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ORIGINAL ARTICLE

Effects of tau on Aβ-induced synaptic damage in a *Drosophila* model of Alzheimer's disease

Qi Wang[1#], Li Ying[2#], Fude Huang[3,4], Jingya Lin[5], Wenan Wang[2,6]

1 Department of Neurology, Tengzhou Central People's Hospital, Tengzhou, Shandong Province, 277599, China
2 Department of Neurology, Xinhua Hospital Chongming Branch, Shanghai, 202150, China
3 Nuo-Beta Pharmaceutical Technology (Shanghai) Co. Ltd, Shanghai, 201210, China
4 University of the Chinese Academy of Sciences, Sino-Danish College, Chinese Academy of Sciences, Beijing, 100049, China
5 Department of Neurology, Tongren Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, 200050, China
6 Department of Neurology, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, 200092, China
# These two authors are co-first authors.

Correspondence to: Wenan Wang, PhD
Department of Neurology, Xinhua Hospital Chongming Branch, No.25, Nanmen Road, Chengqiao Town, Chongming District, 202150, Shanghai, China
TEL: +86 021-69692702, E-MAIL: wangwenanpeace@126.com

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Abstract

OBJECTIVES: The etiology and pathologic mechanism underlying Alzheimer's disease (AD) are not clear. This study determined the effects of tau on amyloid-beta peptide(Aβ)-induced synaptic damages in a *Drosophila* model of AD.
METHODS: Galactose-regulated upstream promoter element 4(Gal4) and an upstream active sequence system was used to establish four kinds of Aβ transgenic *Drosophila* models of AD. Behavioral evaluation and immunohistochemical localization were performed in Aβ transgenic *Drosophila* models. Tau mutants were introduced into arctic mutant Aβ1-42 (arctic mutant Aβ [Aβarc]) *Drosophila*. The P{Gal4}A307 *Drosophila* strain was used as a control group; 12 strains were obtained to determine the effects of tau with or without Aβarc. Electrophysiologic records of the tau mutant groups were created.
RESULTS: The flight and crawling ability of Aβ transgenic *Drosophila* were gradually weakened compared to the control group, and the life span was significantly shorter than the control group. Aβ was specifically expressed in the *Drosophila* giant fiber pathway and further accumulated in neuronal cell bodies based on immunohistochemistry. The percentage of the excitatory junctional potential (EJP) response in transgenic *Drosophila* expressing Aβarc was significantly decreased, which was approximately 40% lower than the control group. The tau deletion mutation alleviated the synaptic transmission disorder caused by Aβ and improved the viability of *Drosophila*.
CONCLUSION: The tau deletion mutation significantly improved the synaptic damage caused by Aβ, and tau protein played an indispensable role in the synaptic dysfunction caused by Aβ, suggesting that Aβ and tau have close interactions in the pathogenesis of AD.

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disorder and the leading cause of dementia in the elderly, with no treatment that can prevent or block disease progression (Piccialli *et al.* 2020). AD manifest clinically as a progressive decline in cognitive and memory functions, and further decline in the activities of daily living, which may be accompanied by psychiatric symptoms and emotional disturbances (Azocar *et al.* 2021; Shea *et al.* 2021; Silva *et al.* 2019). The onset of AD is more common in the elderly, and the course of the disease is slow and irreversible (Azocar *et al.* 2021; Shea *et al.* 2021; Silva *et al.* 2019).

Extracellular deposits of the amyloid-beta peptide (Aβ) are considered the main pathologic hallmark of AD (Deng *et al.* 2020). Aβ production is influenced by intracellular trafficking of secretases and amyloid precursor protein (APP) (Park *et al.* 2012). Selkoe *et al.* (Sze *et al.* 1997) proposed the "Aβ caused synaptic failure hypothesis" on the basis of the "Aβ cascade signaling hypothesis." The hypothesis purports that the abnormal synapse structure and function caused by Aβ, and the final synapse loss are the root causes of cognitive memory impairment and neuronal death (Sze *et al.* 1997). Studies have shown that the affected cortex and hippocampus in patients with AD have a significant decrease in synaptic density and loss of synaptic components (Kawas 2003; Scheff and Price 2006).

Tau protein, a microtubule-associated protein, has a high specific expression in neurons and axons (Tang *et al.* 2019). The tau protein contains phosphate groups, and the tau protein molecule in the normal mature brain contains 2-3 mol of phosphate groups (Pevalova *et al.* 2006). The tau protein in the brains of AD patients is abnormally hyperphosphorylated, and each molecule of tau protein contains 5-9 mol of phosphate groups and lose normal biological functions (Pevalova *et al.* 2006).

Animal models used in AD research include nematodes, fruit flies, mice, rats, and monkeys, and each model animal exhibits characteristics of AD (Crowther *et al.* 2005). The *Drosophila* model of AD has the following advantages: 1) Most of the genes of *Drosophila* are highly conserved (Rubin *et al.* 2000). 2) The passage cycle is approximately 10 days and the life span is short, which is conducive to the study of age-related diseases. 3) Powerful gene editing capabilities facilitate gene mutation in a specific location and within a specific time.

In this study, using the principles of the galactose-regulated upstream promoter element 4 and upstream active sequence (Gal4/UAS) system, a human-derived Aβ gene was transferred into the *Drosophila* genome and crossed with Gal4 transgenic *Drosophila* strains to achieve specific expression of Aβ in the nervous system of *Drosophila*. In the established Aβ transgenic *Drosophila*, a deletion mutation of tau was introduced to study the effect of tau expression on the neurotoxicity caused by Aβ. The electrophysiologic records of the *Drosophila* giant fiber neural pathway were created to study the changes in *Drosophila* synaptic transmission function, and behavioral evaluation of the transgenic *Drosophila* was performed to observe the impact of the introduction of tau mutation on the crawling and survival ability of *Drosophila*.

MATERIALS AND METHODS

Drosophila model

The specific expression of the Aβ transgene in the nervous system of *Drosophila* was achieved through the Gal4/UAS system. An AD model in *Drosophila*, which is more conducive to the study of synaptic structure and function, was established. The selected UAS *Drosophila* includes the following two types: the wild-type Aβ gene (Aβ1-40 or Aβ1-42) that is translated as a 40aa or 42aa peptide fragment; and the arctic Aβ (arctic Aβ [Aβ1-42E22G]) with a single point mutation. The arctic mutant Aβ1-42 (arctic mutant Aβ [Aβarc]) was donated by Dr. Crowther at the University of Cambridge in the United Kingdom (Crowther *et al.* 2005). The P{Gal4} A307*Drosophila* strain was donated by Dr. Allen at the University of Cambridge. The GAL4 strain can drive gene-specific expression in the giant fiber system (GF) and other related sensory nervous systems (Allen *et al.* 1999). The arctic Aβ gene was first discovered in patients with early-onset familial Alzheimer's disease (FAD); the expression product has stronger aggregation ability and cytotoxicity than the wild-type Aβ1-42 peptide (Nilsberth *et al.* 2001). In the current study 4 kinds of Aβ transgenic *Drosophila* models were established, including 1copy of Aβ1-40, 1copy of Aβ1-42, 2copies of Aβ1-42, and 1copy of Aβ1-42E22G, referred to as Aβ40x1, Aβ42x1, Aβ42x2, and Aβarc, respectively.

Behavioral evaluation of Drosophila

The living conditions of the experimental *Drosophila* were effectively adjusted and controlled through an intelligent artificial climate box. The temperature was set to 28.5°C, the relative humidity was 70%, and the light was cycled for 12 h each day and night. The behavioral evaluation experiment was based on the basic principle of gentle operation. Prior the test, the fruit flies will be placed in a test environment to balance and adapt for a period of time. The behavioral evaluation included survival curve drawing, flight ability, and crawling ability. The time of each experiment was guaranteed to be carried out at a fixed time every day, and the operator fixed and assigned double-blind groups to process the data.

Immunohistochemical localization of Aβ in transgenic Drosophila

A small amount of CO_2 was used to anesthetize the *Drosophila*. The *Drosophila* were fixed on silica gel

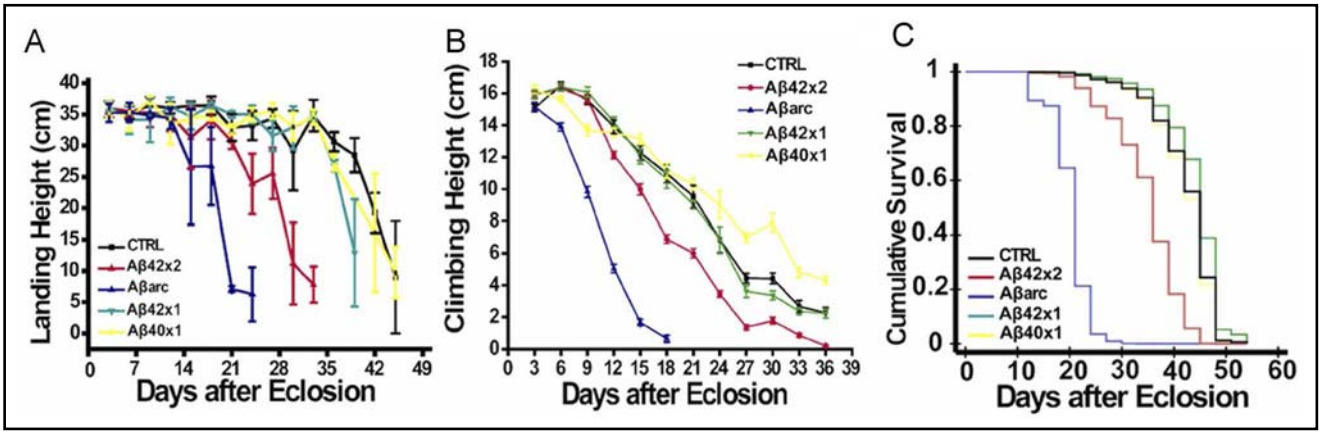

Fig. 1. Aβ expression induced age-dependent decline of flight ability (A), climbing ability(B), and decrease of lifespan(C) in different groups.

in dissecting dishes with a dissecting needle. A sufficient amount of PBS buffer was added to immerse the *Drosophila* and the *Drosophila* were kept in the PBS buffer environment. The *Drosophila* were pre-fixed in 4% paraformaldehyde (PFA) for 10 min. The separated *Drosophila* head and thoracic segments were placed in 4% PFA fixative solution for an additional 30-50 min, and a small part of the thoracic muscle was reserved for easy clamping. After the fixation, the separated *Drosophila* head and thoracic segments were transferred to PBS solution for rinsing 3 times for 15 min each time. After rinsing, the *Drosophila* nerve tissue was placed on a glass slide, a circle was drawn with a diameter of approximately 1cm on the periphery with a water-blocking pen, and approximately 50μL of PBS + 5% BSA + 0.5% Triton X (PBST) was added to rupture the membrane. After 2 h, the PBST was gently aspirated with a pipette, and 70% formic acid was added to expose the epitope of the sample. Then, the sample was rinsed gently with PBS 3 times for 15 min each time. The primary antibody (anti-Aβ monoclonal antibody, 6E10, 1:200) was added and refrigerated overnight (12h)

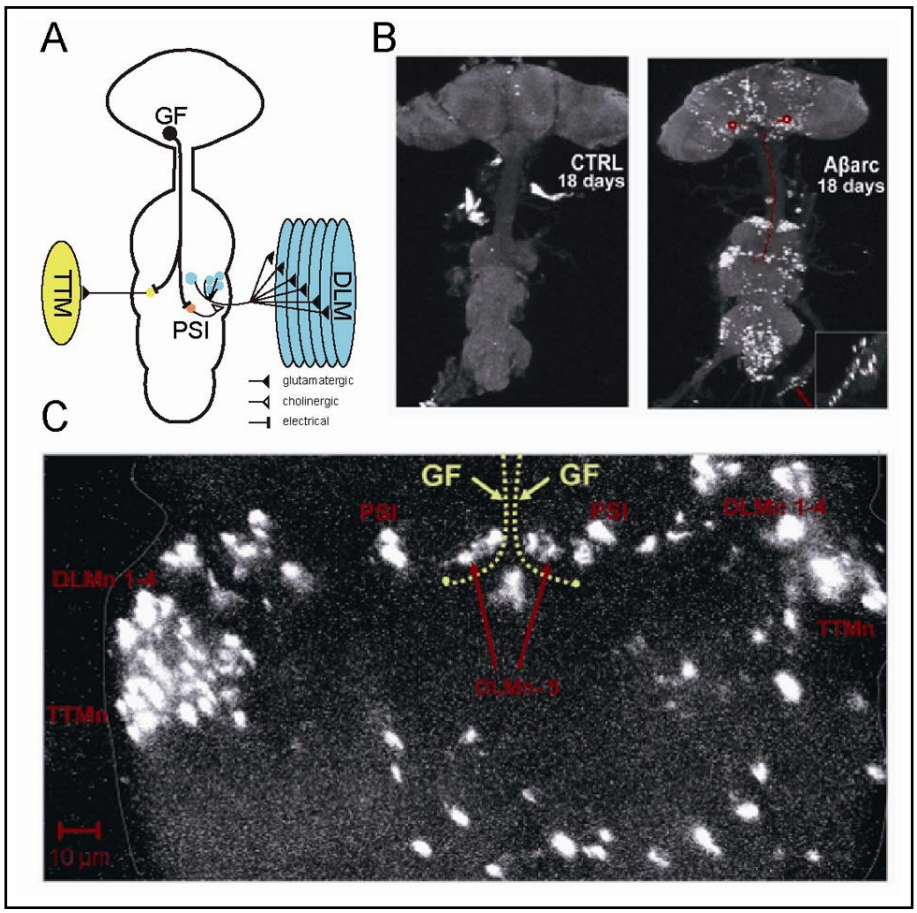

Fig. 2. Aβ expressed in the giant fiber system of Drosophila. (A) Schematic diagram of the Drosophilagiant fiber pathway structure. (B) Aβ immunohistochemical location; the left panel is the control group and the right panel is the Aβarc transgenic Drosophila. (C) Enlarged display of the local details of the thoracic ganglion of Drosophila in B. GF: giant fiber neuron; PSI: peripheral synapsing interneuron; TTM: tergotrochanteral muscle cell; DLM: dorsal longitudinal muscle cell; TTMn: TTM motor neuron; DLMn: DLM motor neuron.

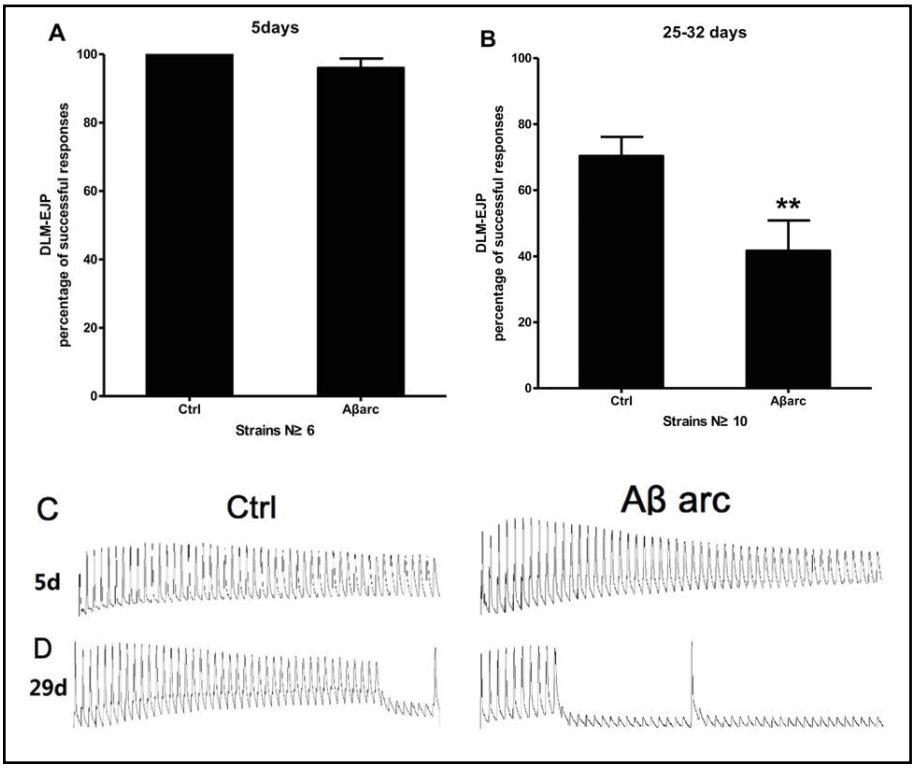

Fig. 3. Aβ expression in Drosophila caused an age-dependent obstacle of synaptic transmission in the giant fiber pathway. (A) DLM-EJP percentage of successful responses in the control and Aβarc Drosophila at 5 days of age. (B) DLM-EJP percentage of successful responses in the control and Aβarc Drosophila from 25-32 days of age. (C) EJP records of Aβarc and control Drosophila at 5 days of age. (D) EJP records of Aβarc and control Drosophila at 29 days of age.

at 4°C, then rinsed with PBS 3 times for 15 min each time. The secondary antibody (anti-mouse IgG-FITC, 1:1000) was added under irradiation of a redlight source. The sample was placed in a Petri dish wrapped in aluminum film to avoid light. After 2 h in the dark at room temperature, the sample was rinsed with PBS 3 times under a red light source for at least 15 min each time. After removing the remaining thoracic segment, the nervous system of the *Drosophila* was placed on a glass slide, and mounting glycerin was added. The sample was stored in a refrigerator at 4°C away from the light. Sections were observed with a ZEISS two-photon microscope (LSM 510 META NLO; ZEISS, Germany) with 20x and 40x objectives, and the pictures were saved.

Introduction of tau mutants

In the previously established *Drosophila* model, transgenic *Drosophila* expressing the Aβarc were selected. In this Aβ *Drosophila* model, the tau mutants (Bloomington *Drosophila* Stock Center; Bloomington, Minnesota, USA) were purchased. The insertion of tau fragments (stock numbers 17098& 15608), the RNA interference of tau (stock number 28891), and the deletion of tau fragments (stock number 9530). The GAL4 strain can drive gene-specific expression in the giant fiber (GF) system and other related sensory nervous systems. Therefore, if the A307-Aβ strain, which is driven by the GAL4-UAS system, specifically expresses Aβ in the escape pathway (GF) as the experimental group, the simple P{GAL4}A307 strain can be used as a suitable control group. The mutants were labeled, as follows: 17098, mutant1; 15608, mutant 2; 28891, mutant3; 9530, mutant4; and 17098/9530, mutant1/mutant4 (i.e., tau mutants on both chromosomes). The P{GAL4}A307 strain and Aβarc were combined with different tau mutants, and 12 strains were obtained, as follows: A307; A307+ mutant1; A307+ mutant2; A307+ mutant3; A307+ mutant4; A307+ mutant1/mutant4; Aβarc; Aβarc+mutant1; Aβarc+ mutant2; Aβarc+ mutant3; Aβarc + mutant4; and Aβarc + mutant1/mutant4.

Electrophysiologic records of Drosophila

The synaptic electrophysiologic records were created and life span assessments for each strain of *Drosophila* were performed. The electrophysiologic recordings on the synapses of the GF neural pathway in *Drosophila* were created to observe the changes in the excitatory junctional potential (EJP) of the flight loop in *Drosophila*, and compared with control *Drosophila* that only expressed P{GAL4}A307. The *Drosophila* were divided into two groups to test the effect of Aβ expression on *Drosophila* synaptic transmission function: the early *Drosophila* group that were < 1 week old; and the late *Drosophila* group that were between 3 and 5weeks old. The electrodes were filled with 3M KCl as a conductive solution. A pair of stimulating electrodes was inserted into the eyes of the fruit fly in the direction perpendicular to the eyes of the fruit fly, and the insertion depth was approximately 0.5mm. The tip of the electrode was near the cell body of the GF interneuron, and the electrode was fixed. The stimulus generator (Master 8; A.M.P.I.) generated a stimulus signal, which

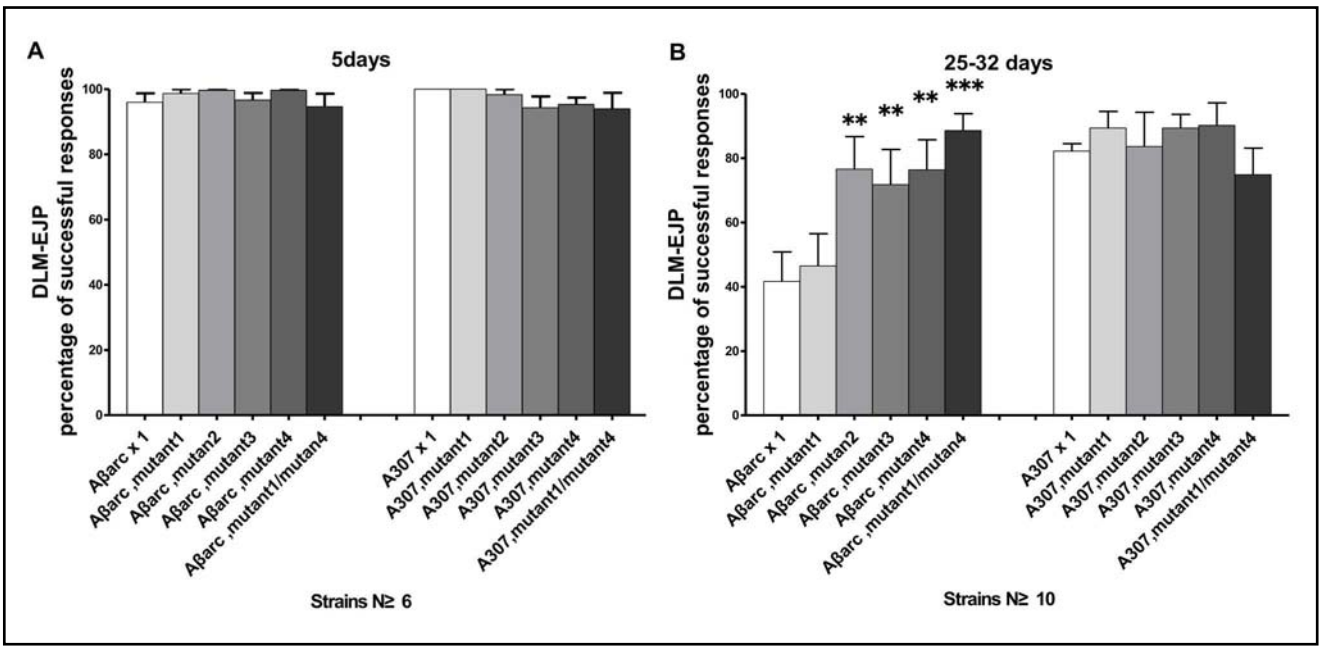

Fig. 4. (A) DLM-EJP percentage of successful responses in the different groups at 5 days of age. (B) DLM-EJP percentage of successful responses in the different groups from 25-32 days of age.
P{GAL4}A307×1 as control without Aβarc. Mutant 1: insertion mutation; mutant 2: insertion mutation; mutant 3 RNA interference; mutant4: deletion mutation; mutant1/mutant4: heterozygote of two mutations.

was processed by astimulus isolator (A.M.P.I.) and transmitted to the stimulation electrode. The angle between the inserted electrode and the dorsal longitudinal muscle (DLM) was approximately 45-60 degrees. When the electrode pierces the muscle cell, a potential change of -50 to -70mV occurs, which represents the membrane potential of the muscle cell. The number of EJPs produced by the DLM of *Drosophila* under a stimulus with a frequency of 100 Hz was recorded, and the percentage of EJPs produced by the DLM was obtained in the first 50 stimuli. The recorded signal was transmitted to an amplifier (multiple clamp 700B; Molecular Devices, Axopatch™, USA) through the recording electrode and the preamplifier. The isolated noise (10-20 kHz) was amplified several times, then input to a digital-to-analog converter (Digidata 1440A; Molecular Devices) and transmitted to a computer. The recorded signal was analyzed by pClamp 10.0 (AXON, USA).

Statistical analysis

The experimental data are expressed as the mean±standard error of the mean (mean±SEM), and paired or between-group t-tests were performed, as indicated. All data were analyzed with SPSS13.0 statistical software. The survival time data was not normally distributed, thus the non-parametric Kruskal-Walls H test was used to compare the means between multiple groups. A $p<0.05$ was considered a significant difference, and a $p<0.01$was considered a statistically significant difference. GraphPad Prism software was used for the overall composition.

RESULTS

Behavioral changes of Aβ transgenic Drosophila

The behavioral evaluation of Aβ transgenic *Drosophila* was performed and compared with a control group (transgenic *Drosophila* with only Gal4 or transgenic flies with only Aβ1-42 UAS) to observe the behavioral changes of Aβ transgenic *Drosophila*. The behavioral evaluation included three modules: flight ability test; crawling ability test; and survival life assessment. The flight and crawling abilities of Aβ transgenic *Drosophila* were gradually weakened with the increase in age, which was significantly earlier than the control group (Figure 1A and 1B). The life span of Aβ transgenic *Drosophila* was also shown to be significantly shorter than the control group (Figure 1C). In the Aβ transgenic *Drosophila* model, the Aβ arc group first showed behavioral differences, followed by Aβ42x2, then Aβ42x1 and Aβ40x1 (Figure 1A-C), which further showed that Aβ toxicity was dose-dependent.

Aβ specifically expressed in the nervous system of Drosophila

The Aβ monoclonal antibody, 6E10, was used to detect Aβ expressed in the neural pathway of *Drosophila* with immunohistochemistry. Aβ expression and accumulation were demonstrated in the neural pathways of Aβ40 X1, Aβ42X1, Aβ42X2, and Aβarc in *Drosophila*, and the expression distribution was completely consistent with the driving range of P{GAL4}A307 (Figure 2). In addition, the expression of Aβ was not only found in the cell body of the neuron but was also strongly expressed

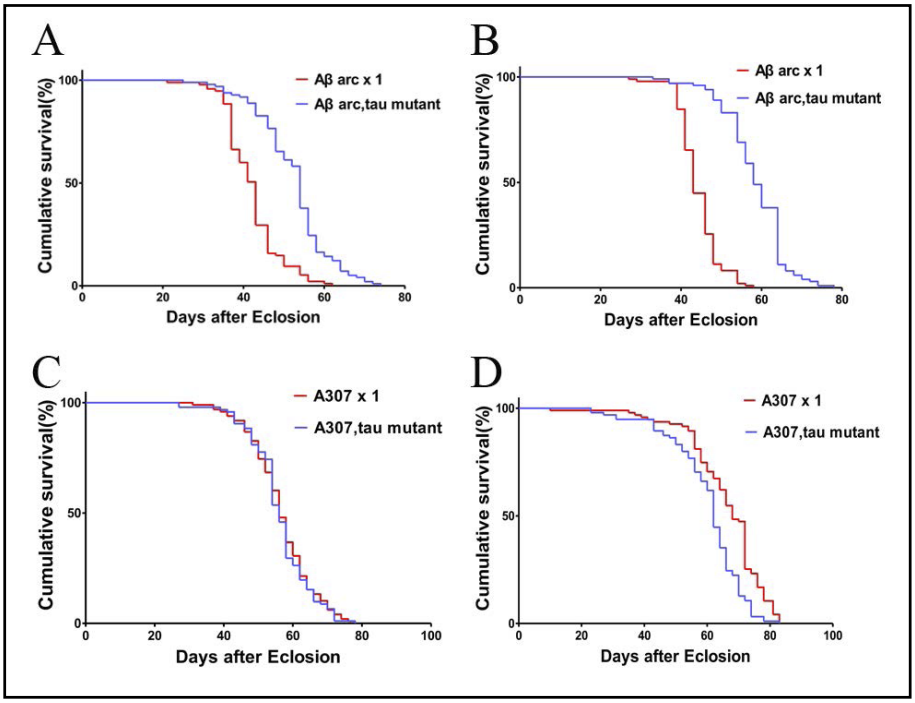

Fig. 5. Survival time analysis of Aβarc and tau mutant Drosophila (A: male, B: female). Survival time analysis of P{GAL4}A307 control and tau mutant Drosophila (C: male, D: female).

in the axon. Using the GAL4/UAS system driven by P{GAL4}A307, Aβ was specifically expressed in the *Drosophila* GF pathway and further accumulated in neuronal cell bodies.

Synaptic function changes in Aβ transgenic Drosophila

Compared with the control group, the Aβ transgenic *Drosophila* that were < 1 week old had no significant EJP changes (Figure 3A). As the age increased in the 3–5-week-old *Drosophila*, the EJP of the control group decreased, but the percentage of EJP response in transgenic *Drosophila* expressing Aβarc was significantly decreased, which was approximately 40% lower than the control group (Figure 3B-D).

Effect of tau mutation on synaptic transmission disorder caused by Aβ

Whether or not there was a decrease in tau expression at 5 days of age, there was no significant difference between the Aβarc transgenic *Drosophila* and the control group (Figure 4A). In the late *Drosophila* group (25-32 days), other than mutant1, significantly improved synaptic transmission barriers occurred in other Aβarc *Drosophila* with tau mutants compared with *Drosophila* that simply expressed Aβarc, and no similar changes were observed in the control *Drosophila* (Figure 4B).

Effect of tau mutation on the change in life span caused by Aβ

Survival of Aβarc transgenic *Drosophila* was significantly improved after the tau mutation, and the survival life was significantly prolonged (Figure 5A and 5B). No significant difference existed between the control *Drosophila* expressing P{GAL4}A307 and tau mutants (Figure 5C and 5D). The reduction of tau protein expression will not cause a significant change in the life span of *Drosophila* without Aβ expression.

DISCUSSION

The Gal4/UAS system drives the specific expression of Aβ in the GF neural pathway of *Drosophila*. Using immunohistochemistry, it was observed that Aβ was deposited and accumulated in the cell bodies of the GF pathway neurons. The flight and crawling abilities of the transgenic *Drosophila* were significantly reduced, and the life span was shortened compared with the control group. The transgenic AD model can be better used to study the specific mechanism underlying Aβ neurotoxicity on synapses. By observing the synaptic transmission function of the neuromuscular junction in Aβ transgenic *Drosophila*, we showed that Aβ damaged synaptic transmission in *Drosophila*, confirming that Aβ is toxic to the synapse and causes synaptic dysfunction. Further research showed that the tau deletion mutation alleviated the synaptic transmission disorder caused by Aβ and improved the viability of *Drosophila*, while the only tau deletion mutation in the absence of Aβ expression did not have a significant effect on synaptic transmission.

To more accurately study the influence of Aβ on synapses, we used the P{GAL4}A307 driver to drive the specific expression of Aβ42 in the GF neural pathway. Aβ42 was accumulated and gathered in the neurons of the GF escape pathway, and the Aβ42 transgenic *Drosophila* had a significant decline in flight ability, tube climbing ability, and survival ability. These results

were consistent with previous results(Collins and Diantonio 2007; Khatoon *et al.* 2019; Lv *et al.* 2017). By specifically expressing Aβ in the GF neural pathway of *Drosophila*, an AD model was established that was more conducive to the study of synaptic structure and function, and the Neuromuscular junction (NMJ) of *Drosophila* was used as the synapse observation site. The AD model has the following advantages: 1) Various experimental methods, such as electrophysiology, electron microscopy, immunofluorescence labeling, and behavioral evaluation, can be used to observe the structure and function of synapses. 2) The NMJ of *Drosophila* is mediated by a glutamatergic neurotransmitter, and this is very similar to the glutamatergic synapses in the vertebrate central nervous system with respect to molecular composition. 3) There are specific motor neurons innervating the NMJ of *Drosophila* and the structure is stable (Fa *et al.* 2019).

The Gal4/UAS system includes two parts: GAL4; and UAS. GAL4 expression can specifically bind to UAS to regulate the expression of genes related to galactose metabolism. When GAL4 and UAS are expressed in *Drosophila*, Gal4 activates the transcription of downstream genes linked to UAS in a tissue-specific manner (Fischer *et al.* 1988), while the simple expression of Gal4 in *Drosophila* has no clear toxic effects. The transcription of UAS-linked genes can only be initiated when GAL4 and UAS co-exist and combine. Brand *et al.* (Brand and Perrimon 1993) systematically explained the application of Gal4/UAS system regulation gene-specific expression in *Drosophila*. The Gal4/UAS system has subsequently been widely used in *Drosophila* gene-related research and has become a powerful tool for studying *Drosophila* gene expression. In another study, Aβ42 was shown to be expressed in the brains of *Drosophila* through transgenes, and Aβ amyloid deposition was detected in the brain tissue (Iijima *et al.* 2004). The Aβ42 transgenic *Drosophila* also had an age-dependent decline in olfactory learning ability and a significant decline in the ability to climb tubes. The survival life was approximately 40% shorter than the control group, and neuron loss was noted in the brains of transgenic *Drosophila*.(Iijima *et al.* 2004) These results indicated that overexpression of Aβ42 in *Drosophila* brains lead to decreased learning ability and neuron loss, which is similar to the clinical symptoms of AD in humans. The molecular mechanism underlying Aβ42 neurotoxicity is relatively conservative in humans and *Drosophila*.

The exact etiology and pathogenesis of AD are unknown, but it is widely accepted that Aβ plays a key role in the pathogenesis of AD (Matsuzaki 2020). The level of enzymes related to cholinergic metabolism in patients with AD are significantly decreased (Davies 1976), and it has been shown that the levels of neurotransmitters are closely related to synaptic function, such as somatostatin, GABA, and serotonin, undergo significant changes (Selkoe and D. 2002). On the basis of these studies, researchers have further observed that the onset of AD is inseparable from the loss of synapses in the study of morphology and ultrastructure (Kashyap *et al.* 2019; Sokolova *et al.* 2021). The pathologic slices of the brain tissues of AD patients showed that 25w-30% of synapses are lost in the cerebral cortex, thus when compared with other pathologic changes, such as senile plaques, neurofibrillary tangles, neuron loss, and glial hyperplasia, synaptic loss is more closely related to cognitive impairment (Terry *et al.* 1991). Synapse damage or loss is a very important feature of the pathogenesis of AD, and may be the leading cause of cognitive impairment (Mir and Rizvi 2019; Petrache *et al.* 2019).

It is now widely accepted that tau protein is a microtubule-associated protein that can regulate the stability of microtubules and participate in the transport of microtubules (Benítez *et al.* 2021). The microtubule-associated protein tau has a certain relationship with the pathogenesis of AD, but the specific mechanism underlying neurotoxicity caused by tau is still unknown. Wittmann *et al.* (Wittmann and C. 2001) expressed wild-type tau and tau mutants in transgenic flies and found that these flies had functional abnormalities similar to AD patients, including progressive neurodegeneration, shortened lifespan, and accumulation of abnormal expression of tau(Wittmann and C. 2001). Aβ promotes the phosphorylation and neurotoxicity of tau. Phosphorylation of serine at position 262 of tau plays a key role in this process, and *Drosophila* does not show neurodegeneration after inhibiting the phosphorylation of this site (Iijima and Gatt 2010). The paired nerve fiber filaments formed by the accumulation of hyperphosphorylated tau in neurons are thought to play a key role in the pathogenesis of AD. Compared with amyloid deposition, the research on tau protein in the pathologic mechanism underlying AD is relatively late, but is more closely related to neurodegeneration and cognitive dysfunction in AD (Goedert and Spillantini 2019).

Tau hyperphosphorylation and neuronal death are thought to be the subsequent effects of Aβ deposition (Hardy and J. 2002); however, how the neurotoxicity of Aβ causes tau-related pathologic changes and whether there is a causal relationship between the two has not been confirmed. Treatment of neurons with Aβ oligomers mediates the phosphorylation of tau, but in patients with frontotemporal dementia in whom tau gene mutations are detectable, tau accumulates and deposits in the absence of Aβ (Ballatore *et al.* 2007). Overexpression of tau protein causes age-dependent neurofibrillary tangles, synaptic dysfunction, neuronal death, and even behavioral abnormalities in mice (Santacruz *et al.* 2005). Of greater interest, when the overexpression of tau is completely suppressed by gene interference technology, although there are neurofibrillary tangles formed in the brain, the recovery

of memory function in mice is still observed and neurodegeneration is also prevented, which suggests that the persistent soluble tau protein may be the main cause of neurotoxicity compared with the neurofibrillary tangles (Santacruz *et al.* 2005).

The main function of synapses is to transmit signals from pre- to post-synapses through the release of transmitters. Impairment of synapse function can lead to synapse destruction and loss, as well as abnormal function of the brain neural network, which is manifested as memory impairment and cognitive dysfunction in AD. Based on our results, we are of the opinion that when abnormal expression of the gene encoding tau causes a decrease in the expression of tau protein, the neurotoxicity mediated by Aβ is disturbed, and damage to the synapses are suppressed, which appears in Aβ transgenic *Drosophila*. The age-dependent synaptic transmission dysfunction is significantly improved when the expression of tau is reduced.

There were some limitations in this study. First, this study was an animal model study, and the results need to be further confirmed in clinical studies. Second, our results revealed a possible correlation between tau and Aβ, but there are still many questions that require answers, including whether the expression of Aβ or the absence of tau will have an impact on synaptic transmission. When interfering with the expression of Aβ or tau through experimental methods, such as genetic manipulation of transgenes, what mechanism will affect synaptic function is unknown, thus further study is warranted.

In conclusion, the flight and crawling abilities of transgenic *Drosophila* were significantly reduced, and the life span was shortened in an AD model, indicating that the transgenic AD model can be used to study the specific mechanism underlying Aβ neurotoxicity on synapses and Aβ damage to the synaptic transmission in *Drosophila*. Further research has shown that the tau deletion mutation significantly improves the synaptic damage caused by Aβ and tau protein and may play an indispensable role in the synaptic dysfunction caused by Aβ, indicating that Aβ and tau have a close interaction in the pathogenesis of AD.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

None declared.

REFERENCES

1 Allen MJ, Shan X, Caruccio P, Froggett SJ, Moffat KG, Murphey RK. (1999). Targeted expression of truncated glued disrupts giant fiber synapse formation in *Drosophila*. J Neurosci. **19**(21): 9374–9384.
2 Azocar I, Livingston G, Huntley J. (2021). The Association Between Impaired Awareness and Depression, Anxiety, and Apathy in Mild to Moderate Alzheimer's Disease: A Systematic Review. Front Psychiatry. **12**: 633081.
3 Ballatore C, Lee VM, Trojanowski JQ. (2007). Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat Rev Neurosci. **8**(9): 663–672.
4 Benítez MJ, Cuadros R, Jiménez JS. (2021). Phosphorylation and Dephosphorylation of Tau Protein by the Catalytic Subunit of PKA, as Probed by Electrophoretic Mobility Retard. J Alzheimers Dis. **79**(3): 1143–1156.
5 Brand AH, Perrimon N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development. **118**(2): 401–415.
6 Collins CA, Diantonio A. (2007). Synaptic development: insights from *Drosophila*. Curr Opin Neurobiol. **17**(1): 35–42.
7 Crowther DC, Kinghorn KJ, Miranda E, Page R, Curry JA, Duthie FA, et al. (2005). Intraneuronal Abeta, non-amyloid aggregates and neurodegeneration in a *Drosophila* model of Alzheimer's disease. Neuroscience. **132**(1): 123–135.
8 Davies P. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet. **2**: 1403.
9 Deng L, Haynes PA, Wu Y, Amirkhani A, Kamath KS, Wu JX, et al. (2020). Amyloid-beta peptide neurotoxicity in human neuronal cells is associated with modulation of insulin-like growth factor transport, lysosomal machinery and extracellular matrix receptor interactions. Neural Regen Res. **15**(11): 2131–2142.
10 Ali F, Rahul, Jyoti S, Naz F, Ashafaq M, Shahid M, Siddique YH (2019). Therapeutic potential of luteolin in transgenic *Drosophila* model of Alzheimer's disease. Neurosci Lett. **692**: 90–99. doi: 10.1016/j.neulet.2018.10.053.
11 Fischer JA, Giniger E, Maniatis T, Ptashne M. (1988). GAL4 activates transcription in *Drosophila*. Nature. **332**(6167): 853–856.
12 Goedert M, Spillantini MG. (2019). Ordered Assembly of Tau Protein and Neurodegeneration. Adv Exp Med Biol. **1184**: 3–21.
13 Hardy, J. (2002). The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics. Science. **297**(5580): 353–356.
14 Iijima K, Gatt A. (2010). Tau Ser262 phosphorylation is critical for Aβ42-induced tau toxicity in a transgenic *Drosophila* model of Alzheimer's disease. Hum Mol Genet. **19**(15): 2947–2957.
15 Iijima K, Liu HP, Chiang AS, Hearn SA, Konsolaki M, Zhong Y. (2004). Dissecting the pathological effects of human A beta 40 and A beta 42 in *Drosophila*: A potential model for Alzheimer's disease. Proc Natl Acad Sci U S A. **101**(17): p. 6623–6628.
16 Kashyap G, Bapat D, Das D, Gowaikar R, Amritkar RE, Rangarajan G, et al. (2019). Synapse loss and progress of Alzheimer's disease -A network model. Sci Rep. **9**(1): 6555.
17 Kawas CH. (2003). Early Alzheimer's Disease. N Engl J Med. **350**(11): 419–420.
18 Khatoon R, Rasheed MZ, Rawat M, Alam M, Parvez S. (2019). Effect of melatonin on Aβ42 induced changes in the mitochondrial function related to Alzheimer's disease in *Drosophila* melanogaster. Neuroscience Letters. **711**: 134376.
19 Lv F, Yang X, Cui C, Su C, Hemachandra R. (2017). Exogenous expression of Drp1 plays neuroprotective roles in the Alzheimer's disease in the Aβ42 transgenic *Drosophila* model. Plos One. **12**(5): e0176183.
20 Matsuzaki K. (2020). Aβ-ganglioside interactions in the pathogenesis of Alzheimer's disease. Biochim Biophys Acta Biomembr. **1862**(8): 183233.
21 Mir FA, Rizvi ZA. 2019. Neurobiological Mechanisms Involved in the Pathogenesis of Alzheimer's Disease. Biological, Diagnostic and Therapeutic Advances in Alzheimer's Disease.
22 Nilsberth C, Westlind-Danielsson A, Eckman CB, Condron MM, Axelman K, Forsell C, et al. (2001). The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. Nat Neurosci. **4**(9): 887–893.

23 Park HJ, Shabashvili D, Nekorchuk MD, Shyqyriu E, Jung JI, Ladd TB, et al. (2012). Retention in endoplasmic reticulum 1 (RER1) modulates amyloid-β (Aβ) production by altering trafficking of γ-secretase and amyloid precursor protein (APP). J Biol Chem. **287**(48): 40629–40640.

24 Petrache AL, Aarib R, Shi A, Andrea W, Takashi S, Saido TC, et al. (2019). Aberrant Excitatory–Inhibitory Synaptic Mechanisms in Entorhinal Cortex Microcircuits During the Pathogenesis of Alzheimer's Disease. Cerebral Cortex.(4): 4.

25 Pevalova M, Filipcik P, Novak M, Avila J, Iqbal K. (2006). Post-translational modifications of tau protein. Bratislavske Lekarske Listy. **107**: 346–353.

26 Piccialli I, Ciccone R, Pannaccione A. (2020). The new K(V)3.4 inhibitor BDS-I[1-8] as a potential pharmacological opportunity in Alzheimer's disease therapy. Neural Regen Res. **15**(7): 1255–1256.

27 Rubin GM, Yandell MD, Wortman JR, Gabor Miklos GL, Nelson CR, Hariharan IK, et al. (2000). Comparative genomics of the eukaryotes. Science. **287**(5461): 2204–2215.

28 Santacruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingelsson M, et al. (2005). Tau suppression in a neurodegenerative mouse model improves memory function. Science. **309**(5733): 476–481.

29 Scheff SW, Price D. (2006). Alzheimer's disease-related alterations in synaptic density: neocortex and hippocampus. Journal of Alzheimer\"s Disease. **9**(s3): 101–115.

30 Selkoe, D. J. (2002). Alzheimer's Disease Is a Synaptic Failure. Science. **298**(5594): 789–791.

31 Shea Y, Pan Y, Mak H, Bao Y, Lee S, Chiu K, et al. (2021). A systematic review of atypical Alzheimer's disease including behavioural and psychological symptoms. Front Psychiatry. **12**: 633081.

32 Silva M, Loures C, Luan C, Souza L, Carvalho M. (2019). Alzheimer's disease: Risk factors and potentially protective measures. J Biomed Sci. **26**(1).

33 Sokolova D, Childs T, Hong S. (2021). Insight into the role of phosphatidylserine in complement-mediated synapse loss in Alzheimer's disease. Fac Rev. **10**: 19.

34 Sze CI, Troncoso JC, Kawas C, Mouton P, Price DL, Martin LJ. (1997). Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. J Neuropathol Exp Neurol. **56**(8): 933–944.

35 Tang Y, Liu HL, Min LX, Yuan HS, Guo L, Han PB, et al. (2019). Serum and cerebrospinal fluid tau protein level as biomarkers for evaluating acute spinal cord injury severity and motor function outcome. Neural Regen Res.

36 Terry RD, Masliah E, Salmon DP, Butters N, Katzman R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. Ann Neurol. **27**(4): 457–464.

37 Wittmann, C. W. (2001). Tauopathy in *Drosophila*: Neurodegeneration Without Neurofibrillary Tangles. Science. **293**(5530): 711–714.