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A Summary of Current Research into Neurodegenerative Disorders Using Drosophila melanogaster

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A Summary of Current Research into Neurodegenerative Disorders Using *Drosophila melanogaster*

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Abstract

Neurodegenerative disorders such as Alzheimer's disease are characterized by damage to neural cells causing widespread neurological deficits. Historically, these diseases were deemed as inevitable declines in cognitive and neurological function that occurred with aging. Recent research has revealed, however, that these diseases have molecular bases allowing potential clinical intervention. The groundbreaking discoveries have been made using differing model organisms, which can replicate the phenotype and physiological cause of these disorders. One major model organism that has been used is the fruit fly, *Drosophila melanogaster*. They have served various roles in elucidating gene pathways and neurophysiological bases seen in these diseases. Current research using *Drosophila* is attempting to further characterize the molecular basis along with potential clinical treatments. These new in-depth studies are in part a result of advancing methodologies, which allows greater flexibility in the types of experiments that can be done with *Drosophila*.

Introduction

1.1 Summary of major neurodegenerative disorders

Some of the major neurodegenerative diseases researched today are Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). They each have their own known pathophysiology along with symptomatic presentation.

AD is a disease that normally appears in those over the age of 65. One of its initial symptoms is memory deficit. As the disease progresses, other symptoms begin to develop such as aphasia, motor dysfunctions, emotional liability, etc. The accumulations of amyloid beta proteins and tau proteins are considered to be the primary causes of AD. The toxic amyloid beta originates from cleavage of its precursor protein, APP, by gamma secretase. The amyloid beta fragments then stick together to form plaques, which activate the immune system causing inflammation and thus neural cell death. Tau protein, which normally plays a role in regulating microtubules in brain cells, can become hyperphosphorylated and lose its biological function. This lack of microtubule regulation can lead to degradation of the synapses along with other cellular issues[1].

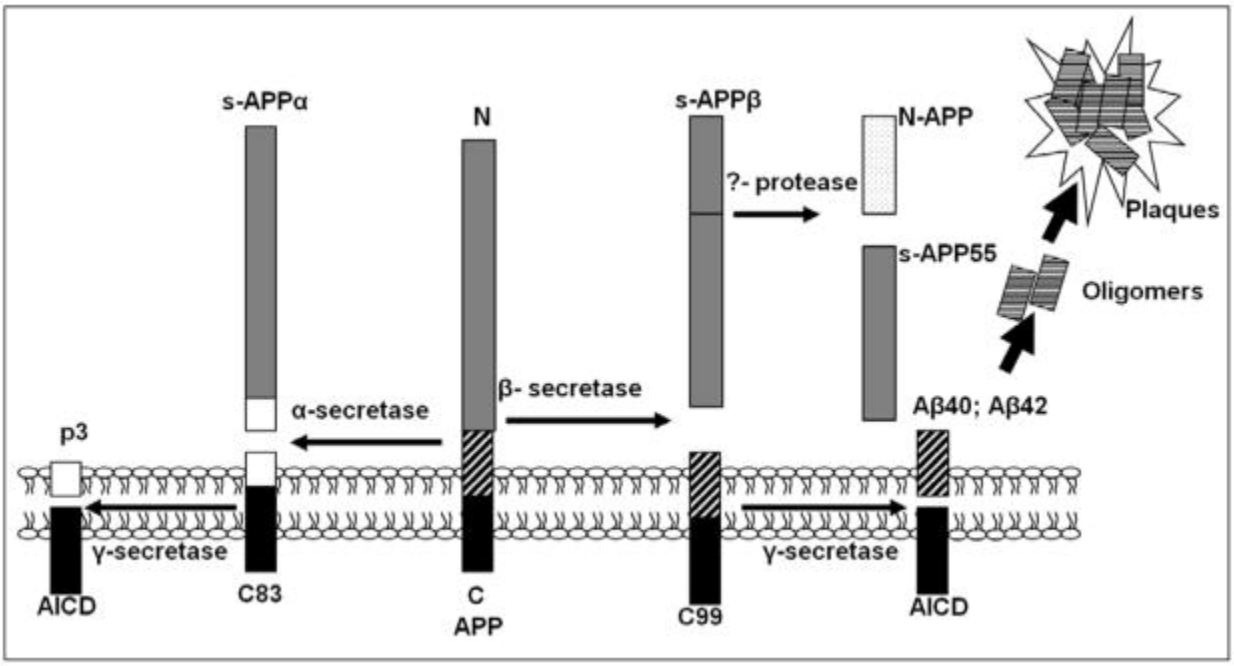

Figure 1. Figure demonstrating cleavage of APP by beta, and then gamma secretase leading to amyloid beta fragments. Image obtained from Zhang et al [2]

PD is a disease characterized by motor deficits that also typically appears in elderly individuals. The motor defects are one of the primary symptoms that have been researched and observed, but there are multiple other non-motor symptoms as well. These include cognitive declines, sleep disruptions and problems with autonomic body functions. The cause of PD is not well documented, but the loss of dopaminergic neurons in the substantia nigra tends to be observed along with lewy body deposition. Some factors that are thought to play a role in the development of PD include genetic factors, environmental toxins, and other damages to neural tissues causing potential protein misfolding. The two major genes believed to be implicated are PINK1 and PARKIN, which play a role in triggering autophagy due to damaged mitochondria[3,4].

HD is another neurodegenerative disease characterized by movement deficits that usually develops in people in their 30's to 50's. The primary symptoms are motor deficits that include loss of coordination and involuntary movements. Similar to PD, non-motor symptoms also begin to appear such as depression and other cognitive effects. The genetic basis of HD is known to involve more than 36 CAG trinucleotide repeats in the huntingtin (HTT) gene, which interferes with protein function and the subsequent neural degeneration. Not much is currently known about the normal function of huntingtin protein[5].

1.2 The *Drosophila* model

Drosophila melanogaster is an arthropod that is most commonly known as the fruit fly. In the wild, they seek out rotting fruit or other plant matter, which they spend time feeding on, and is the location where females eventually lay hundreds of eggs. The eggs then hatch into a larval state after a period of 18-24 hours. The larva move around and feed on where they were lain for about 4 days, and then subsequently enter a pupal stage. The pupal stage lasts another 4 days, and then an adult emerges from the pupae and lives around 40 to 120 days [6].

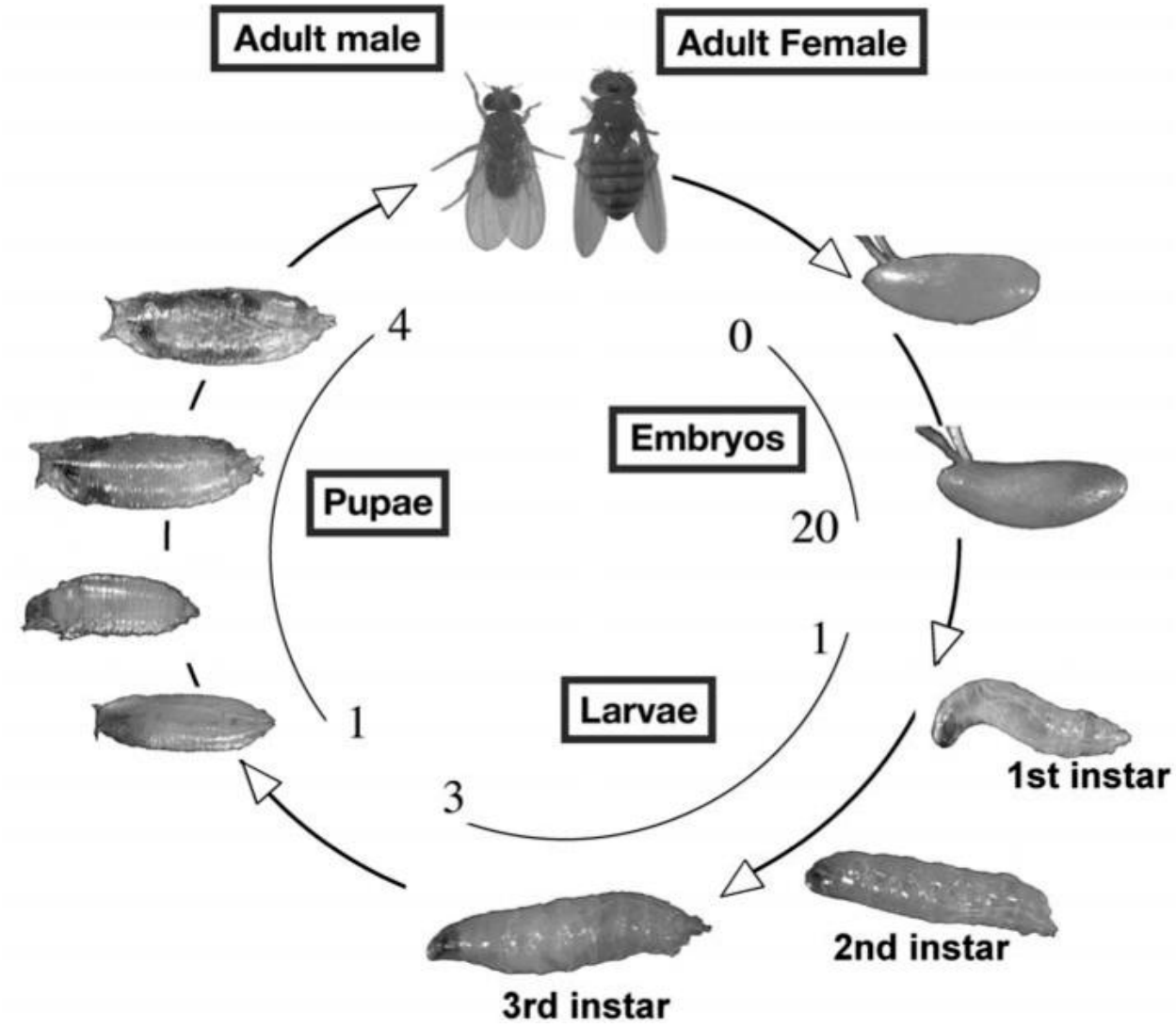

Figure 2. Figure demonstrating images of different stages of the lifecycle as obtained from Fernández-Moreno et al[6]

D. melanogaster contain 4 chromosomes along with 13,600 genes. 2 of the chromosomes which *D. melanogaster* contain are sex chromosomes, with XY resulting

in a sex of male, and XX resulting in a sex of female. *Drosophila* show sexual dimorphism, with males being slightly smaller, having a dark bottom, and containing hairs on their forearms called “sex combs.” Females are larger and are striped all the way down their bottom. Unlike in mammals, where females undergo random X-chromosome inactivation to compensate for their double X chromosomes, in *D. melanogaster,* males undergo X hyperactivation in which their x chromosomes produces twice as much transcript[7].

Drosophila are commonly used in the study of genetics, pathology, and neuroscience due to the reasons listed above. They are cheap, and easy to maintain (only needing to be kept in vials containing a source of food). Their means of reproduction and small number of chromosomes enables easy genetic manipulation and a large group of offspring containing a desired genotype. Their relatively short lifespan allows for phenotypes to be observed throughout development, including degenerative phenotypes in later stages of development. Due to *D. melanogaster’s* widespread use as a model organism, much of their anatomy and genes are well characterized, allowing in-depth studies on specific functions[8].

1.3 *Drosophila* Neurodegenerative models

Drosophila have additionally emerged as a predominant model organism used for the study of neurodegenerative diseases along with *C. elegans.* Anatomically, the nervous system of *D. melanogaster* is well documented, with different cellular lineages such as the mushroom body (aids in learning and memory) having specific genes and protein products that are well known. Additionally, the neurophysiology is relatively similar to humans, with communication between neural synapses being a result of neurotransmitters such as GABA, acetylcholine, etc. There are also behaviors that *Drosophila* exhibit relative to learning and memory that serve as parallel to human behaviors, allowing for observations of specific deficits. Finally, there are many *Drosophila* homologs to human genes observed in diseases. This allows for the manipulation of the paralogs and subsequent phenotype observation[9].

Their genetic makeup, along with various human homologs, are commonly exploited for the use of neural research. The genotype can be manipulated to observe the resulting phenotype via a change in the expression or mutation of already existing genetic homologs, the insertion of human genes known to cause neural disease, or through the insertion of RNA*i* suppressors to modify gene expression. The insertion of human disease genes can be used to create many neurodegenerative models. For example, there can be an amyloid beta gene inserted to model AD, or an HTT mutant inserted to model HD[9].

One of the major ways this is achieved is through the elav-Gal4/UAS system. Elav (embryonic lethal abnormal visual) is a RNA-binding protein that is present in the nervous system of *Drosophila* throughout its development[10]. Gal4 is a yeast derived transcriptional activator protein that contains no normal targets in flies and is inserted

into Gal4/UAS models. UAS (upstream activation sequence) is an enhancer region that is activated by the transcriptional activator protein Gal4. In this transgenic setup, one parental line will have the elav-Gal4 gene, whereas the other line will have a UAS paired with the gene being expressed. When the lines are crossed, the ELAV protein will drive the Gal4 expression in the nervous system. The Gal4 will subsequently bind to the UAS enhancer causing the expression of the desired gene. These transgenic lines are usually produced at lab supply centers such as Bloomington stock, but can also be generated in lab via insertion of the desired vector into embryos[11].

The neurodegenerative *D. melanogaster* models demonstrate various phenotypes that can be indicative of the resulting neurodegeneration. One of the most general observable phenotypes is a reduced lifespan, which is caused by increased levels of toxic proteins that lead to neurodegeneration. Another observable phenotype is a decrease in motor function, caused by motor regions of the CNS being deteriorated. This can be demonstrated in *Drosophila* via the use of "negative geotaxis assays," which take advantage of *Drosophila's* tendency to move against gravity. When significant motor degeneration has occurred, the flies can be observed failing to move up to the top of their containers as they normally would. Another observable phenotype from neurodegeneration is a loss of memory and learning. This can be observed in *Drosophila* models via courtship assay's, which measure a male's ability to remember and learn from rejection[12].

To conduct further research to elucidate functions related to specific neurodegenerative pathologies, additional manipulation of models will usually take place. To develop a further understanding of genetic interactions, additional genes can be inserted or manipulated into well-established models. For example, a gene expected of preventing neurodegeneration can be upregulated in an AD model and compared with the control AD model to observe its effect. To check for potential external factors involved in pathology, lifestyle changes can be enacted on the *Drosophila* such as prevention of sleep in a PD model. Additionally, potential therapeutics can be tested by adding them to food and then observing phenotype changes of the experimental group versus the model. These manipulations are a few of the examples that are currently being used by researches in the field to find the biological basis behind neurodegeneration.

2. Current Research Breakthroughs Using *Drosophila*

2.1 Autophagy

Autophagy is one of the mechanisms through which damaged cells and tissues are removed in multicellular organisms. This process has been demonstrated to have an important relationship with neurodegeneration, as cells containing toxins, damage, or misfolded proteins can be safely discarded in non-pathological tissue. The general mechanism of autophagy involves lysosomal digestion of the cell through 3 differing mechanisms. The first is chaperone-mediated autophagy, where specific proteins with

defects are identified and then marked and sent to the lysosome for digestion. This mechanism allows for specific proteins with deficits to be disposed of. The next major form of autophagy is microautophagy, which is characterized by the lysosome directly taking up specific parts of the cell. This allows for specific areas of the cell that may contain toxins to be disposed of. The final major type of autophagy is macroautophagy, which is characterized by the formation of the autophagosome which fuses with the cell's lysosome. The resulting autolysosome then allows for its contents to be broken down. This process is the major one that research is currently being done on in relation to neurodegeneration[13].

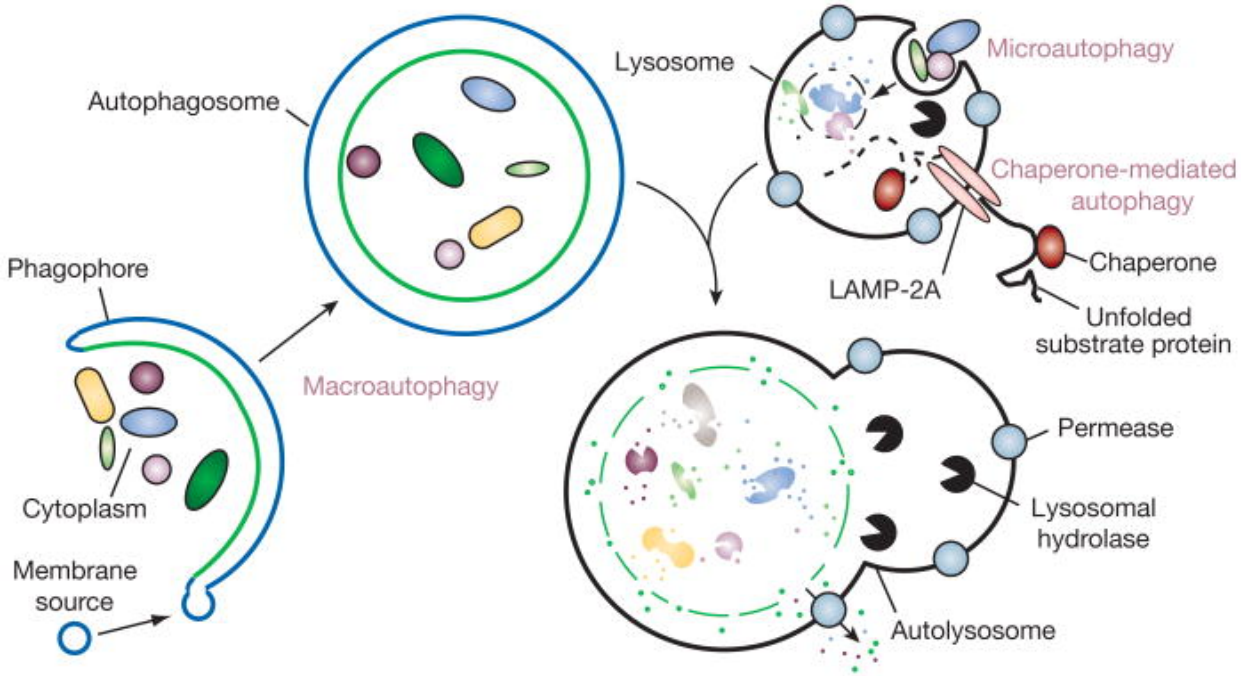

Figure 3. A figure demonstrating the major autophagic processes as described above. Obtained from Mizushima et al[14]

It is currently being discovered that autophagy plays a key role in the maintenance of the nervous system. Some of the major aspects of neurons that are maintained by autophagy and found to be important in the degradative process are defective mitochondria and proteins found in the axon and synapse. The defective proteins found in the synapse and axons can be observed in neurodegenerative disease such as AD, where the mutant Amyloid beta and tau protein accumulate[13].

As genes that are critical for autophagy in the nervous system are being investigated, they are being found to play a role in neuronal aging as well as degeneration. One of the major classes of genes being looked into are the Atg genes[13]. This family of genes contains proteins that form complexes with each other that play a role in the development of the autophagosome. Deficits in these genes can hinder the formation of the autophagosome and cause neuronal degradation and shortened lifespan, with one known example being Atg1[15]. Additionally the loss of Atg5 or Atg7 in *Drosophila* leads to

a similar histology (protein aggregates, neurodegeneration) and phenotype (climbing defect, memory defects, and shortened lifespan) to that which is typically seen in neurodegenerative models[16].

Another gene pathway involved in autophagy that is currently being demonstrated to have a relationship with neuronal maintenance are SNARE proteins[13]. SNARE proteins are proteins that play an important role in vesicle fusion. One of the SNARE genes investigated to play a role in autophagy is Syx17 (Syntaxin 17). The *Drosophila* Syx17 mutants were found to have reduced locomotive ability along with shortened lifespans, similar to that seen in neurodegenerative models. With the Syx17 mutants, there was an observed accumulation of autophagosome along with protein aggregates seen in the CNS[17].

Another study demonstrating the role of autophagy in neuronal maintenance looks at the effects of upregulating autophagy on nervous system health. It was found that enhancing the level of Atg8 protein in adult *Drosophila* resulted in effects such as enhanced lifespan and a reduced presence of protein aggregates. The average adult lifespan increased about 56% as compared to a non Atg upregulated control, and a 12 fold reduction in protein aggregates was observed. Additionally, upregulated autophagy was found to increase the neural resistance to reactive oxidative species, which is a major known risk factor for neural degeneration[18].

2.2 Mitochondrial changes

Mitochondria have been found to function in the pathology of various neurodegenerative diseases, with some type of mitochondrial dysfunction being noted in the 3 that are discussed. Mitochondria typically function in neurons by carrying out energy production, metabolism of nutrients, senescence, and eventual apoptosis or necrosis of neurons[19]. Some of the mechanisms being investigated related to a mitochondrial role in neurodegeneration include a problem with mitochondrial fusion/fission, alterations to mitochondrial metabolism, calcium homeostasis, and regulation of cell death/autophagy[20].

In PD, an impairment to the complex I region of the mitochondria has been observed. This affects the mitochondrial ability to carry out dopaminergic cell death, as well as increases the amount of free radicals that are found in neurons. The defect with the complex I region of the mitochondria leads to a higher number of free radicals within the cell, requiring heightened activation of the antioxidant system to reduce the levels to normal. The increased presence of ROS can cause reactions and change the formation of important cellular molecules like lipids that constitute the membrane along with various cellular proteins. The damage to these cellular components leads to the neural degeneration seen in PD and other similar diseases[21].

Calcium homeostasis is an important part of normal cellular functions. Additionally, high levels of calcium found in the cell can lead to cell death. The mitochondria play an important role in buffering intracellular calcium levels through calcium uptake.

Mitochondrial dysfunction along with calcium dysregulation has been observed in various neurodegenerative disorders such as AD, PD, and HD. Defects in the ER-mitochondrial contact sites are also frequently observed in these diseases. In AD, problems with calcium maintenance and mitochondria appear before any obvious protein aggregates. Additionally, neurons in AD models were shown to have an increase in ER/Mitochondrial connections. For PD, some of the major genes such as Parkin and PINK1 have roles in maintaining calcium homeostasis. While there is shown to be a relationship between calcium homeostasis and neurodegeneration, an exact mechanism is still unknown[22]. Current research is looking into the specific mechanisms through which calcium dysregulation causes the aforementioned changes in neurodegeneration. For example, Popugaeva et al[23] delves deeper into the specific proteins that require proper calcium levels for homeostasis and the resulting effects of dysregulation. In AD, these include the development of toxic protein plaques, synaptic weakening, etc.[23].

As discussed above in 2.1, autophagy and cell death can serve important roles in preventing the build-up of dysfunctional cells and toxic proteins. However, if the process of cell death is not regulated properly, healthy cells and tissue can die. This can be in part due to the failure of the mitochondria in regulating calcium levels, leading to an increase in reactive oxygen species, and thus cell death. It appears that defects with the mitochondrial calcium uniporter has a relation to neurodegeneration from oxidative stress[24].

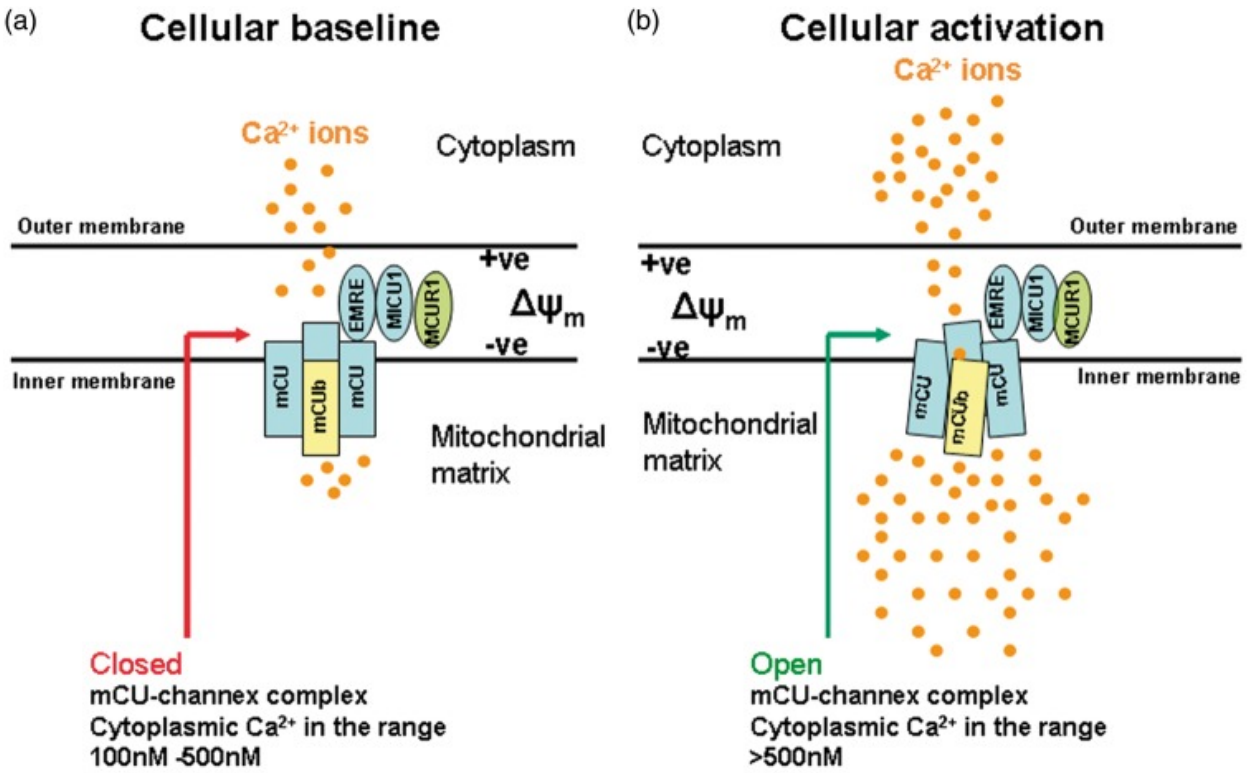

Figure 4. A figure demonstrating the role of the mitochondrial calcium uniporter in the cell. This is one of the mechanisms by which mitochondria regulate calcium in a healthy cell. Image obtained from Kannurpatti[25]

2.3 Synaptic degeneration

One of the major hallmarks seen in any neurodegenerative disease is a decline is synaptic processes. Synapses in a healthy CNS work to allow communication between neurons and thus allow the CNS to function as a whole. In most neurodegenerative illnesses, even before cell death is observed, there are changes seen in synapses. These include a decrease in the amount of individual synapses, alterations to synaptic structure, and changes to both pre and post synaptic structures. These changes can be seen in various diseases such as AD, PD, and HD[26].

Synaptic function plays a key role in memory, which is one of the most obvious deficits in AD patients and models. One of the proposed mechanisms by which synaptic function can be dysregulated in AD models involves the improper activation of microglial cells. These cells normally play an important role in the neurological immune system and function to remove damaged neurons. When they are exposed to amyloid beta, it is thought that they function incorrectly and can degrade important synaptic structures[27]. Another mechanism for the early development of memory deficits is the reduction in gene expression of proteins important for synaptic transmission. This is thought to occur with the expression of amyloid beta[28].

In non-pathological neurons, the proteins PINK1 and PARKIN work together to cause the degradation of damaged mitochondria. When PINK1 is mutated, as seen in PD, the failing mitochondria cannot be removed. As mentioned in 2.2, mitochondrial defects can lead to an accumulation of ROS, dysregulation of calcium homeostasis, along with energetic deficits of the cell. While extreme accumulations of defective mitochondria can result in inappropriate neuronal death, synaptic function can also be hindered with minor levels of mitochondrial dysfunction. This is due to the high reliance of synaptic release of neurotransmitters on mitochondria-regulated processes. The packaging of neurotransmitters into vesicles and eventual exocytosis requires a large amount of ATP. Additionally, calcium is the ionic trigger which signals the release of neurotransmitters. When there is calcium dyshomeostasis and energetic deficits due to dysfunctional mitochondria, communication is hindered at the synapses. In Doktór et al[3], they further demonstrate that *Drosophila* PINK1 mutations lead to a decrease in the presynaptic protein BRP, which aids in the accumulation of calcium channels for neurotransmitter release[3].

The Htt protein’s primary functions in non-pathological tissues are not well documented. It is known that HTT can be found on some membranes and plays a role in endocytosis. In Akbergenova and Littleton[29], an increase in synaptic connections was noted due to an increase in BMP signaling. They attributed the increase in BMP signaling to

pathogenic Htt blocking the termination of the BMP receptor. The pathologic Htt is seen to prevent proper exchange of vesicles at the synapse in *Drosophila* models. This leads to an inability for communications between neurons or at the neuromuscular junction[29].

2.4 Summary of current research direction

The bulk of neurodegenerative research in *Drosophila* is looking into genes related to specific disease processes. These include studies into the prevalence of certain phenotypic deficits, histological changes, and molecular changes. While these studies typically attempt to elucidate specific disease mechanisms, there are some commonalities found between the different neurodegenerative disorders. These common traits present in different ways in each disease as noted above. Despite the differences, the connections between the mechanisms of disease progression can be used to draw conclusions about overall neurodegenerative disease progression.

There are extensive studies in *Drosophila* to expand upon the exact mechanisms and genetic involvement seen in the varying neurodegenerative disorders. One of these areas of research involves the progression of cell death, which is a crucial hallmark of all neurodegenerative diseases. This has been demonstrated to occur via various processes in the major neurodegenerative diseases. In AD, one of the primary causes of neuronal cell death is related to build ups of toxic protein. In PD, damaged mitochondria can abnormally signal the process of cell death. Finally, in HD there is some dysregulation of the autophagic process observed causing inappropriate cell death[30]. In addition to neuronal death being observed in these diseases, there is similar mechanistic pathways through which they progress. One of the most common vectors of progression involves dysregulated autophagy. This can cause an excessive amount of cell death in cells with accumulated toxic proteins or mitochondrial deficits. It can also cause a lack of autophagy in cells that are damaged, leading to a buildup of defective cells. These pathways of degeneration are linked to the mitochondrial dysfunction and calcium dyshomeostasis as outlined above.

Research on *Drosophila* is also currently looking into mitochondrial function in neurodegenerative diseases. Again, the specific mechanisms vary between the different diseases in the cause of mitochondrial-based degeneration. The major processes that are thought to be involved are alterations to aerobic metabolism causing ROS buildups, calcium dysregulation, and also their role in autophagy. Current research is looking into the specific gene pathways that are involved in the pathologic processes. The general findings that are noted include the discovery that the mitochondria can be the key source of the problems found in the other areas of pathological problem. The mitochondria play key roles in mediating cell death. Calcium, which is regulated by mitochondria, plays a role in abnormal neural death as well as autophagy. Additionally, mitochondria are being researched due to their importance in proper synaptic function.

When there is a dysfunction in mitochondria, synapses are one of the first areas of the nervous system to begin showing deficits.

Synaptic function is an additional major area of research currently being conducted. This is partially due to the importance of synapses to the function of memory and neural health, which is shown to decline in the different neurodegenerative diseases. Many separate studies into differing sources of neurodegeneration demonstrate some form of synaptic dysfunction. Current research is looking into the different regions of the synapses that are the source of the issue. It has been found that presynaptic structures, and release of vesicles cannot function properly with dysfunctional mitochondria. Also, specific genes have found to be important in the release of the synaptic vesicles, and the receiving of neurotransmitters from the previous neuron. Dysfunctions in any of these processes can lead to improper neuronal communication.

2.5 Therapeutic applications

Treatments and therapeutics have historically been limited in neurodegenerative disorders due to a limited understanding of the diseases' molecular mechanisms. Recent research is helping to break down this barrier, and open the doors for studies into different therapeutics. While for most neurodegenerative diseases a complete remedy is still far off, different therapeutics are still being developed now. These therapeutics would work by interfering with the pathological processes that are being discovered in currently evolving research.

In AD, there has been a large amount of focus on the amyloid beta pathway of neural degeneration. This has led to a focus on development of anti-amyloid beta drugs, which have not demonstrated much success in symptomatic relief. One major therapeutic possibility involves intervention in the calcium pathway. The excessive calcium presence leads to weakened synapses through long term depression mechanisms. This is responsible for some of the memory loss observed in AD patients, and is an early hallmark for AD. Popugaeva et al[23] proposes several different therapeutic approaches based on the different levels of calcium dyshomeostasis as a result of ER leak channels. One of these includes direct inhibitor treatments of the membrane protein responsible for the calcium leak. It also proposed potential treatments to the downstream proteins that are affected by the abnormal calcium levels[23].

Understanding the function of certain proteins that are found in neurodegenerative diseases is crucial for furthering research into therapeutic possibilities. One protein that is currently being researched for its relation to Lewy Body Dementia, AD, and PD is alpha synuclein. Alpha synuclein has been shown to aggregate and form Lewy bodies, which are found in the progression of PD. Accumulations of this protein are additionally found in various types of neurons in PD patients and models[31]. Alpha synuclein, along with the other known PD genes such as PINK1 and Parkin, have been demonstrated to participate in mitochondrial function and clearance. Specifically, alpha synuclein has

recently been observed interacting with Miro, which is a protein that is required to be removed from the outer mitochondrial membrane before mitophagy can be initiated. Mitophagy is an important process for disposing of damaged mitochondria. This is important for neurons due to the reasons discussed in 2.2, as damaged mitochondria can quickly cause problems with proper neural functioning. When alpha synuclein beings to accumulate, Miro protein has been found to be upregulated. When the expression of Miro is downregulated in *Drosophila* PD models, a significant decline in both locomotive defects and neurodegeneration was observed. This demonstrates that a therapeutic that works to reduce alpha synuclein (and thus Miro) could aid in reducing degradation seen in PD[32].

In HD, mutant HTT plays a large role in the progression of the disease. It has a toxic effect on neurons, causing eventual neurodegeneration. After mutant HTT is produced, it undergoes many changes that play a role in its toxicity and function in the neurons. One of these changes include ubiquitination, a process that normally functions in marking the degradation of a protein within the cell. Ubiquitin proteasome system is a protein that plays a role in ubiquitination and the autophagy clearance pathway. When this is protein is stimulated, autophagy can occur in cells containing toxic proteins. This allows for neurons to clear toxic cells that can spread neurodegenerative proteins. Other proteins that play a role in the autophagic process could serve to aid in treating HD, along with other neurodegenerative disorders[33].

3. Current Research Methodology in *Drosophila* research

3.1 Transgenic lines

Drosophila have been used to obtain a large portion of the above information, and are one of the crucial models for neurodegenerative research. Before determining specific pathways by which the diseases progress, a common model had to be established. *Drosophila* research is heavily dependent on having a common model for which to base studies on particular diseases. These models are achieved through various types of transgenic lines. These transgenic lines can be generated in a lab via vector insertion and crossing, or more commonly received from a stock center that creates specific models. One of the frameworks through which neurodegenerative *Drosophila* models are typically created is the Elav-gal4 uas line which is discussed above in 1.3. This general outline of the processes of transgenic lines include inserting a gene that produces proteins known to be heavily involved in the production of neural deficits. These genes' expression are then driven by tissue specific transcription factors, which allow expression in desired tissues. Key genes have generally been used for each disease model, however there are variants of each gene which produce slightly different pathologies[34].

The primary type of gene insertion for HD models include HTT genes with CAG repeats. The main types of variation that is found in the inserted genes in transgenic HD lines

involves varying amount of glutamine repeats. The different amounts of variations cause phenotypic variations in severity, onset, as well as differences in the eye structure. Overall, *Drosophila* HD models tend to display a decrease in motor function, and shortened lifespan. In addition to just the HTT protein, some common models include different protein expressions that are thought to interact with the HD pathway. These include chaperone proteins that can either alleviate or worsen the phenotype, as well as proteins that play a role in autophagy[35].

Models for AD in *Drosophila* typically include some variation of the amyloid beta protein or tau combined with the Elav-gal4 model. The amyloid beta gene that is included in the transgenic lines is typically a directly toxic variant of amyloid beta such as amyloid beta 42 or 40. It can also be the precursor of which this toxic protein is formed. This includes an APP protein and the gene that encodes for the enzyme that cleaves it into its toxic variant, beta-secretase or BACE. This can be used to observe pathways that involve the formation of amyloid beta. Another gene that is inserted to generate the transgenic lines is tau, which can be included with the amyloid beta genes. Additional variant models include genes thought to have some relationship to the development including ApoE and presenilin. When these genes are inserted or crossed into the Elav-gal4 model, similar neurodegenerative declines to the amyloid beta models are observed[36].

Similar to the other neurodegenerative models, PD in *Drosophila* is modeled through inserted genes that produce a similar phenotype to human PD. These genes include multiple mutant variants of “PARK” with some of the significant ones being SNCA, parkin, Pink1, DJ-1, and LRRK2. They all induce similar phenotypes and cause increased ROS and mitochondrial damage, but with differences among the mechanisms through which they function. The phenotypes of these different PD models usually include locomotive defects, shortened lifespan, wing muscle weakening, dopaminergic neuron loss, and sometimes retinal degeneration[21].

3.2 Further Manipulation of Models

The above models are the origin of how to replicate the diseases in *Drosophila*. In order to conduct research on molecular mechanisms in *Drosophila*, additional manipulation of the models is required. Researches do this via physical manipulation of the model, the introduction of external substances, or further genetic manipulation. Further physical manipulation could include exposure to exercise or increased darkness. The introduction of external substances could include the addition of alcohol in the food of a PD model. Finally, further genetic manipulation could include the insertion of an RNAi vector to observe the downregulation of another gene. All of these methods help to elucidate further processes behind the disease. These manipulations are then paired with subsequent observation of molecular and phenotypic changes to demonstrate what processes are being affected.

Varying physical factors has long been used as a method to observe phenotypic changes in model organisms. These factors are important as they represent normal behavioral variations in disease progression that would normally be seen in humans. Researchers have developed creative methodologies to achieve these same variation in animal models. One example of this type of manipulation is seen in the blue light experiments of Nash et al[37]. In this study, *Drosophila* were exposed to a different light conditions and subsequent neurodegeneration was observed. Another example of physical factor manipulation is seen in the exposure to exercise as seen in Berlandi et al[38]. This study utilized a “swing boat” to expose groups of *Drosophila* to exercise, and then observed a subsequent relation to changes in neurodegeneration.

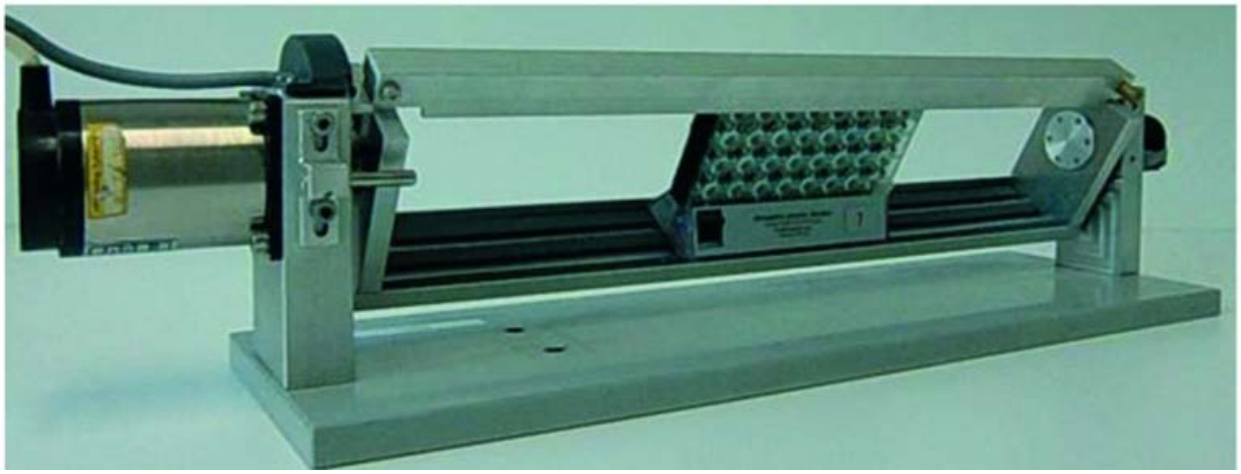

Figure 5. Figure depicting the Swing Boat as used in Berlandi et al[38]

The inclusion of external substances to neurodegenerative *Drosophila* models is another important consideration. These inclusions are important to test the effects of chemicals in disease progression that humans are normally exposed to. These can include chemical exposures to substances such as pesticides in PD, or a study looking to connect alcohol with neurodegeneration. These types of experimental modifications are also important for the testing of potential therapeutics. The addition of potential therapeutics and subsequent observation can be used to further expand upon disease processes, or demonstrate substances that could be beneficial. These external substances are typically given to the models through their food. An example of a study that uses this type of manipulation is Mattioli et al[39], in which *Drosophila* are given food containing a plant extract containing polyphenols.

Another type of alteration made to *Drosophila* models is further genetic manipulation, where genetic expressions in the model are modified to help elucidate cellular pathways and related genes. This is achieved through direct changes to genes, insertion of additional genes, or alteration of gene expression through RNAi. Changes to genes could include site directed mutagenesis in which individual nucleotides can be altered.

Including additional genes in the lines can elicit phenotypic changes with interactions between the inserted genes, such as including tau in the amyloid beta models. Finally, alterations of gene expression through RNAi allows the monitoring of specific native genes relating to disease progression. This method is used in Abul Khair et al[40], in which an RNAi knockdown of a glucocerebrosidase gene is included in a PD model. The resulting molecular and phenotypic changes in relation to control PD models was then observed.

3.3 Methods for Observing Effects of Manipulation

After the additional changes have been made to the neurodegenerative *Drosophila* models, procedures are carried out to view the changes to the model. These include both methods of observing phenotypic changes and molecular changes. To observe neurological phenotypic changes, the most common assays done measure the model's locomotive ability, lifespan, and memory. To observe the molecular changes, typical molecular lab procedures are carried out including protein analysis, nucleic acid analysis, and immunohistochemistry.

The different assays to observe phenotypic changes are done by taking advantages of some of *Drosophila's* unique properties. As discussed in 1.3, locomotor assays are done to observe any changes to the models motor ability. This is accomplished by taking advantage of *Drosophila's* negative geotaxis, or movement against gravity. Locomotor assays take place by trapping a fly in a container, and then observing the amount that can make it to the top within a limited time. In PD, HD, and advanced AD models, the locomotive ability of the flies tends to decrease. Memory assays are done by taking advantage of the male flies' ability to learn during courtship. When male flies are rejected by female flies that have already mated, they "learn" to stop trying for a period of time after, even when exposed to virgin female flies. In models where memory loss is present, such as AD models, this learning process is stunted and male flies will persist. While these are the fundamentals behind the assays, certain adjustments can be made by researchers. These phenotypic assays are performed to observe the effects of the model changes discussed in 3.2[12].

The molecular procedures are done to directly observe changes to the model's genome, gene expression, or protein presence. The common methods of nucleic acid observation include gel electrophoresis of DNA or cDNA from RT-PCR (reverse-transcriptase-polymerase chain reaction). These are done to verify gene presence. Gene expression can then be measured through qPCR or western blots. A combination of these methods are typically carried out to observe the relationship between inserted genes and their effect on other gene expression or protein presence. This can also be taken a step further in immunohistochemistry. In neurodegenerative *Drosophila* models, this is typically done by creating thin slices of the brain and then staining with specific antibodies. Proper imaging can then show the protein of interest's presence in the brain,

and potential neurodegeneration. These, along with other methods of observing molecular changes, are used to verify changes caused by additional factors in the disease pathway[41].

4. Future Direction of Neurodegenerative Research in *Drosophila*

Up to this point, research in *Drosophila* has greatly expanded the understanding of specific neurodegenerative diseases along with the general neurodegenerative process. The current research direction consists of an expanding understanding of the processes of these diseases and other involved genes. Additionally, there is a push to test for potential therapeutics that can help with neurodegeneration and even potentially repair damages done. This is a major goal in neurodegenerative disease research among all models, as currently used therapeutics only work to target symptoms, rather than disease origin. Going forward, there will be further work done to develop a clear cellular pathway for the different diseases. Understanding the genes and proteins involved is vital for a therapeutic which can hinder or halt disease progression. Although each disease has its own genes and origins, current research is demonstrating there may be similar pathways among them. One such recently published study reveals that mutant HTT protein in HD consists of similar aggregation and properties to the amyloid beta protein in AD[42]. Further studies demonstrating these similar properties will be important for a holistic understanding of disease progression. Another future direction of neurodegenerative research involves the reversal of neuronal damages. While neurogenesis in adult brains only occurs in certain areas[43], neuronal processes can grow and change throughout adulthood. One aspect of this, axonal regeneration, is currently being discussed as a means to maintain synapses despite damages[44].

5. Conclusion

A significant portion of what is known today about the nervous system and the diseases that affect it have been obtained from research on *D. melanogaster.* At a glance, HD, PD, and AD may seem to occur via differing mechanisms that occur from various genetic causes. From the many studies on neurodegeneration being done by researchers from around the world, commonalities are beginning to be drawn between the different degenerative processes. These include defects in the neurons ability to carry out autophagy, proper mitochondrial function, and synaptic degeneration. While a clear connection is not well understood at the time, research is currently being conducted to clarify these processes and how to halt them. As *Drosophila* has proven to be vital in past research into these diseases, they will also play a key role in the road ahead.

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