

# Studying Alzheimer's Disease Using *Drosophila melanogaster* as a Powerful Tool

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## Abstract

**Background:** Alzheimer's disease (AD) is an increasingly prevalent neurodegenerative disease characterized by protein aggregation in the form of amyloid plaques containing beta-amyloid peptides and neurofibrillary tangles containing hyperphosphorylated tau protein. The central molecular events underlying AD pathogenesis remain controversial and poorly defined. *Drosophila melanogaster* has emerged as an important genetic resource for studying the pathology of AD. Many AD models have been created using *Drosophila*, taking advantage of its short generation times, sophisticated genetic tools, and abundance of homologs to human genes. **Purpose:** This review summarizes different models for studying AD in *Drosophila melanogaster*, including the full-length APP, C99, A $\beta$ 42 and Tau models, explaining how the models were built and what we have learned from them. **Conclusion:** Four main AD *Drosophila* models are introduced in this review, which can serve as a future method to investigate genes and drugs that can modify symptoms.

## Keywords

*Drosophila*, Alzheimer's Disease, APP, C99, A $\beta$ 42, Tau

## 1. Introduction

Alzheimer's Disease (AD) is the most common type of dementia that affects over 6 million people in the United States [1]. In the 2020 US Census, the prevalence of AD jumped from 5.3% in people 65 to 74 years old to 34.6% in people aged 85 and older [2]. Globally, the number is projected to rise from 57.4 million in 2019 to 152.8 million in 2050 [3].

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The first symptom of AD is a gradual memory decline. In later stages, as memory degenerates, language skills and basic body functions such as swallowing and bladder control also deteriorate [4]. At the neuropathological level, there are two main hallmarks of AD which were noted by Dr. Alois Alzheimer upon his discovery of AD back in 1906: amyloid plaques and neurofibrillary tangles [5].

Amyloid plaques are chiefly composed of amyloid-beta peptides ( $A\beta$ ), created from the cleavage of a transmembrane protein called the amyloid precursor protein (APP). APP is cleaved by  $\beta$ -secretase, which yields sAPP $\beta$  and C99. The C99 domain is then cleaved by  $\gamma$ -secretase, breaking off into an intracellular fragment, AICD, and different forms of  $A\beta$  due to imprecise cleavage by  $\gamma$ -secretase (Figure 1). One of these forms is called  $A\beta_{42}$ , which is considered significantly toxic.  $A\beta_{42}$  gives rise to  $A\beta$  amyloid fibril formation. These  $A\beta$  amyloid fibrils later form amyloid plaques, eventually leading to cell death [6]. The theory that this accumulation of  $A\beta$  is the chief cause of AD is called the amyloid hypothesis, first introduced by Dr. John Hardy and Dr. David Allsop in 1991 [7]. This has been the main hypothesis driving AD research and drug development for decades.

Neurofibrillary tangles are made up of hyperphosphorylated tau proteins. In the healthy brain, tau proteins play a critical role in stabilizing microtubules (MTs), which support the growth of neurons [8]. In AD patients, however, the phosphorylation of tau leads to its detachment from MT. These tau proteins then stick to each other, creating neurofibrillary tangles around neurons. The number of neurofibrillary tangles correlates with the severity of the clinical symptoms of

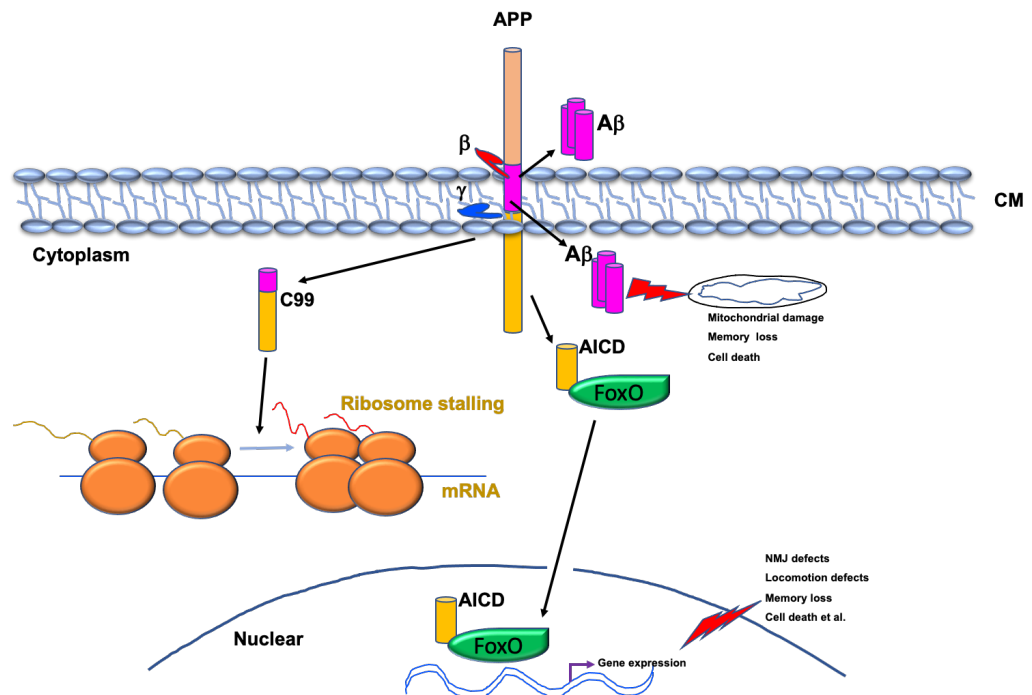


Figure 1. The procession of APP and its function.

AD [4]. The theory that hyperphosphorylated tau proteins are the leading cause of AD is called the tau hypothesis.

AD has been studied in many different model organisms, most notably the mouse [9] [10] and *Drosophila melanogaster*. This review summarizes the different models for studying AD in *Drosophila melanogaster*, explaining the models and what was found employing them.

## 2. Why Use *Drosophila*?

*Drosophila melanogaster* has been used for over 120 years and has emerged within the past five decades as a preeminent model for genetics research [11]. There are many benefits to using this complex organism, the most noteworthy of which is its homology to humans at the gene and genomic levels. We share around 75% of our genes with *Drosophila* [12] [13]. Importantly, when homologs of human disease genes are introduced into *Drosophila*, they can cause a range of disease phenotypes, including cancers and neurodegenerative diseases such as AD [14]. *Drosophila* can also be a great research tool because of its complexity, as behaviors such as memory and learning can be studied [15]. Another thing that sets *Drosophila* apart from other organisms used to study genetics is that it is easy to be manipulated in genetics. In addition to being a robust genetic tool, *Drosophila* is easy to be used because of its short life cycle. *Drosophila* is an organism that has a four-stage life cycle: egg, larva, pupa, and adult. Egg production by a female fly can reach up to approximately 100 eggs per day [16], and it only takes ten days at 25°C for *Drosophila* to mature from an embryo to adulthood [11]. On average, *Drosophila* has a life span of 2 - 3 months [17], making it easier to be used to study age-related diseases such as AD.

Specific to neurodegeneration, *Drosophila* is an effective tool because it responds strongly to factors such as A $\beta$ 42 and tau protein implicated in AD. Neurons die from overexpression of these disease-associated factors, and this neuronal loss manifests as distinctive phenotypes such as reduced locomotion, rough eye texture, or abnormal wing posture [18] [19] [20], which are easy to be scored by the naked eye or under dissecting microscope. For these reasons, *Drosophila* is an optimal model for studying genetics, specifically neurodegenerative diseases such as AD.

## 3. *Drosophila* AD Models

### 3.1. Full-Length APP Model

The amyloid precursor protein (APP), or in *Drosophila* the amyloid precursor-like protein (APPL), is a single-pass transmembrane protein with a large extracellular domain [21]. The normal physiological function of APP is still not well understood, but it has been shown to regulate synaptic structure [22] and neurogenesis [23] [24]. Animal model studies have supported its role in the development of AD [25] [26].

In *Drosophila*, one way to study APP pathobiology is to overexpress it in the

nervous system. Pirooznia *et al.* used a *Drosophila* AD model to study the effect Tip60 HAT on APP, targeting the developing central nervous system [27]. Tip60 (Tat-interactive protein-60 KDa) is a member of the MYST family of Histone Acetyltransferases (HATs) [28]. Tip60 plays a role in DNA repair and apoptosis [29] and has been linked to the development of AD [30]. In a fly model that overexpressed human APP full length or human APP without the C-terminal domain (APP-deltaCT), the authors expressed wild type Tip60 or HAT-defective dominant negative Tip60 and found that Tip60 loss heightened the lethality effect of APP, whereas wild type Tip60 overexpression suppressed these lethal effects. It was also found that Tip60 and APP interacted with each other to mediate nervous system-specific development, a process that was dependent on Tip60 interaction with the C-terminal domain of APP. As mentioned above, Tip60 plays a role in apoptosis. Interestingly, coexpression of Tip60 suppressed neuronal apoptosis induced by APP. Collectively, these findings suggest that Tip60 and APP interact with each other to play a role in neuronal development and neuronal apoptosis in *Drosophila*.

Another study that employed the nervous system to study APP in *Drosophila* was reported by Wang *et al.* [31]. They investigated FoxO (forkhead box O), a protein that helps maintain cellular quality control and modulate homeostatic processes including apoptosis [32] [33]. Wang *et al.* first established that APP could induce cell death in *Drosophila* not only in the nervous system but also in developing non-neural tissues. They also showed that cell death from APP was dependent on APP intracellular domain (AICD) and that dFoxo was required for such APP-induced behavior. They concluded that through the release of AICD, APP induced FoxO-mediated cell death. Consistently, Wang *et al.* looked at the effect of AICD on the transcription of FoxO target genes and found that AICD served as a transcriptional cofactor of FoxO to activate the expression of Bim, a gene involved in apoptosis [34] (**Figure 1**).

For studying the function of APP in *Drosophila*, the wing is also a helpful model. Peng *et al.* studied Polo kinases and their effect on APP-induced phenotypes [35]. Polo kinases are involved in cell division and cell cycle progression [36] [37], and specific Polo-like kinases (Plk1 and Plk2) have been shown to play a role in the pathology of AD [38] [39]. Through a genetic modifier screen, the authors found Polo as a line that suppressed the wing phenotype induced by APP. They further investigated Polo/APP relationship by studying the neuromuscular junction (NMJ) and found that the depletion of Polo suppressed an increase of boutons and branches at the NMJ caused by overexpressing APP, and that Polo ameliorated larval locomotion defects induced by APP expression at the NMJ. Next, the authors tested a series of behavioral defects induced by APP: locomotion decline, shortened lifespan, and male courtship choice dysfunction (used as a model for cognitive ability). They repeatedly found that a loss of Polo ameliorated these deficits. They lastly targeted the eye, where APP induces a rough eye phenotype, and found that a knockdown of Polo partly suppressed the APP-induced retina neurodegeneration. Together, these data demonstrate that

Polo is required for APP induced NMJ and locomotion defects.

### 3.2. C99 Model

APP.C99 is an important APP fragment for studying AD pathology, as there is much evidence demonstrating that C99 accumulation is involved in AD. Different AD models from mice [40] [41] [42] to monkeys [43] have shown heightened C99 accumulation. C99 can also cause AD in an  $A\beta$ -independent manner in mammalian models [44] [45]. There is a paucity of studies on AD pathogenesis by the C99 fragment using *Drosophila* as a model. In a recent study [46], Rimal *et al.* used *Drosophila* to express APP.C99 driven by *Mhc*-Gal4 in the muscle, which resulted in a droopy wing or a held-up wing posture. These postures indicated muscle degeneration. A series of tests of *Drosophila* expressing C99 in the muscle and in postmitotic neurons showed that C99 increased mitochondrial fragmentation, reduced locomotor activity, led to synapse loss, and impaired aversive taste memory. These data showed that the fly C99 model displays many of the same features as AD. Rimal *et al.* next overexpressed and knocked down different genes, identifying what rescued or worsened the abnormal wing posture. They found that the ABCE1 protein, a ribosome recycling factor, rescued translational stalling of C99 and the abnormal wing posture when overexpressed. These results supported the notion that ABCE1 is involved in the stalled translation of C99. Rimal *et al.* next tested ZNF598, a sensor of ribosome stalling and collisions, to test the effect of ribosome-mediated quality control on C99 translation. ZNF598 RNAi reduced C99 stalling and rescued the abnormal wing posture that was a consequence of C99 toxicity. Moreover, ZNF598 rescued endosomal and lysosomal deficits in the muscle tissues of flies expressing C99. Lastly, Rimal *et al.* tested how different proteins impacted the CAT-tailing of C99, a ribosome collision/stalling product. This is important because CAT-tailed C99 leads to degeneration of the neuronal cell body, which was also observed by Udagawa *et al.* [47]. They found that ZNF598 RNAi, overexpression of ABCE1, and RQC factors decreased the number of CAT-tailed species. Collectively, the study by Rimal *et al.* showed that stalled translation of C99 and resultant CAT-tailed C99 led to proteostasis failure, leading to the development of amyloid plaques and neuronal death.

### 3.3. $A\beta$ 42 Model

$A\beta$ 40 and  $A\beta$ 42 have been known to be the primary components of the senile plaques of the AD brain for decades [48]. Cellular studies have shown that  $A\beta$ 42 may be more toxic than  $A\beta$ 40. Whether an accumulation of amyloid plaques is the root cause of AD has been debated since the hypothesis was first formed [49]. Up to now, even with the recent approval of Aducanumab [50], a drug that targets amyloid-beta plaques, a consensus has not been made. In *Drosophila*, pan-neuronal expression of  $A\beta$ 42 can lead to locomotor deficits, neurodegeneration of brain regions, and premature death in aged animals [51]. Various studies

have used *Drosophila* as a model to investigate A $\beta$ 42 in AD. Some of the most prevalent tissues used as targets to study A $\beta$ 42 in AD are the eye and the nervous system.

The eye is a popular region to target for neurodegenerative diseases as it is complex and accessible [52] [53]. A common theme is that the more A $\beta$ 42 is expressed, and the older the fly, the more severe the eye phenotype becomes. Using the *Drosophila* eye, Hua *et al.* studied the impact of zinc and copper on A $\beta$ 42 expression [54]. They tested this by raising or keeping flies in zinc or copper-enriched food and measuring the survival rates of flies expressing A $\beta$ 42 with copper and zinc supplements as well as a copper and zinc chelator, DP-109, and found that survival rates of A $\beta$ 42 expressing flies were decreased further with zinc but not copper and that DP-109 increased the number of A $\beta$ 42 expressing flies reaching adulthood. They also tested locomotion using a climbing assay, which worsened with age in A $\beta$ 42 expressing flies, and found that zinc further deteriorated locomotion. Another critical finding was with overexpression or removal of a regulator of metal homeostasis called MFT-1 (Metal Responsive Transcription Factor), where they found that overexpression of MFT-1 alleviated A $\beta$ 42 toxicity, whereas removal of MFT-1 (in a null mutant background) led to the eye phenotype getting significantly more severe.

Another study that utilized the *Drosophila* eye to study A $\beta$ 42 is by Sarkar *et al.* on the effects of the protein Lunasin to reverse the toxicity of A $\beta$ 42 [55]. Lunasin is a soy protein with an antioxidant and anti-cancer effect [56] [57] [58]. They used *GMR*-Gal4, which drives gene expression in the eye, and found that the neurodegenerative phenotype of *GMR* > A $\beta$ 42, which includes axon targeting defects in the eye, was significantly rescued by the misexpression of Lunasin (*GMR* > A $\beta$ 42 + Lun).

Another important target tissue for AD study is the mushroom body, which is part of the central brain regions of *Drosophila*. The mushroom body is associated with a multitude of complex behaviors such as taste memory and learning as well as locomotor activity [59] [60], and therefore it is widely used to study neurodegenerative diseases [61]. Iijima-Ando *et al.* used the mushroom body structures of *Drosophila* to express A $\beta$ 42 to study the effects of Nephilysin (NEP), an amyloid-degrading enzyme that can slow the progression of AD [62] [63] [64]. Iijima-Ando *et al.* expressed A $\beta$ 42 in the ER of the mushroom body of *Drosophila* and then coexpressed NEP proteins in these A $\beta$ 42 fly brains. From this, they found that NEP significantly reduced the level of A $\beta$ 42 in the A $\beta$ 42 expressing flies. They also found that NEP suppressed the neuronal loss induced by A $\beta$ 42 expression. However, they found that overexpression of NEP did not rescue the A $\beta$ 42 induced premature fly death. In fact, the expression of NEP shortened the lifespan of *Drosophila* relative to the control. Overexpression of NEP also caused axon degeneration and decreased CREB-mediated transcription in the fly.

Iijima-Ando performed another interesting study employing mushroom body structures to test the brains of A $\beta$ 42 flies [65]. In this study, Iijima-Ando *et al.*

expressed mito-GFP and A $\beta$ 42 in the mushroom body structure and found that the number of mitochondria is reduced in the axons and dendrites of the A $\beta$ -42 fly brain but not in the cell body, showing that expressing A $\beta$ 42 does not result in a global decline in mitochondria but rather in mitochondrial mislocalization. A lack of mitochondria in the axons has been shown to disrupt the cAMP/PKA signaling, which reduced synaptic strength. They found that decreasing the cAMP levels led to A $\beta$ 42 induced locomotor defects occurring earlier. Additionally, neuronal knockdown of PKA intensified the locomotor defects induced by A $\beta$ 42. From these results, they concluded that neuronal dysfunctions in A $\beta$ 42 flies could be a product of cAMP/PKA signaling reduction along axons and dendrites.

A more recent study used the mushroom body of A $\beta$ 42 expressing *Drosophila* to investigate the involvement of mitochondrial dysfunction [66]. Just like Iijima-Ando, the authors expressed A $\beta$ 42 and mito-GFP to look at the effect of A $\beta$ 42 on mitochondria in the neurons of *Drosophila*. They found that A $\beta$ 42 causes fragmentation of somatic mitochondria in flies starting on their first day of life. They then investigated whether the mitochondrial fragmentation observed was neuron-wide or specific to the soma of the mushroom body neurons and found that mitochondrial fragmentation first occurred in the soma, then in the dendrites, and lastly in the distal axons. The authors next studied the effect A $\beta$ 42 had on mitochondria, measuring the calcium import of the mushroom body neurons. They found that by day 5, mitochondrial calcium import decreased significantly in A $\beta$ 42 expressing flies, indicating an impairment of the somatic mitochondria in the mushroom body neurons. They also looked at how A $\beta$ 42 impacted apoptosis since mitochondria play a critical role in programmed cell death [67], and found that as time passed, there was a heightened level of apoptosis in the soma of the A $\beta$ 42 *Drosophila*. Lastly, they investigated the effect of expressing A $\beta$ 42 in the mushroom body neurons on memory and found that after 15 days, a significant memory impairment was observed. This study establishes the *Drosophila* model as a useful *in vivo* system for probing the mechanisms by which A $\beta$ 42 produces mitochondrial toxicity that contributes to memory dysfunction.

### 3.4. Tau Model

Tau is a microtubule-associated protein, typically found in the axons, that works to maintain the stability of microtubules [68]. In AD, tau becomes hyperphosphorylated 3 to 4 times more than in a normal adult brain [69] [70] and disrupts microtubule integrity [71] [72]. The free tau molecules then aggregate into helical patterns [73]. In *Drosophila*, expressing human tau has been shown to impair olfactory and motor learning and disrupt the mushroom body neurons [74] [75].

One of the most common models to study tau in *Drosophila* is the eye. Nishimura *et al.* studied the effects that PAR-1 kinase had on tau toxicity in a *Drosophila* AD model [76]. In this study, they looked at the interactions between

PAR-1 and tau and found that photoreceptor degeneration caused by tau was partially suppressed by the removal of PAR-1. In addition, they found that overexpression of PAR-1 enhanced the toxicity of a mutant form of human tau (h-tau) associated with frontotemporal dementia. They used the 12E8 antibody, which recognizes p-S262 and p-S256 sites of h-tau, and they found that overexpression of PAR-1 can lead to increased phosphorylation of human and fly tau at the residues detected by 12E8. They also found that the phosphorylation by PAR-1 at the S262 and S256 sites are prerequisites for the phosphorylation by kinases such as GSK-3 and CDK5 at other sites. Their data suggested that PAR-1 phosphorylation at the 12E8 sites provides docking sites for kinases such as GSK-3 and CDK5 to prime the phosphorylation of other nearby sites.

Another group that used the eye to study tau in the *Drosophila* AD model was Zhang *et al.* [77]. This study specifically examined the effect of Salidroside (Sal) on a *Drosophila* tau model. Sal is a glycoside with a strong antioxidative, anti-inflammatory, and anti-apoptosis effects [78] [79]. Zhang *et al.* expressed tau in the CNS and the eyes and examined the effects of Sal. They fed Sal or Donepezil (a clinically approved drug for AD) to tau transgenic *Drosophila* in different concentrations and tested for survival rate. They found that Sal treatment increased both survival rate and time comparably to Donepezil. They also found that Sal treatment improved the climbing ability of tau transgenic flies after 30 days. Vacuoles in the brain are hallmarks of *Drosophila* neurodegeneration, so they also fed tau transgenic flies with Sal and observed vacuoles. They found that the Sal treatment prevented the histological abnormalities in the vacuoles. Lastly, Zhang *et al.* investigated the pathways affected by Sal and found that Sal increased the level of p-GSK-3 $\beta$ , which is part of a pathway that prompts neuronal survival and plays a role in the pathogenesis of AD [80] [81], while decreasing levels of p-tau that is a downstream target of GSK-3 $\beta$ .

Anupama *et al.* also studied a tau induced AD model of *Drosophila* [82]. They examined the effect of Jatamansinol on the neurotoxicity induced by tau. *N. jatamansi* is an herb whose root extract can decrease A $\beta$ 42 toxicity in *Drosophila* [83]. They gave tau expressing flies different doses of jatamansinol-supplemented food and found that compared to the control flies fed with normal food, flies fed with Jatamansinol supplements showed increased survival rates and improved locomotor activity. They also found that tau expressing flies fed with jatamansinol supplements showed a significant decrease in the amount of tau protein compared to the control in all age groups. They then tested for learning and memory and found that flies fed with jatamansinol food had a significantly higher memory performance index than the control. Next, Anupama *et al.* examined tau induced eye degeneration and found that there was a significant reduction in the amount of eye degeneration in flies fed with jatamansinol-supplemented food. Next, they tested jatamansinol treatment of the control flies and found that compared to the regular food group, there was a reduction of reactive oxygen species, suggesting that jatamansinol improves antioxidant de-



fense. Lastly, they found that jatamansinol also inhibits the cholinesterase enzymes, which are disturbed in AD [84].

#### 4. Conclusion

*Drosophila melanogaster* is an excellent tool for studying the pathology of AD, as it shares 75% of our genes. In addition, neurodegeneration in *Drosophila* manifests in obvious phenotypic changes. Furthermore, *Drosophila* is a great model system because of its short generation times and facile genetic analysis. Given that *Drosophila* is such an effective tool for studying AD, in this paper, we examine some of the models for studying this disease, going over how they were performed and what was found. The first model examined is APP Full Length, focusing on the two popular targets: the nervous system and the wing. Next is the C99 model, which has been employed recently to reveal the importance of ribosome-associated protein quality control in AD pathogenesis. Then the A $\beta$ 42 *Drosophila* models of the eye and the mushroom body are discussed, which are popular for studying AD since the eye is complex and accessible, and the mushroom body can be used to study learning and memory. Last, the tau protein *Drosophila* model is discussed, focusing on the eye, which is a common target. It is anticipated that the continued use of these models will deepen our understanding of AD pathogenesis and uncover novel drug targets for combating AD.

#### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

#### References

- [1] Barker, W.W., Luis, C.A., Kashuba, A., Luis, M., Harwood, D.G., Loewenstein, D., Waters, C., Jimison, P., Shepherd, E., Sevush, S., Graff-Radford, N., Newland, D., Todd, M., Miller, B., Gold, M., Heilman, K., Doty, L., Goodman, I., Robinson, B., Gary, P., Dennis, D. and Duara, R. (2002) 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*, **16**, 203-212. <https://doi.org/10.1097/00002093-200210000-00001>
- [2] Rajan, K.B., Weuve, J., Barnes, L.L., McAninch, E.A., Wilson, R.S. and Evans, D.A. (2021) Population Estimate of People with Clinical Alzheimer's Disease and Mild Cognitive Impairment in the United States (2020-2060). *Alzheimer's & Dementia*, **17**, 1966-1975. <https://doi.org/10.1002/alz.12362>
- [3] Nichols, E., Steinmetz, J.D., Vollset, S.E., Fukutaki, K., Chalek, J., Abd-Allah, F., Abdoli, A., Abualhasan, A., Abu-Gharbieh, E., Akram, T.T., Al Hamad, H., Alahdab, F., Alanezi, F.M., Alipour, V., Almustanyir, S., Amu, H., Ansari, I., Arabloo, J., Ashraf, T., Bezabih, Y.M., et al. (2022) Estimation of the Global Prevalence of Dementia in 2019 and Forecasted Prevalence in 2050: An Analysis for the Global Burden of Disease Study 2019. *The Lancet Public Health*, **7**, e105-e125. [https://doi.org/10.1016/S2468-2667\(21\)00249-8](https://doi.org/10.1016/S2468-2667(21)00249-8)
- [4] Schultz, C., Del Tredici, K. and Braak, H. (2004) Neuropathology of Alzheimer's Disease. In: Richter, R.W., Richter, B.Z., Eds., *Alzheimer's Disease Current Clinical*

- Neurology*, Humana Press, Totowa, NJ.
- [5] Hippus, H. and Neundörfer, G. (2003) The Discovery of Alzheimer's Disease. *Dialogues in Clinical Neuroscience*, **5**, 101-108. <https://doi.org/10.31887/DCNS.2003.5.1/hhippius>
  - [6] Kametani, F. and Hasegawa, M. (2018) Reconsideration of Amyloid Hypothesis and Tau Hypothesis in Alzheimer's Disease. *Frontiers in Neuroscience*, **12**. Article 25. <https://doi.org/10.3389/fnins.2018.00025>
  - [7] Liu, P.-P., Xie, Y., Meng, X.-Y. and Kang, J.-S. (2019) History and Progress of Hypotheses and Clinical Trials for Alzheimer's Disease. *Signal Transduction and Targeted Therapy*, **4**, Article No. 29. <https://doi.org/10.1038/s41392-019-0063-8>
  - [8] Barbier, P., Zejneli, O., Martinho, M., Lasorsa, A., Belle, V., Smet-Nocca, C., Tsvetkov, P.O., Devred, F. and Landrieu, I. (2019) Role of Tau as a Microtubule-Associated Protein: Structural and Functional Aspects. *Frontiers in Aging Neuroscience*, **11**, Article 204. <https://doi.org/10.3389/fnagi.2019.00204>
  - [9] Demattos, R., Lu, J., Tang, Y., Racke, M., Delong, C., Tzaferis, J., Hole, J., Forster, B., McDonnell, P., Liu, F., Kinley, R., Jordan, W. and Hutton, M. (2012) A Plaque-Specific Antibody Clears Existing  $\beta$ -Amyloid Plaques in Alzheimer's Disease Mice. *Neuron*, **76**, 908-920. <https://doi.org/10.1016/j.neuron.2012.10.029>
  - [10] Kiani Shabestari, S., Morabito, S., Danhash, E.P., Mcquade, A., Sanchez, J.R., Miyoshi, E., Chadarevian, J.P., Claes, C., Coburn, M.A., Hasselmann, J., Hidalgo, J., Tran, K.N., Martini, A.C., Chang Rothermich, W., Pascual, J., Head, E., Hume, D.A., Pridans, C., Davtyan, H., Swarup, V. and Blurton-Jones, M. (2022) Absence of Microglia Promotes Diverse Pathologies and Early Lethality in Alzheimer's Disease Mice. *Cell Reports*, **39**, Article ID: 110961. <https://doi.org/10.1016/j.celrep.2022.110961>
  - [11] Jennings, B.H. (2011) *Drosophila*—A Versatile Model in Biology & Medicine. *Materials Today*, **14**, 190-195. [https://doi.org/10.1016/S1369-7021\(11\)70113-4](https://doi.org/10.1016/S1369-7021(11)70113-4)
  - [12] Pandey, U.B. and Nichols, C.D. (2011) Human Disease Models in *Drosophila melanogaster* and the Role of the Fly in Therapeutic Drug Discovery. *Pharmacological Reviews*, **63**, 411-436. <https://doi.org/10.1124/pr.110.003293>
  - [13] Reiter, L.T., Potocki, L., Chien, S., Gribskov, M. and Bier, E. (2001) A Systematic Analysis of Human Disease-Associated Gene Sequences in *Drosophila melanogaster*. *Genome Research*, **11**, 1114-1125. <https://doi.org/10.1101/gr.169101>
  - [14] Bier, E. (2005) *Drosophila*, the Golden Bug, Emerges as a Tool for Human Genetics. *Nature Reviews Genetics*, **6**, 9-23. <https://doi.org/10.1038/nrg1503>
  - [15] Mcguire, S.E., Deshazer, M. and Davis, R.L. (2005) Thirty Years of Olfactory Learning and Memory Research in *Drosophila melanogaster*. *Progress in Neurobiology*, **76**, 328-347. <https://doi.org/10.1016/j.pneurobio.2005.09.003>
  - [16] Stocker, H. and Gallant, P. (2008) Getting Started. *Methods in Molecular Biology*, **420**, 27-44. [https://doi.org/10.1007/978-1-59745-583-1\\_2](https://doi.org/10.1007/978-1-59745-583-1_2)
  - [17] Sun, Y., Yolitz, J., Wang, C., Spangler, E., Zhan, M. and Zou, S. (2013) Aging Studies in *Drosophila melanogaster*. *Methods in Molecular Biology*, **1048**, 77-93. [https://doi.org/10.1007/978-1-62703-556-9\\_7](https://doi.org/10.1007/978-1-62703-556-9_7)
  - [18] Fossgreen, A., Brückner, B., Czech, C., Masters, C.L., Beyreuther, K. and Paro, R. (1998) Transgenic *Drosophila* Expressing Human Amyloid Precursor Protein Show  $\gamma$ -Secretase Activity and a Blistered-Wing Phenotype. *Proceedings of the National Academy of Sciences*, **95**, 13703-13708. <https://doi.org/10.1073/pnas.95.23.13703>
  - [19] Sang, T.-K. and Jackson, G.R. (2005) *Drosophila* Models of Neurodegenerative Disease. *NeuroRX*, **2**, 438-446. <https://doi.org/10.1602/neurorx.2.3.438>

- [20] Prüßing, K., Voigt, A. and Schulz, J.B. (2013) *Drosophila* Melanogaster as a Model Organism for Alzheimer's Disease. *Molecular Neurodegeneration*, **8**, Article No. 35. <https://doi.org/10.1186/1750-1326-8-35>
- [21] O'Brien, R.J. and Wong, P.C. (2011) Amyloid Precursor Protein Processing and Alzheimer's Disease. *Annual Review of Neuroscience*, **34**, 185-204. <https://doi.org/10.1146/annurev-neuro-061010-113613>
- [22] Tyan, S.-H., Shih, A.Y.-J., Walsh, J.J., Maruyama, H., Sarsoza, F., Ku, L., Eggert, S., Hof, P.R., Koo, E.H. and Dickstein, D.L. (2012) Amyloid Precursor Protein (APP) Regulates Synaptic Structure and Function. *Molecular and Cellular Neuroscience*, **51**, 43-52. <https://doi.org/10.1016/j.mcn.2012.07.009>
- [23] López-Toledano, M.A. and Shelanski, M.L. (2007) Increased Neurogenesis in Young Transgenic Mice Overexpressing Human APP<sub>Sw,Ind.</sub> *Journal of Alzheimer's Disease*, **12**, 229-240. <https://doi.org/10.3233/JAD-2007-12304>
- [24] Zhou, Z.-D., Chan, C.H.-S., Ma, Q.-H., Xu, X.-H., Xiao, Z.-C. and Tan, E.-K. (2011) The Roles of Amyloid Precursor Protein (APP) in Neurogenesis. *Cell Adhesion & Migration*, **5**, 280-292. <https://doi.org/10.4161/cam.5.4.16986>
- [25] Zheng, H. and Koo, E.H. (2011) Biology and Pathophysiology of the Amyloid Precursor Protein. *Molecular Neurodegeneration*, **6**, Article No. 27. <https://doi.org/10.1186/1750-1326-6-27>
- [26] Coronel, R., Bernabeu-Zornoza, A., Palmer, C., Muñiz-Moreno, M., Zambrano, A., Cano, E. and Liste, I. (2018) Role of Amyloid Precursor Protein (APP) and Its Derivatives in the Biology and Cell Fate Specification of Neural Stem Cells. *Molecular Neurobiology*, **55**, 7107-7117. <https://doi.org/10.1007/s12035-018-0914-2>
- [27] Pirooznia, S.K., Sarthi, J., Johnson, A.A., Toth, M.S., Chiu, K., Koduri, S. and Elefant, F. (2012) Tip60 HAT Activity Mediates APP Induced Lethality and Apoptotic Cell Death in the CNS of a *Drosophila* Alzheimer's Disease Model. *PLOS ONE*, **7**, e41776. <https://doi.org/10.1371/journal.pone.0041776>
- [28] Utley, R.T. and Côté, J. (2003) The MYST Family of Histone Acetyltransferases. *Current Topics in Microbiology and Immunology*, **274**, 203-236. [https://doi.org/10.1007/978-3-642-55747-7\\_8](https://doi.org/10.1007/978-3-642-55747-7_8)
- [29] Ikura, T., Ogryzko, V.V., Grigoriev, M., Groisman, R., Wang, J., Horikoshi, M., Scully, R., Qin, J. and Nakatani, Y. (2000) Involvement of the TIP60 Histone Acetylase Complex in DNA Repair and Apoptosis. *Cell*, **102**, 463-473. [https://doi.org/10.1016/S0092-8674\(00\)00051-9](https://doi.org/10.1016/S0092-8674(00)00051-9)
- [30] Li, Z. and Rasmussen, L.J. (2020) TIP60 in Aging and Neurodegeneration. *Ageing Research Reviews*, **64**, Article ID: 101195. <https://doi.org/10.1016/j.arr.2020.101195>
- [31] Wang, X., Wang, Z., Chen, Y., Huang, X., Hu, Y., Zhang, R., Ho, M.S. and Xue, L. (2014) FoxO Mediates App-Induced AICD-Dependent Cell Death. *Cell Death & Disease*, **5**, e1233. <https://doi.org/10.1038/cddis.2014.196>
- [32] Webb, A.E. and Brunet, A. (2014) FOXO Transcription Factors: Key Regulators of Cellular Quality Control. *Trends in Biochemical Sciences*, **39**, 159-169. <https://doi.org/10.1016/j.tibs.2014.02.003>
- [33] Zhang, X., Tang, N., Hadden, T.J. and Rishi, A.K. (2011) Akt, Foxo and Regulation of Apoptosis. *Biochimica Et Biophysica Acta (BBA)-Molecular Cell Research*, **1813**, 1978-1986. <https://doi.org/10.1016/j.bbamcr.2011.03.010>
- [34] O'connor, L. (1998) Bim: A Novel Member of the Bcl-2 Family That Promotes Apoptosis. *The EMBO Journal*, **17**, 384-395. <https://doi.org/10.1093/emboj/17.2.384>
- [35] Peng, F., Zhao, Y., Huang, X., Chen, C., Sun, L., Zhuang, L. and Xue, L. (2015) Loss of Polo Ameliorates App-Induced Alzheimer's Disease-Like Symptoms in *Drosophila*. *Scientific Reports*, **5**, Article ID: 16816. <https://doi.org/10.1038/srep16816>

- [36] Zitouni, S., Nabais, C., Jana, S.C., Guerrero, A. and Bettencourt-Dias, M. (2014) Polo-Like Kinases: Structural Variations Lead to Multiple Functions. *Nature Reviews Molecular Cell Biology*, **15**, 433-452. <https://doi.org/10.1038/nrm3819>
- [37] Archambault, V., Lépine, G. and Kachaner, D. (2015) Understanding the Polo Kinase Machine. *Oncogene*, **34**, 4799-4807. <https://doi.org/10.1038/onc.2014.451>
- [38] Song, B., Davis, K., Liu, X.S., Lee, H.-G., Smith, M. and Liu, X. (2011) Inhibition of Polo-Like Kinase 1 Reduces Beta-Amyloid-Induced Neuronal Cell Death in Alzheimer's Disease. *Aging*, **3**, 846-851. <https://doi.org/10.18632/aging.100382>
- [39] Lee, J.S., Lee, Y., André, E.A., Lee, K.J., Nguyen, T., Feng, Y., Jia, N., Harris, B.T., Burns, M.P. and Pak, D.T.S. (2019) Inhibition of Polo-Like Kinase 2 Ameliorates Pathogenesis in Alzheimer's Disease Model Mice. *PLOS ONE*, **14**, e0219691. <https://doi.org/10.1371/journal.pone.0219691>
- [40] Rockenstein, E., Mante, M., Alford, M., Adame, A., Crews, L., Hashimoto, M., Esposito, L., Mucke, L. and Masliah, E. (2005) High  $\beta$ -Secretase Activity Elicits Neurodegeneration in Transgenic Mice despite Reductions in Amyloid- $\beta$  Levels. *Journal of Biological Chemistry*, **280**, 32957-32967. <https://doi.org/10.1074/jbc.M507016200>
- [41] Cavanagh, C., Colby-Milley, J., Bouvier, D., Farso, M., Chabot, J.-G., Quirion, R. and Krantic, S. (2013)  $\beta$ CTF-Correlated Burst of Hippocampal TNF $\alpha$  Occurs at a Very Early, Pre-Plaque Stage in the TgCRND8 Mouse Model of Alzheimer's Disease. *Journal of Alzheimer's Disease*, **36**, 233-238. <https://doi.org/10.3233/JAD-122131>
- [42] Mondragón-Rodríguez, S., Gu, N., Manseau, F. and Williams, S. (2018) Alzheimer's Transgenic Model Is Characterized by Very Early Brain Network Alterations and  $\beta$ -CTF Fragment Accumulation: Reversal by  $\beta$ -Secretase Inhibition. *Frontiers in Cellular Neuroscience*, **12**, Article 121. <https://doi.org/10.3389/fncel.2018.00121>
- [43] Cai, Y., Xiong, K., Zhang, X.-M., Cai, H., Luo, X.-G., Feng, J.-C., Clough, R.W., Struble, R.G., Patrylo, P.R., Chu, Y., Kordower, J.H. and Yan, X.-X. (2010)  $\beta$ -Secretase-1 Elevation in Aged Monkey and Alzheimer's Disease Human Cerebral Cortex Occurs around the Vasculature in Partnership with Multisystem Axon Terminal Pathogenesis and  $\beta$ -Amyloid Accumulation. *European Journal of Neuroscience*, **32**, 1223-1238. <https://doi.org/10.1111/j.1460-9568.2010.07376.x>
- [44] Pera, M., Larrea, D., Guardia-Laguarta, C., Montesinos, J., Velasco, K.R., Agrawal, R. R., Xu, Y., Chan, R.B., Di Paolo, G., Mehler, M.F., Perumal, G.S., Macaluso, F.P., Freyberg, Z.Z., Acin-Perez, R., Enriquez, J.A., Schon, E.A. and Area-Gomez, E. (2017) Increased Localization of App-C99 in Mitochondria-Associated ER Membranes Causes Mitochondrial Dysfunction in Alzheimer Disease. *The EMBO Journal*, **36**, 3356-3371. <https://doi.org/10.15252/embj.201796797>
- [45] Bourgeois, A., Lauritzen, I., Lorivel, T., Bauer, C., Checler, F. and Pardossi-Piquard, R. (2018) Intraneuronal Accumulation of c99 Contributes to Synaptic Alterations, Apathy-Like Behavior, and Spatial Learning Deficits in 3 $\times$ TgAD and 2 $\times$ TgAD Mice. *Neurobiology of Aging*, **71**, 21-31. <https://doi.org/10.1016/j.neurobiolaging.2018.06.038>
- [46] Rimal, S., Li, Y., Vartak, R., Geng, J., Tantray, I., Li, S., Huh, S., Vogel, H., Glabe, C., Grinberg, L.T., Spina, S., Seeley, W.W., Guo, S. and Lu, B. (2021) Inefficient Quality Control of Ribosome Stalling during APP Synthesis Generates Cat-Tailed Species That Precipitate Hallmarks of Alzheimer's Disease. *Acta Neuropathologica Communications*, **9**, Article No. 169. <https://doi.org/10.1186/s40478-021-01268-6>
- [47] Udagawa, T., Seki, M., Okuyama, T., Adachi, S., Natsume, T., Noguchi, T., Matsuzawa, A. and Inada, T. (2021) Failure to Degrade Cat-Tailed Proteins Disrupts Neuronal Morphogenesis and Cell Survival. *Cell Reports*, **34**, Article ID: 108599. <https://doi.org/10.1016/j.celrep.2020.108599>

- [48] Masters, C.L., Simms, G., Weinman, N.A., Multhaup, G., McDonald, B.L. and Beyreuther, K. (1985) Amyloid Plaque Core Protein in Alzheimer Disease and Down Syndrome. *Proceedings of the National Academy of Sciences*, **82**, 4245-4249. <https://doi.org/10.1073/pnas.82.12.4245>
- [49] Selkoe, D.J. and Hardy, J. (2016) The Amyloid Hypothesis of Alzheimer's Disease at 25 Years. *EMBO Molecular Medicine*, **8**, 595-608. <https://doi.org/10.15252/emmm.201606210>
- [50] Woloshin, S. and Kesselheim, A.S. (2022) What to Know About the Alzheimer Drug Aducanumab (Aduhelm). *JAMA Internal Medicine*, **182**, Article No. 892. <https://doi.org/10.1001/jamainternmed.2022.1039>
- [51] Iijima-Ando, K. and Iijima, K. (2009) Transgenic *Drosophila* Models of Alzheimer's Disease and Tauopathies. *Brain Structure and Function*, **214**, 245-262. <https://doi.org/10.1007/s00429-009-0234-4>
- [52] Singh, A. and Irvine, K.D. (2011) *Drosophila* as a Model for Understanding Development and Disease. *Developmental Dynamics*, **241**, 1-2. <https://doi.org/10.1002/dvdy.23712>
- [53] Kumar, J.P. (2017) The Fly Eye: Through the Looking Glass. *Developmental Dynamics*, **247**, 111-123. <https://doi.org/10.1002/dvdy.24585>
- [54] Hua, H., Münter, L., Harmeier, A., Georgiev, O., Multhaup, G. and Schaffner, W. (2011) Toxicity of Alzheimer's Disease-Associated A $\beta$  Peptide Is Ameliorated in a *Drosophila* Model by Tight Control of Zinc and Copper Availability. *Biological Chemistry*, **392**, 919-926. <https://doi.org/10.1515/BC.2011.084>
- [55] Sarkar, A., Gogia, N., Glenn, N., Singh, A., Jones, G., Powers, N., Srivastava, A., Kango-Singh, M. and Singh, A. (2018) A Soy Protein Lunasin Can Ameliorate Amyloid-Beta 42 Mediated Neurodegeneration in *Drosophila* Eye. *Scientific Reports*, **8**, Article No. 13545. <https://doi.org/10.1038/s41598-018-31787-7>
- [56] De lumen, B.O. (2005) Lunasin: A Cancer-Preventive Soy Peptide. *Nutrition Reviews*, **63**, 16-21. <https://doi.org/10.1111/j.1753-4887.2005.tb00106.x>
- [57] Jiang, Q., Pan, Y., Cheng, Y., Li, H., Liu, D. and Li, H. (2016) Lunasin Suppresses the Migration and Invasion of Breast Cancer Cells by Inhibiting Matrix Metalloproteinase-2/-9 via the FAK/Akt/ERK and NF- $\kappa$ B Signaling Pathways. *Oncology Reports*, **36**, 253-262. <https://doi.org/10.3892/or.2016.4798>
- [58] Wan, X., Liu, H., Sun, Y., Zhang, J., Chen, X., and Chen, N. (2017) Lunasin: A Promising Polypeptide for the Prevention and Treatment of Cancer. *Oncology Letters*, **13**, 3997-4001. <https://doi.org/10.3892/ol.2017.6017>
- [59] Davis, R.L. (1993) Mushroom Bodies and *Drosophila* Learning. *Neuron*, **11**, 1-14. [https://doi.org/10.1016/0896-6273\(93\)90266-T](https://doi.org/10.1016/0896-6273(93)90266-T)
- [60] Heisenberg, M. (2003) Mushroom Body Memoir: From Maps to Models. *Nature Reviews Neuroscience*, **4**, 266-275. <https://doi.org/10.1038/nrn1074>
- [61] Pech, U., Dipt, S., Barth, J., Singh, P., Jauch, M., Thum, A.S., Fiala, A. and Riemensperger, T. (2013) Mushroom Body Miscellanea: Transgenic *Drosophila* Strains Expressing Anatomical and Physiological Sensor Proteins in Kenyon Cells. *Frontiers in Neural Circuits*, **7**, Article 147. <https://doi.org/10.3389/fncir.2013.00147>
- [62] Iijima-Ando, K., Hearn, S.A., Granger, L., Shenton, C., Gatt, A., Chiang, H.-C., Hakker, I., Zhong, Y. and Iijima, K. (2008) Overexpression of Neprilysin Reduces Alzheimer Amyloid- $\beta$ 42 (A $\beta$ 42)-Induced Neuron Loss and Intraneuronal A $\beta$ 42 Deposits but Causes a Reduction in cAMP-Responsive Element-Binding Protein-Mediated Transcription, Age-Dependent Axon Pathology, and Premature Death in *Drosophila*. *Journal of Biological Chemistry*, **283**, 19066-19076. <https://doi.org/10.1074/jbc.M710509200>

- [63] Nalivaeva, N., Zhuravin, I. and Turner, A. (2020) Nephrylysin Expression and Functions in Development, Ageing and Disease. *Mechanisms of Ageing and Development*, **192**, Article ID: 111363. <https://doi.org/10.1016/j.mad.2020.111363>
- [64] El-Amouri, S.S., Zhu, H., Yu, J., Marr, R., Verma, I.M. and Kindy, M.S. (2008) Nephrylysin: An Enzyme Candidate to Slow the Progression of Alzheimer's Disease. *The American Journal of Pathology*, **172**, 1342-1354. <https://doi.org/10.2353/ajpath.2008.070620>
- [65] Iijima-Ando, K., Hearn, S.A., Shenton, C., Gatt, A., Zhao, L. and Iijima, K. (2009) Mitochondrial Mislocalization Underlies A $\beta$ 42-Induced Neuronal Dysfunction in a *Drosophila* Model of Alzheimer's Disease. *PLOS ONE*, **4**, e8310. <https://doi.org/10.1371/journal.pone.0008310>
- [66] Wang, X. and Davis, R.L. (2020) Early Mitochondrial Fragmentation and Dysfunction in a *Drosophila* Model for Alzheimer's Disease. *Molecular Neurobiology*, **58**, 143-155. <https://doi.org/10.1007/s12035-020-02107-w>
- [67] Brand, M.D., Orr, A.L., Perevoshchikova, I.V. and Quinlan, C.L. (2013) The Role of Mitochondrial Function and Cellular Bioenergetics in Ageing and Disease. *British Journal of Dermatology*, **169**, 1-8. <https://doi.org/10.1111/bjd.12208>
- [68] Mandelkow, E. (1998) Tau in Alzheimer's Disease. *Trends in Cell Biology*, **8**, 425-427. [https://doi.org/10.1016/S0962-8924\(98\)01368-3](https://doi.org/10.1016/S0962-8924(98)01368-3)
- [69] Iqbal, K., Liu, F., Gong, C.-X. and Grundke-Iqbal, I. (2010) Tau in Alzheimer Disease and Related Tauopathies. *Current Alzheimer Research*, **7**, 656-664. <https://doi.org/10.2174/156720510793611592>
- [70] Mandelkow, E.-M., Biernat, J., Drewes, G., Gustke, N., Trinczek, B. and Mandelkow, E. (1995) Tau Domains, Phosphorylation, and Interactions with Microtubules. *Neurobiology of Aging*, **16**, 355-362. [https://doi.org/10.1016/0197-4580\(95\)00025-A](https://doi.org/10.1016/0197-4580(95)00025-A)
- [71] Alonso, A.C., Zaidi, T., Grundke-Iqbal, I. and Iqbal, K. (1994) Role of Abnormally Phosphorylated Tau in the Breakdown of Microtubules in Alzheimer Disease. *Proceedings of the National Academy of Sciences*, **91**, 5562-5566. <https://doi.org/10.1073/pnas.91.12.5562>
- [72] Li, B., Chohan, M.O., Grundke-Iqbal, I. and Iqbal, K. (2007) Disruption of Microtubule Network by Alzheimer Abnormally Hyperphosphorylated Tau. *Acta Neuropathologica*, **113**, 501-511. <https://doi.org/10.1007/s00401-007-0207-8>
- [73] Medeiros, R., Baglietto-vargas, D. and Laferla, F.M. (2010) The Role of Tau in Alzheimer's Disease and Related Disorders. *CNS Neuroscience & Therapeutics*, **17**, 514-524. <https://doi.org/10.1111/j.1755-5949.2010.00177.x>
- [74] Beharry, C., Alaniz, M.E. and Alonso, A.D.C. (2013) Expression of Alzheimer-Like Pathological Human Tau Induces a Behavioral Motor and Olfactory Learning Deficit in *Drosophila Melanogaster*. *Journal of Alzheimer's Disease*, **37**, 539-550. <https://doi.org/10.3233/JAD-130617>
- [75] Mershin, A., Pavlopoulos, E., Fitch, O., Braden, B.C., Nanopoulos, D.V. and Skoulakis, E.M.C. (2004) Learning and Memory Deficits upon TAU Accumulation in *Drosophila* Mushroom Body Neurons. *Learning & Memory*, **11**, 277-287. <https://doi.org/10.1101/lm.70804>
- [76] Nishimura, I., Yang, Y. and Lu, B. (2004) PAR-1 Kinase Plays an Initiator Role in a Temporally Ordered Phosphorylation Process That Confers Tau Toxicity in *Drosophila*. *Cell*, **116**, 671-682. [https://doi.org/10.1016/S0092-8674\(04\)00170-9](https://doi.org/10.1016/S0092-8674(04)00170-9)
- [77] Zhang, B., Li, Q., Chu, X., Sun, S. and Chen, S. (2016) Salidroside Reduces Tau Hyperphosphorylation via Up-Regulating Gsk-3 $\beta$  Phosphorylation in a Tau Transgenic *Drosophila* Model of Alzheimer's Disease. *Translational Neurodegeneration*, **5**, Article No. 21. <https://doi.org/10.1186/s40035-016-0068-y>

- [78] Shi, K., Wang, X., Zhu, J., Cao, G., Zhang, K. and Su, Z. (2015) Salidroside Protects Retinal Endothelial Cells against Hydrogen Peroxide-Induced Injury via Modulating Oxidative Status and Apoptosis. *Bioscience, Biotechnology, and Biochemistry*, **79**, 1406-1413. <https://doi.org/10.1080/09168451.2015.1038212>
- [79] Liu, S., Yu, X., Hu, B., Zou, Y., Li, J., Bo, L. and Deng, X. (2015) Salidroside Rescued Mice from Experimental Sepsis through Anti-Inflammatory and Anti-Apoptosis Effects. *Journal of Surgical Research*, **195**, 277-283. <https://doi.org/10.1016/j.jss.2015.01.021>
- [80] Darshit, B.S. and Ramanathan, M. (2015) Activation of AKT1/GSK-3 $\beta$ /Catenin-TRIM11/Survivin Pathway by Novel GSK-3 $\beta$  Inhibitor Promotes Neuron Cell Survival: Study in Differentiated SH-SY5Y Cells in OGD Model. *Molecular Neurobiology*, **53**, 6716-6729. <https://doi.org/10.1007/s12035-015-9598-z>
- [81] Ma, T. (2014) GSK3 in Alzheimer's Disease: Mind the Isoforms. *Journal of Alzheimer's Disease*, **39**, 707-710. <https://doi.org/10.3233/JAD-131661>
- [82] Anupama, K.P., Antony, A., Shilpa, O., Raghu, S.V. and Gurushankara, H.P. (2022) Jatamansinol from *Nardostachys jatamansi* Ameliorates Tau-Induced Neurotoxicity in *Drosophila* Alzheimer's Disease Model. *Molecular Neurobiology*, **59**, 6091-6106. <https://doi.org/10.1007/s12035-022-02964-7>
- [83] Liu, Q.F., Jeon, Y., Sung, Y.-W., Lee, J.H., Jeong, H., Kim, Y.-M., Yun, H.S., Chin, Y.-W., Jeon, S., Cho, K.S. and Koo, B.-S. (2018) *Nardostachys jatamansi* Ethanol Extract Ameliorates A $\beta$ 42 Cytotoxicity. *Biological and Pharmaceutical Bulletin*, **41**, 470-477. <https://doi.org/10.1248/bpb.b17-00750>
- [84] Reinikainen, K.J., Soininen, H. and Riekkinen, P.J. (1990) Neurotransmitter Changes in Alzheimer's Disease: Implications to Diagnostics and Therapy. *Journal of Neuroscience Research*, **27**, 576-586. <https://doi.org/10.1002/jnr.490270419>