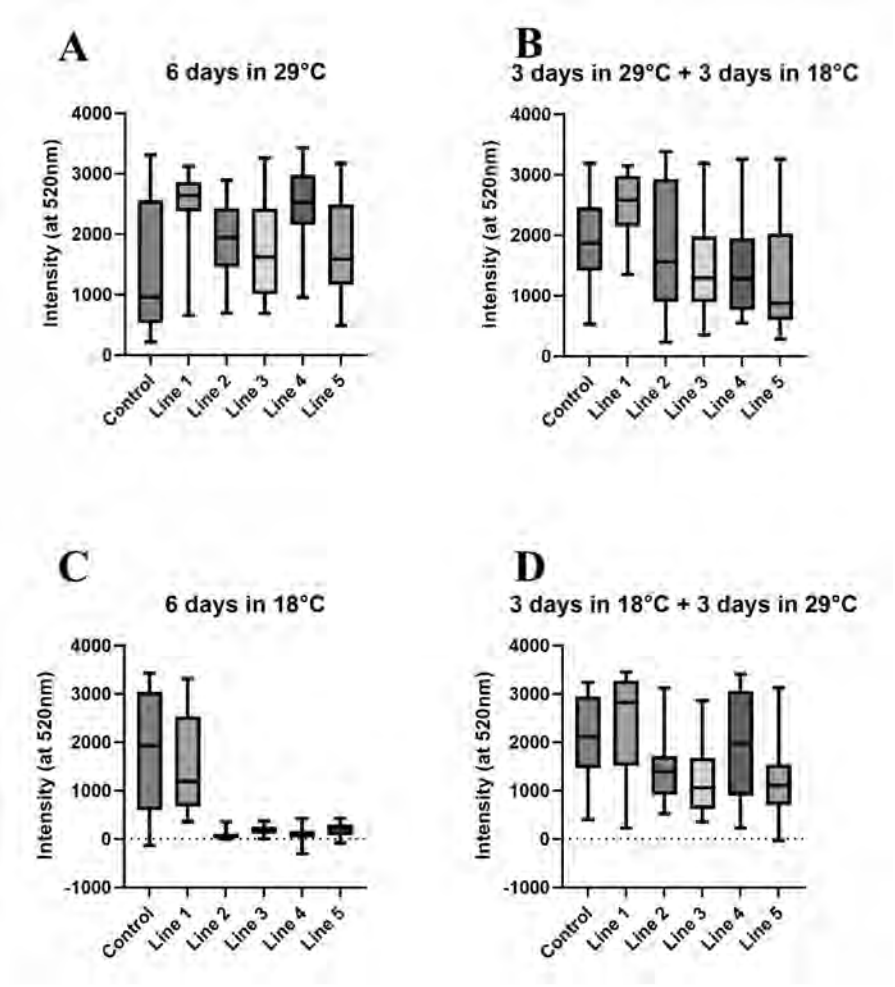

Figure 17: Plots of data gathered from images on Drosophila head sections. The data comes from the ROI chosen for each image at wavelength 520 nm. Control are Gal80$^-$FC, Line 1 are Gal80$^+$FL1, Line 2 Gal80$^+$FL2 and so on. In A, plots of the data gathered from flies born and kept in 29° C for six days are shown. In B, plots of the data gathered from flies born in 29° C, transferred to 18° C after three days and kept there for an additional 3 days, are shown. In C, plots of the data gathered from flies born and kept in 18° C for six days are shown. The negative values in this plot is due to the background-ROI, which is subtracted from each ROI intensity, are greater than the ROI-data. This can be considered as zero. In D, plots of the data gathered from flies born in 18° C, transferred to 29° C after three days and kept there for an additional 3 days, are shown.

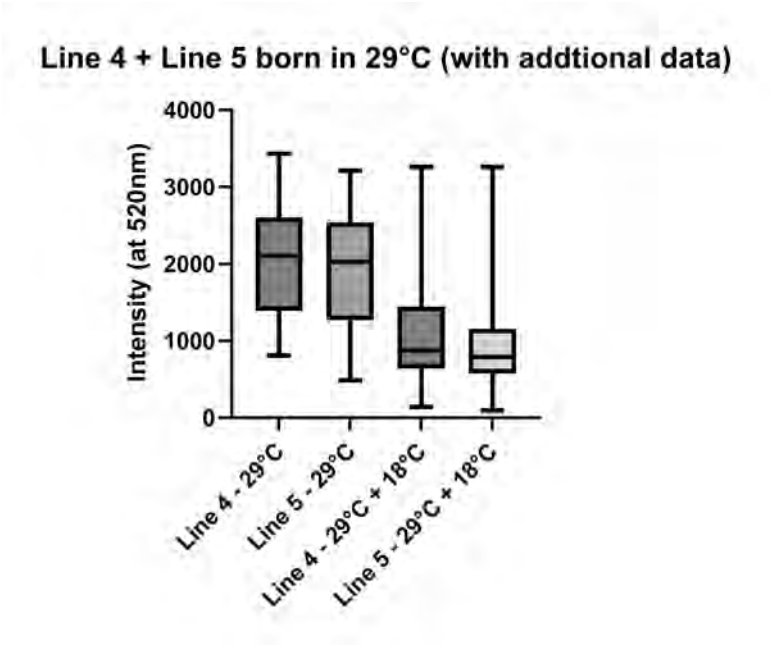

Figure 18: Plots of data gathered from images on Drosophila head sections from Gal80$^+$FL4 and Gal80$^+$FL5. The data shown are both the first set of data (shown in figure 17A-B) and data from another set of fly heads which was imaging at a later stage. The data comes from the ROI chosen for each image at wavelength 520 nm. Line 4 – 29° C are Gal80$^+$FL4 that were in 29° C for six days, Line 5 – 29° C + 18° C are Gal80$^+$FL5 that were in 29° C for three days and in 18° C for three days, and so on.

5.2 Lifespan assay

In figure 19-21, the results from the lifespan assay are shown. Females and males were separated in different vials, but the results shown here are combined.
In figure 19 the two groups that were either kept in 29°C (A) or 18°C (B) only are shown. In 29°C only it can be seen that the Empty line have a slow and steady decrease of survival percentage over time, and that this decrease rate are much slower compared to Gal80$^-$ and Gal80$^+$. Gal80$^-$ have a quick drop within the first 10 days, a very similar result to that reported by Jonson et al.[26]. Gal80$^+$ do however have clearly better survival than Gal80$^-$, an indication of that Gal80^{ts} might not be completely deactivated in 29°C. For the Empty line in 18°C only (see figure 19B) a quick drop in survival percentage can be seen, but are after this rather stable with few deaths. Gal80$^+$ does not show the same drop, and actually survived slightly better in the end of the assay. Gal80$^-$ has a significantly higher decrease rate of survival. However, all three lines survived longer in 18°C compared to 29°C.

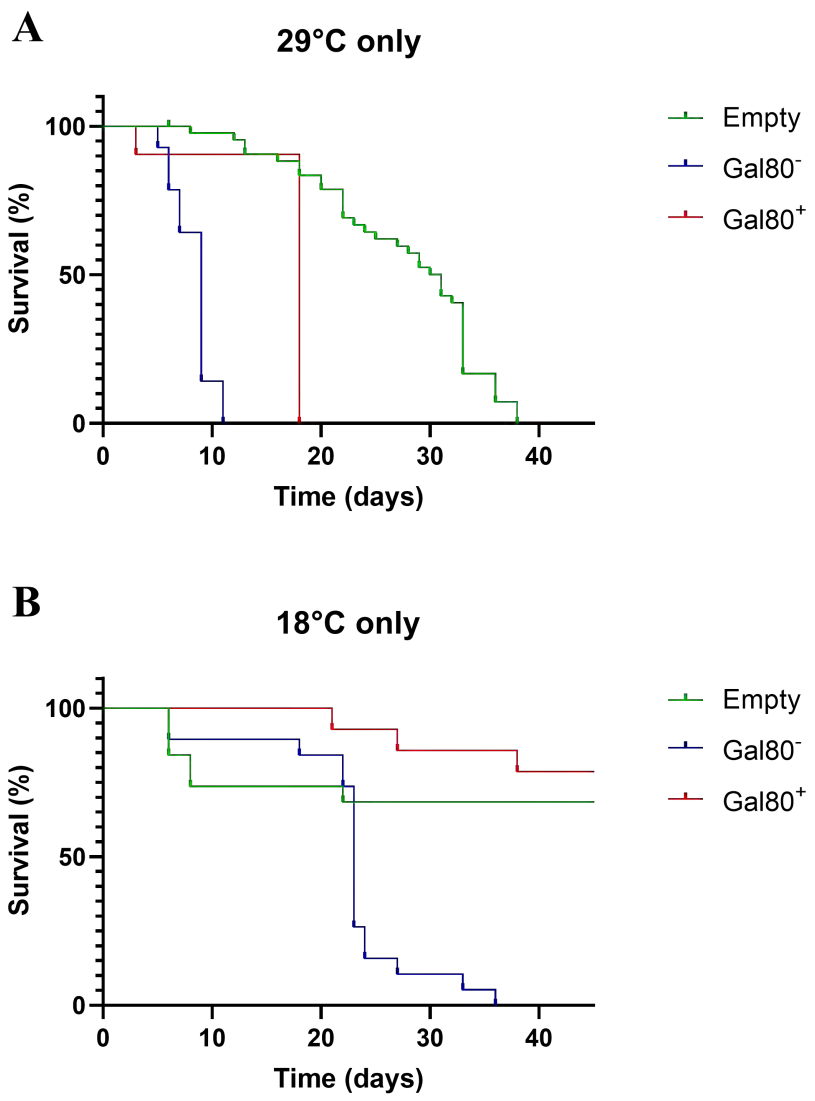

Figure 19: Plots of lifespan assay for the lines Empty (green), Gal80$^-$ (blue) and Gal80$^+$ (red) kept in 29° C only (A) and 18° C only (B).

For the Empty line born in 29°C and later transferred to 18°C (see figure 20) it can be concluded that no major difference was spotted between the flies that had been in 3, 5 or 10 days in 29°C. For the Gal80$^-$ line, there is a more distinct difference between the flies that had been in 3, 5 or 10 days in 29°C. The flies that had been in 29°C for 10 days are here very similar to the flies that only were in 29°C (see figure 19A), although the last flies that had been in 29°C for 10 days only survived one additional day after being transferred to 18°C. For Gal80$^+$, there are a major difference between flies kept in 29°C for 3 and 5, compared with those kept in 29°C for 10 days. In fact both 3 days in 29°C and 5 days in 29°C only dropped a few percent throughout the assay. 5 days in 29°C also had better survival rate than 3 days in 29°C, due to a quick drop in the beginning of the assay for 3 days in 29°C. 5 days in 29°C had an almost identical curve as both 18°C only (see figure 19B) and the Empty line that were for 5 days in 29°C.

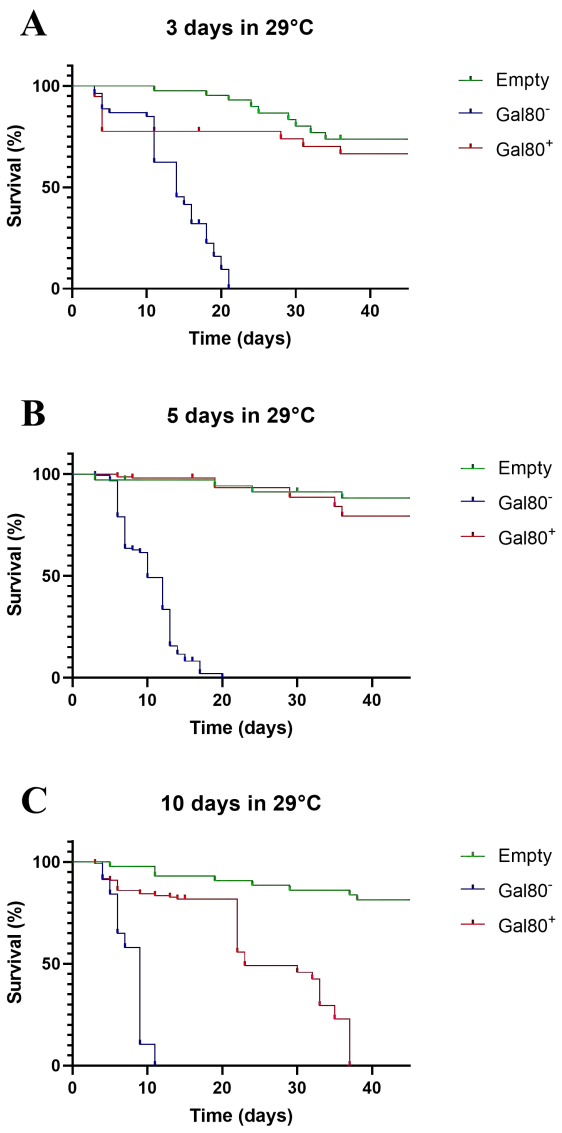

Figure 20: Plots of lifespan assay for the lines Empty (green), Gal80$^-$ (blue) and Gal80$^+$ (red) born in 29° C and transferred to 18° C after 3 (A), 5 (B) or 10 (C) days.

In figure 21 the survival data for the Gal80$^-$ and Gal80$^+$ lines that were born in 18°C and transferred to 29°C are shown. A clear difference between the three groups can be seen here, but with a relationship between being longer in 18°C and surviving longer. All groups also have a lag phase until switched to 29°C, were the two curves (from the two *Drosophila* lines) starts to separate. After this separation, it is clear that Gal80$^+$ survives longer, also in 29°C.

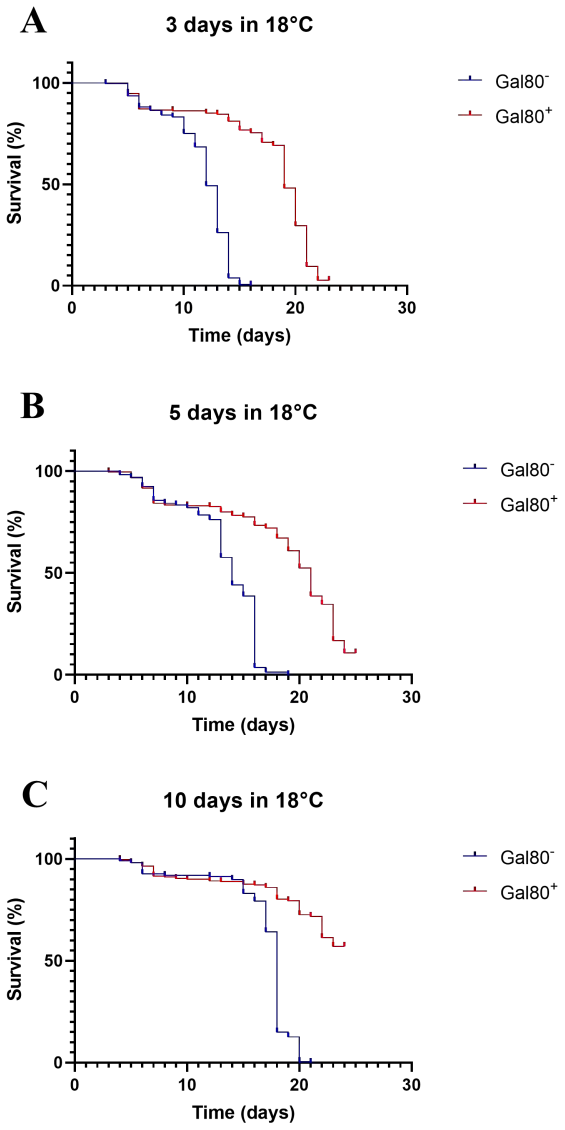

Figure 21: Plots of lifespan assay for the lines Empty (green), Gal80$^-$ (blue) and Gal80$^+$ (red) born in 18° C and transferred to 29° C after 3 (A), 5 (B) or 10 (C) days.

5.3 Activity assay

In figure 22 the resulting plots from the iFly assay is shown. In all velocity plots (i.e. figure 22A, 22C and 22E) there is a distinct difference between Gal80$^+$ and Gal80$^-$. However, no significant difference can be seen between the plots, i.e. no significant difference depending on how long each group of flies were in 18°C before being transferred to 29°C.
In the right hand plots in figure 22 (figure 22B, 22D and 22F), the difference in angle of movement between Gal80$^+$ and Gal80$^-$ are shown. No major differences can be seen between Gal80$^+$ and Gal80$^-$ for the flies kept in 18°C for 3 and 5 days. A minor difference in angle of movement could however be seen at the last day of the Gal80$^-$ recordings. In the group of flies that were kept in 18°C for 10 days, there is a significant difference however, even though Gal80$^+$ and Gal80$^-$ only had 4 and 2 days of recording (respectively) in this group. It should be said though, that the data for both 3 days in 18°C and 10 days in 18°C had major variance in each data point, which causes uncertainty about the exact differences. This causes the same uncertainty when comparing the plots from the three groups, even though the means in 3 days in 18°C shows a little lower angles.

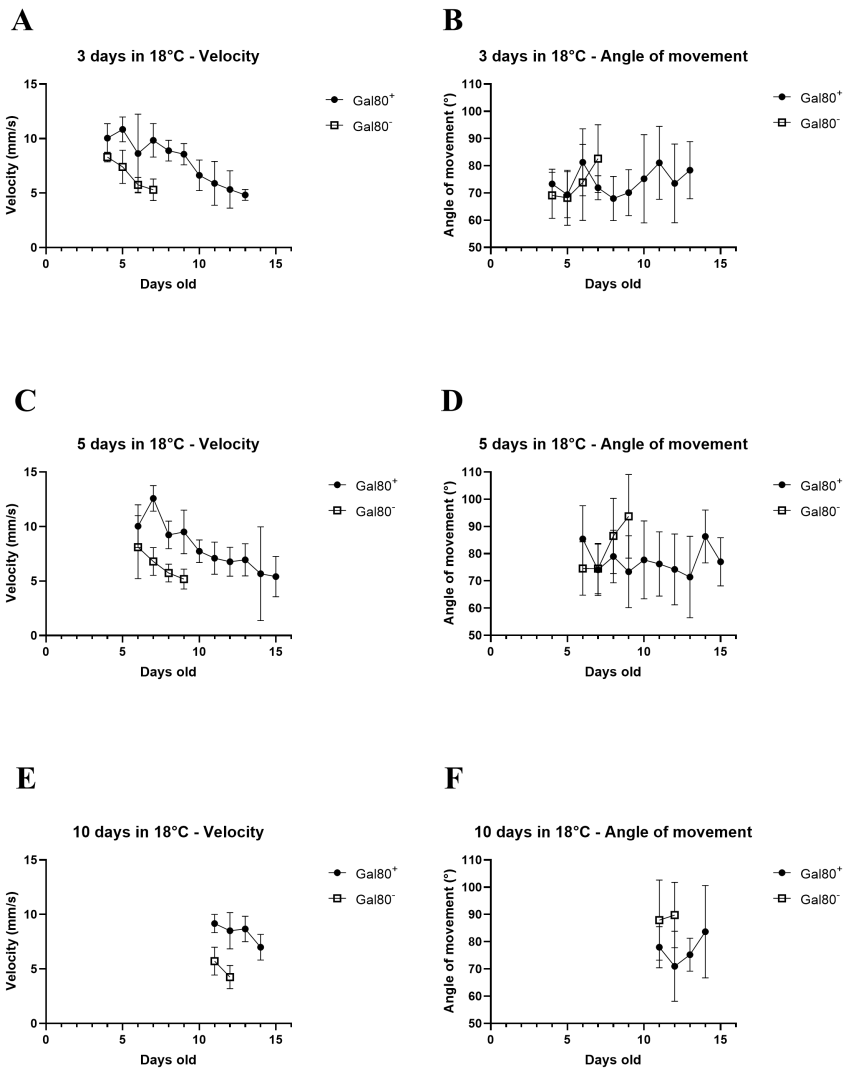

Figure 22: Plots generated from iFly data, both from velocity (left plots) and angle of movement (right plots). Circles corresponds to $Gal80^+$ and squares to $Gal80^-$. Plots for group of flies that where 3 days in 18° C and then transferred to 29° C (A-B), group of flies that where 5 days in 18° C and then transferred to 29° C (C-D), and group of flies that where 3 days in 18° C and then transferred to 29° C (E-F) are shown here. The first recorded data of each plot are when that group had been in 29° C for one day.

6 Process analysis

In this section, the process of the project will be discussed. More specifically, the planning which was discussed in section 3 and how it compares to how the actual project was performed. In figure 23, an updated GANTT chart is presented. The key difference is the lab work activity. This activity was planned to be finished about the same time. First of all, the line selection got delayed several weeks. This was mainly due to preparatory steps for the fluorescence microscopy of the fly heads, which both took more time than expected and had to be remade. Also, as discussed in section 5.1.3, additional data was added to distinguish two of the lines observed for a more confident line selection. This delay also had consequences on the rest of planned lab work, with both the activity assay and analysis of data being delayed. Moreover, more lab work was initially discussed, if time was available. This included protein detection methods as western blot, a second activity assay, called *Drosophila* Activity Monitor (DAM) system, and qPCR. The latter was actually performed in a late stage of the project, but unsuccessfully and was therefore disregarded from this report. Because of all mentioned changes, milestone 3 was divided into several different weeks.
Besides the lab work, the plan was more or less followed. Some minor changes were made to the chart regarding the final report and presentation, but an exact date for this was not yet set on the first two weeks of the project when the chart was made.
In table 3, all milestones, there planned calendar weeks and their actual calendar weeks are presented.

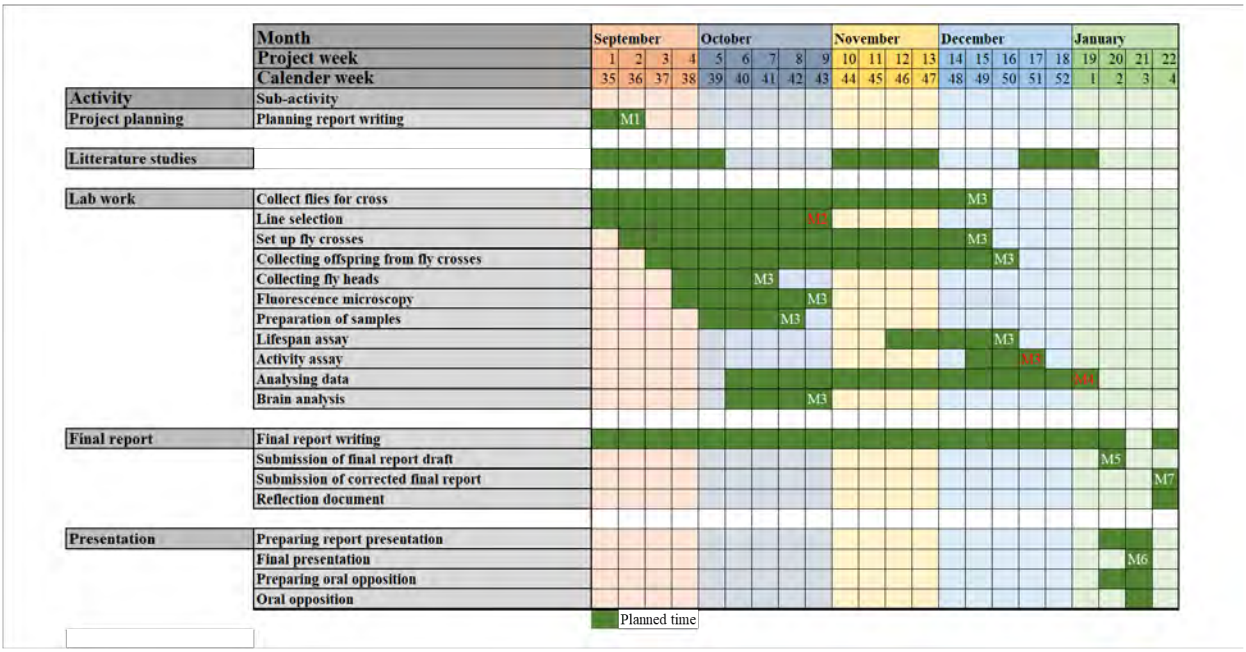

Figure 23: Updated version of the GANTT chart (first presented in figure 8), with milestones (M1-M6) marked. Delayed milestones are marked in red.

Table 3: Table with description of the projects milestones with its planned calendar week and the actual calendar weeks.

Milestones	Description	Planned calendar week	Actual calendar week
M1	Submission of planning report	36	36
M2	Line selection finished	37	43
M3	Lab work finished	50	41, 42, 43, 49, 50, 51
M4	Analysing data finished	52	1
M5	Submission of final report draft	1	2
M6	Final presentation	3	3
M7	Submission of corrected final report	4	4

7 Discussion

7.1 Discussion of results

In these results it has been shown that Gal80^{ts} can work highly effective together with the UAS/Gal4 system and an Aβ1-42 transgene. In this section, both results from the line selection process and the rest of the assays will be discussed together. This is because the line selection process not only could be seen as a step to select the correct line but can also be seen as further evidence of the effectiveness of Gal80^{ts}. The selection itself were more or less straightforward. Instead focus should here be on Gal80^{+}FL4 (the line selected) and Gal80^{-}FC (the control). Both in figures 11-12 and in figures A1-A2, i.e. the figures showing images on the larvae, there is a clear difference in green fluorescence intensity between the two temperatures when comparing the Gal80^{+}FL4 images. The Gal80^{-}FC still had clear green intensity (at 520 nm) in images from both temperatures. The presence of this intensity in as young larvae as 1st instar, shows that expression of Aβ1-42 starts at an even younger stage, presumably in the embryonic stage. Not only did the larvae show green fluorescence in the brain, clear fluorescence intensities could also be seen scattered along the body in shapes indicating that high degree of aggregates also were present. This is an important difference when comparing *Drosophila* with humans. Aβ aggregation has a lag phase (see figure 3). In humans this is also clear in Aβ mediated dementia, which also have a lag phase when in young ages, and dementia (and AD) usually reaches a clinical disease stage at older ages [27]. It is important to remember here that in this project, Aβ1-42 were expressed together with the UAS/Gal4 system and in this step also fused with mNG. In humans Aβ peptides is a result of a β-secretase and γ-secretase mediated cleavage of APP. This difference could indicate that the lag phase of Aβ mediated dementia seen in humans could be because of the amyloidogenic pathway, rather than a result of the fibrillation lag phase.
When analysing the data from the fly heads sections, it was also clear that not only were there a difference when having the flies only in 18°C or 29°C. Changing the temperature after three days also had an effect on expression of mNG-Aβ1-42. Surprisingly, Gal80^{-}FC, actually had lower mean intensity when kept in 29°C only, compared to all of the other combinations. This should however not be put in too much focus. The quality of the sections varied, and the ROI could more easily be selected from a complete section, than from a highly disrupted section. The ROI was also selected manually, meaning that there could have been higher intensities that were missed. Lastly, an additional ROI was selected from each image representing the background. In some cases this background-ROI had larger intensity at 520 nm than the the other ROI, leading to some negative values. This was especially true for the images which had very low intensities at 520 nm. This decrease in intensity was also not confirmed in any way in the lifespan and activity assays. It should however be emphasized that the flies in the line selection process expressed Aβ1-42 fused with the fluorescent probe mNG. The probe is 236 residues long, compared with Aβ1-42 that is 42 residues long, i.e. a major difference in size. How this effect the aggregation, and if the probe itself might be aggregation-prone, is not known. Nevertheless, this was not a factor that seemed to play a large role when comparing the fluorescence data with the lifespan and activity assay.
When the line selection process was finished, the lines expressing mNG-Aβ1-42 was no longer used, and so no further consideration needed to be taken regarding the probes effect on aggregation. When comparing the Empty line with the Gal80^{-} line (see figure 19-20), it is clear that expression of Aβ1-42 causes shorter lifespan, regardless of temperature. Comparing the curves from all lines in 18°C and 29°C only (see figure 20) does however point out an important concept: flies tend to survive longer in 18°C compared to 29°C, regardless of transgenes. One final point could also be made regarding the Empty-line: flies dying in the first few days probably has no relation with either temperature or Aβ expression. Tendencies of a quick drop in survival percentage, as seen in figure 19B (in green) is probably due to coincidence or by another factor not discussed here.
When instead focusing on the comparison of Gal80^{-} and Gal80^{+}, a significant difference is clear. This applies to both the number of days in each temperature and between the two lines. Interestingly, Gal80^{+} in the 5 days in 29°C group (see figure 20) still seemed to have had enough of Gal80^{ts} expression to have an almost identical outcome as those in 18°C only (see figure 19B). Here, the concept of an early drop in survival percentage can also be repeated. If it were not for the early drop in Gal80^{+} survival when for 3 days in 29°C, also this curve would have been fairly similar to those in 18°C only and 5 days in 29°C. Still, a distinct difference is seen when flies are kept in 29°C for 10 days, where

the expression of Gal4 and Aβ seemingly "outcompetes" the expression of Gal80^{ts}. The same pattern is naturally seen when flies are kept in 29°C only. The reversed relations can be seen in flies born in 18°C and later transferred to 29°C (see figure 21). The key difference between the two lines in figure 21 is however that Gal80^{+} flies survive significantly longer, with flies in all groups outliving the assays time limitation. The two curves are very similar when kept in 18°C, but starts to branch off each other first when switched to 29°C. This indicate either that Gal80^{ts} is not completely deactivated when in 29°C, or that Aβ has already been expressed in a much higher degree in Gal80^{-} and that the higher temperature accelerates the Aβ aggregation and neurodegenerative process.
The data obtained from iFly (see figure 22) follows the same pattern as the previous plots regarding the difference between Gal80^{+} and Gal80^{-}, at least for the velocity factor. A steady decrease can be observed in all three plots, indicating a progressive neurodegenerative process, presumably caused by Aβ1-42. An initial difference is seen in the first data points of the velocity plots, but the curves is then decreasing at approximately similar rates, indicating a temperature sensitivity, presumably due to Gal80^{ts}. No major difference could be seen between the three groups (3, 5 or 10 days in 18°C). The reason for this might be that all groups are kept in 18°C and only one day in 29°C prior to the recordings. When initially in 18°C, a lag phase can be seen in the lifespan plots (see figure 21). I.e. both Gal80^{+} and Gal80^{-} seem to have a period of time where no major neurodegenerative or lethal effects of Aβ aggregation seem to occur. Still, data from much fewer days could be obtained from the flies that were 11 days old on day one of recording, compared to the other two. The two groups had on the other hand similar number of days where data could be gathered. Not as many conclusions could however be drawn from the angle of movement factor. All three groups had high variance at each day. A difference could be seen in the flies kept in 18°C for 10 days, and in this group the increase was also more obvious, which was expected considering all other data obtained in this project, but more data should be gathered before making any profound statements on this. Also, because of the high variation at each day, if significant outliers were to be subtracted from each data point, there is a possibility that more could have been said about these plots.

7.2 Conclusions

The importance of AD related research cannot be emphasized enough. As mentioned, it is estimated that more than 131 million people will suffer from the disease by 2050. More specifically, research on Aβ can also lead to research progress on other neurodegenerative diseases, as Lewy body dementia. The organism *Drosophila melanogaster* is an excellent model for this kind of research. Low cost, high homology in disease related genes, relatively fast life cycle and mating, to name a few. Moreover it's much larger and complex than many other model organisms with the same regulatory restrictions concerning ethics (or lack of such), like prokaryotes or *Caenorhabditis elegans*. There is thereby a need for making this organism as manageable and as useful as possible. In this project, experimentations have been made to add to the understanding of the effect from Aβ related diseases. Moreover, for the first time, an Aβ1-42 transgene has been inserted into Drosophila together with the UAS/Gal4 system and Gal80^{ts}. The result from this project shows clear effectiveness of Gal80^{ts} when expressed in this context. Not only does it show major effectiveness when expressed in only 18°C or 29°C but switching from one temperature to another seem to have clear effects as well. The results are so clear that even a two day difference show distinct differences in survival percentage, working in both directions of temperature change.

7.3 Further prospects

There are several complementary assays that can be performed to add to the data given in this project. First of all, instead of using a fluorescent probe, staining of the fly head could be done to observe how mNG affects the aggregation and complement these results. Brain dissection could also be performed to work around the inconsistencies that cryosectioning might bring. For a longer project, a full lifespan assay could also be made, where all flies are kept until 0% survival are reached for all groups. Another activity assay (like DAM system) could complement iFly, since this assay is better fitted for younger flies and the flies in this project in one case had not started to be recorded until the flies were eleven days old. Finally, several assays could be performed to confirm that the shorter lifespan and decreased velocity were due to Aβ aggregation. Examples of this being Aβ1-42 quantification by ELISA, western blot and qPCR.

Further complement to the overall research on Aβ and AD (of course) need to be, and are, made. As mentioned in section 1.1.3, several drugs are today used for treatment of AD, both that target β-secretase and γ-secretase, but also that target the Aβ aggregates directly. The latter includes antibody utilization, for example newly FDA approved Aducanumab [11] and Lecanemab [12]. In the light of these two, interesting and important research would be to investigate the degradation of *in vivo* Aβ aggregates, something that today is not fully understood. Hopefully, the project presented here can be of use in such an investigation, and add a small piece of the very complex puzzle that the neurodegenerative diseases in whole present.

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8 References

[1] M. J. Prince, A. Comas-Herrera, M. Knapp, M. M. Guerchet, and M. Karagiannidou, "World alzheimer report 2016-improving healthcare for people living with dementia: Coverage, quality and costs now and in the future.," 2016.

[2] H.-M. Gao and J.-S. Hong, "Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression," *Trends in immunology*, vol. 29, no. 8, pp. 357–365, 2008.

[3] M. A. DeTure and D. W. Dickson, "The neuropathological diagnosis of alzheimer's disease," *Molecular neurodegeneration*, vol. 14, no. 1, pp. 1–18, 2019.

[4] W. W. Barker, C. A. Luis, A. Kashuba, M. Luis, D. G. Harwood, D. Loewenstein, C. Waters, P. Jimison, E. Shepherd, S. Sevush, *et al.*, "Relative frequencies of alzheimer disease, lewy body, vascular and frontotemporal dementia, and hippocampal sclerosis in the state of florida brain bank," *Alzheimer Disease & Associated Disorders*, vol. 16, no. 4, pp. 203–212, 2002.

[5] K. A. Dill and J. L. MacCallum, "The protein-folding problem, 50 years on," *Science*, vol. 338, no. 6110, pp. 1042–1046, 2012.

[6] K. A. Dill, "Dominant forces in protein folding," *Biochemistry*, vol. 29, no. 31, pp. 7133–7155, 1990.

[7] S. Sadigh-Eteghad, B. Sabermarouf, A. Majdi, M. Talebi, M. Farhoudi, and J. Mahmoudi, "Amyloid-beta: a crucial factor in alzheimer's disease," *Medical principles and practice*, vol. 24, no. 1, pp. 1–10, 2015.

[8] C. Priller, T. Bauer, G. Mitteregger, B. Krebs, H. A. Kretzschmar, and J. Herms, "Synapse formation and function is modulated by the amyloid precursor protein," *Journal of Neuroscience*, vol. 26, no. 27, pp. 7212–7221, 2006.

[9] B. Das and R. Yan, "A close look at bace1 inhibitors for alzheimer's disease treatment," *CNS drugs*, vol. 33, no. 3, pp. 251–263, 2019.

[10] T. E. Golde, E. H. Koo, K. M. Felsenstein, B. A. Osborne, and L. Miele, "γ-secretase inhibitors and modulators," *Biochimica et Biophysica Acta (BBA)-Biomembranes*, vol. 1828, no. 12, pp. 2898–2907, 2013.

[11] I. S. Padda and M. Parmar, "Aducanumab," in *StatPearls [Internet]*, StatPearls Publishing, 2021.

[12] C. H. van Dyck, C. J. Swanson, P. Aisen, R. J. Bateman, C. Chen, M. Gee, M. Kanekiyo, D. Li, L. Reyderman, S. Cohen, *et al.*, "Lecanemab in early alzheimer's disease," *New England Journal of Medicine*, vol. 388, no. 1, pp. 9–21, 2022.

[13] E. Karran and B. De Strooper, "The amyloid hypothesis in alzheimer disease: new insights from new therapeutics," *Nature reviews Drug discovery*, vol. 21, no. 4, pp. 306–318, 2022.

[14] E. Mahase, "Alzheimer's disease: Fda approves lecanemab amid cost and safety concerns," 2023.

[15] P. Fernandez-Funez, L. de Mena, and D. E. Rincon-Limas, "Modeling the complex pathology of alzheimer's disease in *Drosophila*," *Experimental neurology*, vol. 274, pp. 58–71, 2015.

[16] M. D. Adams, S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne, P. G. Amanatides, S. E. Scherer, P. W. Li, R. A. Hoskins, R. F. Galle, *et al.*, "The genome sequence of *Drosophila melanogaster*," *Science*, vol. 287, no. 5461, pp. 2185–2195, 2000.

[17] S. Chyb and N. Gompel, *Atlas of Drosophila Morphology: Wild-type and classical mutants*. Academic Press, 2013.

[18] S. E. McGuire, G. Roman, and R. L. Davis, "Gene expression systems in *Drosophila*: a synthesis of time and space," *TRENDS in Genetics*, vol. 20, no. 8, pp. 384–391, 2004.

[19] E. E. Caygill and A. H. Brand, "The gal4 system: a versatile system for the manipulation and analysis of gene expression," *Drosophila*, pp. 33–52, 2016.

[20] J. W. Lichtman and J.-A. Conchello, "Fluorescence microscopy," *Nature methods*, vol. 2, no. 12, pp. 910–919, 2005.

[21] N. C. Shaner, G. G. Lambert, A. Chammas, Y. Ni, P. J. Cranfill, M. A. Baird, B. R. Sell, J. R. Allen, R. N. Day, M. Israelsson, *et al.*, "A bright monomeric green fluorescent protein derived from *Branchiostoma lanceolatum*," *Nature methods*, vol. 10, no. 5, pp. 407–409, 2013.

[22] M. D. Piper, D. Skorupa, and L. Partridge, "Diet, metabolism and lifespan in *Drosophila*," *Experimental gerontology*, vol. 40, no. 11, pp. 857–862, 2005.

[23] B. Biteau, J. Karpac, D. Hwangbo, and H. Jasper, "Regulation of *Drosophila* lifespan by jnk signaling," *Experimental gerontology*, vol. 46, no. 5, pp. 349–354, 2011.

[24] J. Tower, "Transgenic methods for increasing *Drosophila* life span," *Mechanisms of ageing and development*, vol. 118, no. 1-2, pp. 1–14, 2000.

[25] K. J. Kohlhoff, T. R. Jahn, D. A. Lomas, C. M. Dobson, D. C. Crowther, and M. Vendruscolo, "The ifly tracking system for an automated locomotor and behavioural analysis of *Drosophila melanogaster*," *Integrative Biology*, vol. 3, no. 7, pp. 755–760, 2011.

[26] M. Jonson, S. Nyström, A. Sandberg, M. Carlback, W. Michno, J. Hanrieder, A. Starkenberg, K. P. R. Nilsson, S. Thor, and P. Hammarström, "Aggregated Aβ1-42 is selectively toxic for neurons, whereas glial cells produce mature fibrils with low toxicity in *Drosophila*," *Cell chemical biology*, vol. 25, no. 5, pp. 595–610, 2018.

[27] C. R. Jack Jr, D. S. Knopman, W. J. Jagust, R. C. Petersen, M. W. Weiner, P. S. Aisen, L. M. Shaw, P. Vemuri, H. J. Wiste, S. D. Weigand, *et al.*, "Tracking pathophysiological processes in alzheimer's disease: an updated hypothetical model of dynamic biomarkers," *The Lancet Neurology*, vol. 12, no. 2, pp. 207–216, 2013.

Appendix

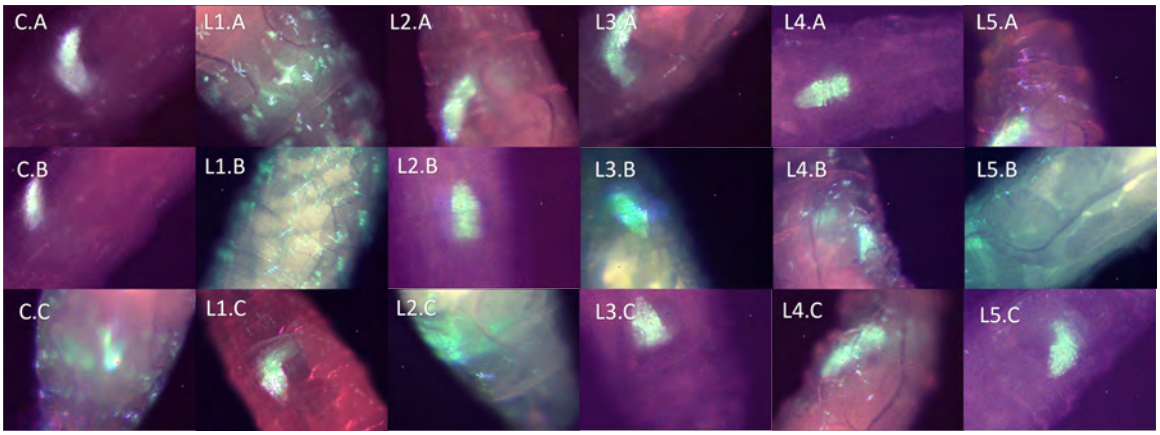

Figure A1: All larvae which was born in 29° C and observed with fluorescence microscopy at wavelength 520nm. L1 is Line 1, L2 is Line 2 and so on, and C is control. A, B and C refers to different larvae imaged within that particular line.

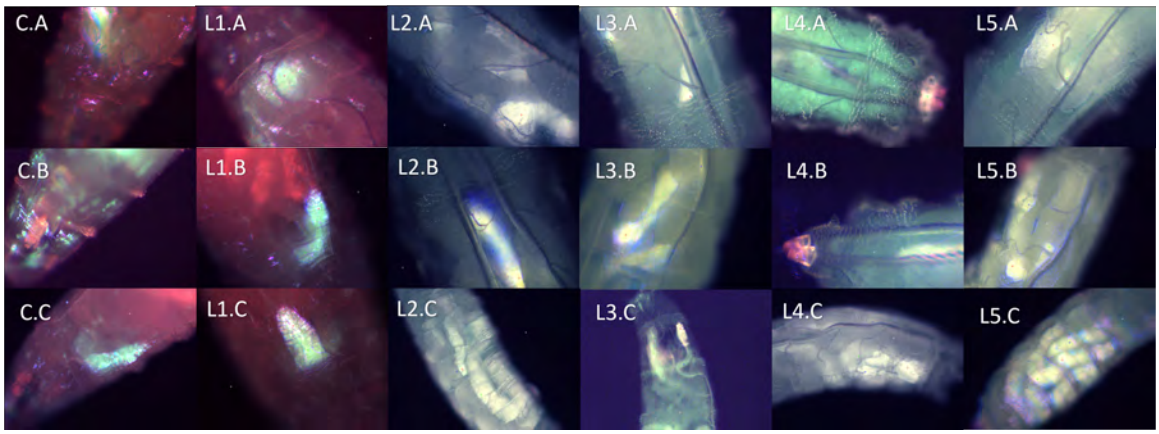

Figure A2: All larvae which was born in 18° C and observed with fluorescence microscopy at wavelength 520nm. L1 is Line 1, L2 is Line 2 and so on, and C is control. A, B and C refers to different larvae imaged within that particular line.