Differential dysregulation of CREB and synaptic genes in transgenic Drosophila melanogaster expressing shaggy (GSK3), TauWT, or Amyloid-beta

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Abstract

Background: Tau, Amyloid-beta ($A\beta_{42}$), and Glycogen synthase kinase 3 (GSK3) contribute to synaptic dysfunction observed in Alzheimer's disease (AD), the most common form of dementia. In the current study, the effect of pan-neuronal expression of Tau^{WT}, $A\beta_{42}$, or shaggy (orthologue of GSK3) in *Drosophila melanogaster* was assessed on the locomotor function, ethanol sensitivity, synaptic genes and *CREB* expression. The effect of Tau^{WT} and $A\beta_{42}$ on the expression of *shaggy* was also determined.

Methods and results: Gene expression analysis performed using quantitative real-time RT-PCR method. While syt1, SNAP25 and CREB (upstream transcription factor of syt1 and SNAP25) were upregulated in flies expressing TauWT or Aβ42, a prominent decline was observed in those genes in shaggy expressing flies. While all transgenic flies showed climbing disability and higher sensitivity to ethanol, abnormality in these features was significantly more prominent in transgenic flies expressing shaggy compared to TauWT or Aβ42. Despite a significant upregulation of shaggy transcription in TauWT expressing flies, Aβ42 transgenic flies witnessed no significant changes.

Conclusions: Tau^{WT}, $A\beta_{42}$, and shaggy may affect synaptic plasticity through dysregulation of synaptic genes and *CREB,* independently. However shaggy has more detrimental effect on synaptic genes expression, locomotor ability and sensitivity to ethanol. It is important when it comes to drug discovery. It appears that CREB is a direct effector of changes in synaptic genes expression due to the same pattern of their alteration and it is likely to be a part of compensatory mechanisms independent of the GSK3/CREB pathway in Tau^{WT} or $A\beta_{42}$ expressing flies.

Introduction

Alzheimer's disease (AD) is one of the most prevalent forms of dementia in the elderly population. It is a progressive disorder that gradually disrupts the function of different brain regions involved in memory formation and cognition. AD is characterized by widespread synaptic and neuronal loss and the generation of two neuropathological lesions, including extracellular amyloid plaques composed of Aβ peptides and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein [1]. In addition, GSK3β expression is dysregulated in the brain of AD patients, and the evidence confirms its pivotal role in AD pathogenesis [2].

AD is mainly a disorder of synaptic dysfunction referred to as synaptopathy [3]. There is a robust correlation between synaptic loss and cognitive decline in AD, and the dysfunction occurs in the very early stages of the disease [4]. Remarkable evidence has demonstrated that increased phosphorylated Tau, Aβ, and GSK3 levels can lead to synaptic dysfunction and loss [reviewed in 2, 4-5]. In addition, these factors are all involved in the dysregulation of various genes in the brain of AD patients and models of Alzheimer's disease [6-9].

Previous studies revealed that two presynaptic proteins, Synaptotagmin 1 (SYT1) and Synaptosomal-associated protein of 25 kDa (SNAP25), undergo expression alteration at both RNA and protein levels in AD brain patients [10-12]. It is noteworthy that there is a close relationship between altered synaptic gene expression and decreased neuronal activity, cognitive impairment, and memory loss during AD pathogenesis [13].

SYT1 is a primary Ca^{2+} sensor for triggering synaptic vesicle exocytosis at central nerve system synapses [14]. It is a potential biomarker of synaptic activity [15] and crucial for regulating synaptic plasticity in different brain regions. This presynaptic protein facilitates learning and memory formation, and its altered levels lead to cognition impairment [16].

SNAP25 is one of the critical components of the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) complex, which is essential for calcium-dependent exocytosis of the synaptic vesicle [17]. This protein is also involved in the regulation of synaptic plasticity and is a biomarker for synaptic plasticity impairment [18]. SNAP25 is necessary for learning and memory formation and consolidation, neuronal survival, and cognitive function [17].

Cyclic AMP response element-binding protein (CREB) plays a pivotal role in learning and memory because it is a central regulator of memory gene expression, including synaptic genes. CREB is activated upon phosphorylation [19], and it has been shown that total and active CREB (pCREB) is downregulated in the postmortem brain of AD patients confirming that the CREB signaling pathway is disrupted in this disease [20-21].

Although many aspects of mechanisms underlying AD pathogenesis have been discovered, the disease is still incurable and current therapies only slow down the progression of the disease. To achieve an appropriate cure for the disease, discovering various mechanisms involved in AD seems necessary [22]. As a powerful invertebrate model for studying human diseases, especially neurodegenerative diseases, *Drosophila melanogaster* was applied in this research [23]. To elucidate the pathogenic mechanisms of Tau^{WT}, $A\beta_{42,}$ and GSK3 on synaptic dysfunction in Alzheimer's disease, we investigated the expression of *syt1* and *SNAP25* synaptic genes and CREB in transgenic drosophila flies expressing Tau^{WT}, $A\beta_{42}$, or shaggy (orthologue of GSK3), independently. In addition, the effect of Tau^{WT} and $A\beta_{42}$ was assessed on the *shaggy* expression. Behavioral studies were also performed to compare the effect of Tau, $A\beta_{42}$, or shaggy on locomotor function and alcohol sensitivity.

Materials And Methods

Flies lines and maintenance

Flies were raised on standard oats-agar media at 24±1 ℃, at 60-70% relative humidity, and a photoperiod of 12h. As $A\beta_{42}$ is the most toxic form of Amyloid-beta peptides and there are no known mutations in Tau associated with AD, we applied $A\beta_{42}$ and Tau^{WT} transgenic flies [24, 25]. UAS-Tau^{WT}, UAS-$A\beta_{42}$

(Bloomington Stock NO.33769), and UAS-shaggy (Bloomington Stock NO.5255) carry human Tau^{WT}, human $A\beta_{42,}$ and S9A shaggy (orthologue of GSK3 in Drosophila) gene, respectively. nSyb-gal4 (Bloomington Stock NO.51945), the neuron-specific transcription driver, was applied for the pan-neuronal expression of transgenes. Human tau expressing flies were a gift from Feany's lab (Harward Medical School, Boston, USA), and all other lines were from the Bloomington drosophila stock center (https://bdsc.indiana.edu/). UAS parental lines were applied as control groups. In all experiments, 15-day-old flies were assessed. RT-PCR was employed using specific primers detecting Tau^{WT}, $A\beta_{42,}$ or shaggy [7] (Fig S1) in order to verify the expression of transgenes in each line of flies.

Climbing assay

The climbing assay was performed according to the procedure described by Ali et al. [26]. The detailed description is given in the supplemental experiment procedure.

Alcohol sedation and recovery assay

The alcohol sedation assay was accomplished according to the protocol explained by Maples and Rottenfluh [27]. The detailed procedure is explained in the supplemental experiment procedure.

RNA extraction and cDNA synthesis

RNA extraction was performed from a homogenate of 100 flies' heads using RNXplus kit (Cinnagene). RNA concentration and purity were measured using Nanodrop (Thermo Fisher Scientific), and the integrity was evaluated by 1.5% agarose gel electrophoresis. 2.5 microgram of each RNA sample was converted to cDNA using Yekta Tajhiz Azma (YKT) cDNA synthesis kit following the provider's protocol. A separate reaction was performed without RT enzyme (noRT control). Three biological replicates were performed for each transgenic and control flies.

Primer designing and Quantitative Real-time PCR

The exon-exon junction primer or intron-inclusion primers were designed using AlleleID software version 7. Designed primers were specific for target mRNA and did not amplify genomic DNA. The primers detected all known isoforms of target genes.

Real-time PCR reactions were performed with 50 nanogram cDNA from each sample using the SYBR green PCR master mix (ampliqon) on an ABI prism 7500 real-time PCR system (Applied Biosystems). A negative control lacking cDNA was also included. A melt curve verified a single product generated for each transcript. All Real-time PCR reactions were run in triplicate. Relative gene expression levels of interested genes were calculated after normalizing against endogenous reference gene *EF1α* by $2^{-\Delta\Delta Ct}$ method [28]. The list of primers' sequences is available in Supplementary Table S1.

Statistical analysis

The normality test, Shapiro-Wilk, was carried out to ensure the normal distribution of all data. Mean comparison between flies expressing transgene and their controls was performed using independent sample *t*-test. Comparative analysis between different transgenes was done using one-way analysis of variance followed by Tukey HSD posthoc test for pairwise group comparison.

All statistical analyses were conducted by IBM SPSS statistic software version 21 (SPSS, Chicago, IL, US). The minimum level of significance was set at p-value of 0.05.

Results

Expression of shaggy caused more locomotor deficits compared to human Tau or human $A\beta_{42}$

Changes in locomotion happen in very early stages of AD [29]. Locomotor assay has been previously described to evaluate neuronal dysfunction in the fly model of alzheimer's disease [30]. Therefore, we compared locomotor activity in our transgenic flies. Flies expressing human Tau, human $A\beta_{42}$, or shaggy in neuronal brain displayed a significant reduction in climbing ability (39%, 29%, and 72%, respectively) compared to their control flies (p values < 0.001) (Fig 1). As it can be seen from Fig 1, shaggy expressing flies showed the highest reduction compared to the other transgenic flies and there was no significant difference between Tau^{WT} and $A\beta_{42}$ expressing flies in reducing locomotor ability (Table S2).

More sensitivity to ethanol was observed in flies expressing shaggy compared to flies expressing human wild-type Tau or human $A\beta_{42}$

The genes involved in alcohol metabolizing pathway are shared between flies and mammals [31]. Moreover, there is an overlapping between genes contributing to alcohol use disorder and Alzheimer's disease [32]. Interestingly, recent studies showed similarity between genes involved in memory formation and ethanol-related behaviors in *Drosophila melanogaster* [reviewed in 33]. So we investigated the influence of overexpression of Tau^{WT}, $A\beta_{42}$, or shaggy on ethanol sensitivity using ethanol sedation assay. The ST50 of flies expressing Tau^{WT}, $A\beta_{42}$, or shaggy was significantly lower (37%, 30%, and 57%, respectively) than the ST50 of control genotypes (p values < 0.001) (Fig 2a), while these transgenic flies showed a significant elevated RC50 value (36%, 27%, and 67%, respectively) compared to their control groups (P-value < 0.001, p-value < 0.01, p-value < 0.001) (Fig 2b). Interestingly, shaggy expressing flies had more impact on the sensitivity to ethanol compared to the other two transgenic groups and Tau^{WT} and $A\beta_{42}$ transgenic flies did not indicate any remarkable difference in increasing sensitivity to ethanol (Table S3 and S4).

While wild-type Tau and $A\beta_{42}$ upregulated the expression of *syt1* and *SNAP25,* shaggy overexpression downregulated these synaptic genes

To elaborate on the effect of Tau^{WT}, $A\beta_{42,}$ or shaggy on synaptic dysfunction in AD, we measured *syt1* and *SNAP25* mRNA levels on 15-day-old adult flies expressing Tau^{WT}, $A\beta_{42}$, or shaggy in the central nerve system (CNS) neurons using relative real-time RT-PCR.

Both *syt1* and *SNAP25* mRNA levels were significantly elevated in Tau^{WT} transgenic flies (Fig 3) (*syt1*: 61%, *SNAP25*: 35%) (p-value < 0.01, p-value < 0.01) and flies expressing $A\beta_{42}$ relative to their controls (Fig 3) (*syt1*: 25%, *SNAP25*: 23%) (p-value < 0.05, p-value < 0.01). In contrast, we observed a significant reduction in mRNA levels of *syt1* and *SNAP25* in flies overexpressing shaggy compared to their control (Fig 3) (*syt1*: 28%, *SNAP25*: 61%) (p-value < 0.05, p-value < 0.001, respectively). No significant difference was observed between Tau^{WT} and $A\beta_{42}$ transgenic flies in terms of *syt1* and *SNAP25* genes expression levels (Table S5).

CREB transcription factor was differently dysregulated in shaggy overexpressing flies compared to wild-type Tau or $A\beta_{42}$transgenic flies

In the next step, we examined the expression of *CREB*, the upstream transcription factor of *syt1* and *SNAP25* in our transgenic flies [34]. We perceived a striking increase of 128% in the expression of *CREB* in Tau^{WT} transgenic flies compared to their control (Fig 4) (p-value < 0.01). Similarly, with regards to $A\beta_{42}$ expressing flies, *CREB* expression was notably elevated by 114% compared to their counterpart control (Fig 4) (p-value < 0.001). By contrast, shaggy transgenic flies witnessed a plummet of 86% in mRNA level of *CREB* in comparison to the control group. (Fig 4) (p-value < 0.01). No remarkable difference was spotted between Tau^{WT} and $A\beta_{42}$ expressing flies concerning *CREB* mRNA level (Table S5).

While wild-type Tau upregulated shaggy expression, no change was observed in shaggy levels in $A\beta_{42}$ transgenic flies

As pointed out before, GSK3 has a detrimental effect on synaptic plasticity and dysregulates in AD [2, 35]. Also, GSK3 is a regulator of CREB activity [36]; therefore, we determined the mRNA level of *shaggy* in Tau^{WT} or $A\beta_{42}$ transgenic flies to investigate if the change in CREB expression is through GSK3/CREB pathway or not. As it can be seen in Fig 5, there was an increase in transcription levels of *shaggy* in Tau^{WT} expressing flies by 28% in comparison to the control group (p-value < 0.05). However, we could not detect any significant difference in *shaggy* levels between $A\beta_{42}$ transgenic flies and their control.

Discussion

In the current research, first, the effect of the transgenes was examined on the locomotor activity and ethanol sensitivity. Then, the impact of these transgenes was assessed on the expression of synaptic genes, *CREB*, and *shaggy*. We expressed Tau^{WT}, $A\beta_{42,}$ or shaggy in *Drosophila melanogaster* using nSyb-Gal4 driver, which drives protein expression only in adult neurons. Therefore, the developmental effects of transgenes were avoided (http://flybase.org/reports/FBtp0041245.html).

Locomotor activity correlates with the efficiency of synaptic transmission in flies [37]. Previous studies highlighted that a number of synaptic proteins are involved in ethanol responses such as ethanol sensitivity, tolerance and preference [reviewed in 38]. In the present study, all 15-day-old transgenic flies exhibited a reduction in locomotion ability and a surge in sensitivity to ethanol. However shaggy revealed

a higher impact, which may implicate that shaggy has a more adverse effect on the efficiency of neurotransmission and synaptic proteins.

As mentioned earlier, Tau^{WT}, $A\beta_{42,}$ and GSK3β play crucial roles in synaptic dysfunction [reviewed in 2, 4–5]. Moreover, changes in synaptic proteins, SYT1 and SNAP25, have been observed in AD patients' brains [10–12]. Therefore, in the current study, the effect of Tau^{WT}, $A\beta_{42,}$ or shaggy overexpression in neurons of *Drosophila* was investigated on *syt1* and *SNAP25* mRNA expression. The data obtained in our research exhibited that Tau^{WT} and $A\beta_{42}$ significantly upregulated *syt1* and *SNAP25* transcriptions. This result was in accordance with the previous results showing that the expression of genes involves in synaptic vesicle trafficking and neurotransmitter release, including *SYT1* and *SNAP25*, are upregulated in individuals with mild cognitive impairment (MCI) compared to age-matched controls or AD patients [10, 11] Our 15-day-old flies are also probably at the early stages of AD, as it was reported by Sofola et al. [39].

The increase in *syt1* and *SNAP25* expression might reflect a compensatory response to synaptic defects and loss. Synaptic dysfunction and loss occur in the very early stages of AD. In MCI, the remaining presynaptic terminals are sprouted and expanded to compensate for the absence of lost synapses [40].

The increased expression of genes involved in synaptic vesicle trafficking and release presumably increases neurotransmitters' release in the brain. The FMRI results confirm the brain activity has increased during the MCI stage [41]. Although increasing the release of neurotransmitters is expected to improve cognitive and perceptual functions, neuronal hyperactivity causes excitotoxicity leading to a number of devastating consequences such as generation of ROS and oxidative stress, and apoptosis which finally causes memory impairment [42].

It can be suggested that the neuronal cells try to compensate for the effects of these pathological lesions in Tau^{WT} and $A\beta_{42}$ transgenic flies by increasing synaptic genes expression (*syt1* and *SNAP25*) 15 days after eclosion. However, it is likely to impair synaptic plasticity and networks and ultimately lead to inability to record new information. Defect in memory impairment observed in 15 days old Tau^{WT} or $A\beta_{42}$ expressing flies in our previous study [7] and deficit in locomotor ability and less sensitivity to ethanol in the current study may testify that the presumed compensatory mechanisms were inefficient.

Next, we assessed the relative expression of *CREB* in the brain of transgenic flies to find out if the observed changes in synaptic genes expression can reflect this transcription factor's dysregulation. CREB positively regulates the expression of genes involved in memory consolidation such as *SYT1* and *SNAP25* [19, 34], and has been dysregulated in AD [20–21].

According to our results, pan-neuronal expression of Tau^{WT} or $A\beta_{42}$ in nerve cells dramatically increased the level of *CREB* mRNA, which may suggest that CREB is likely a part of compensatory mechanisms in our flies. Previous studies reported the reduction of *CREB* expression in cell culture treated with $A\beta_{42}$ [21, 43] which is in contrast with the current results. In addition, investigation of the total and activated form of CREB protein in AD postmortem brain at late stages of the disease revealed a reduction [20–21].

However, to the best of current authors' knowledge, no studies at the early stages or at transcription level of CREB have been yet conducted.

The mechanism underlying gene expression dysregulation in flies expressing shaggy seemed different. *CREB* expression and also *syt1* and *SNAP25* showed downregulation in shaggy transgenic flies. Intriguingly, it has been demonstrated that neuronal overexpression of shaggy leads to a decrease in the number and size of synapses and presynaptic terminals and a decline in neurotransmitter release [44–46]. Our finding is in agreement with earlier research indicated that there is a decrease in transcription of genes involved in exocytosis of neurotransmitters like *SYT1* and *SNAP25* in AD patients compared to age-matched control or MCI [10, 11]. Furthermore, there is a decrease in the protein level of *CREB* in the postmortem brain of individuals suffering from AD at later stages [20–21]. Moreover, it has been demonstrated that overexpression of GSK3β in neurons of mice resulted in downregulation of *Syt1* transcription in the hippocampus [6].

Phosphorylation of CREB on serine 129 by GSK3 leads to its inactivation [36]. As CREB is a self-regulator, its inactivation can lead to a decrease in its mRNA level [19]. Therefore, it can be suggested that shaggy, likely through the inactivation and downregulation of CREB in flies' brains, leads to a decrease in the target genes of this transcription factor.

At last, to examine whether the change in *CREB* transcript level is through GSK3/CREB pathway, we spotted the expression of *shaggy* in Tau^{WT} or $A\beta_{42}$ transgenic flies. The current study showed that ectopic expression of Tau^{WT} in the drosophila nerve system upregulated transcription of *shaggy*, which is consistent with the finding of a preceding study demonstrating that mRNA level of *GSK3β* has been elevated in the hippocampus at the early stages of Alzheimer's disease [33]. However, we observed that $A\beta_{42}$ did not affect *shaggy* expression. Our data proposed that the upregulation of *CREB* as a part of the compensatory mechanism in Tau^{WT} or $A\beta_{42}$ transgenic flies is probably independent of GSK3/CREB pathway.

It has been exhibited that GSK3β can trigger abnormal hyperphosphorylation and aggregation of Tau^{WT} [2], but there is no study on the effect of Tau^{WT} on shaggy levels or activation. So we are the first to report that the expression of Tau^{WT} may affect the expression of *shaggy*, GSK3β orthologue. It would be more interesting to investigate the impact of Tau^{WT} on protein level and activity of GSK3β, as well. In spite of the upregulation of *shaggy* in Tau^{WT} expressing flies, there was an increase in synaptic genes expression, which may put forward that in the early stages of the illness, the compensatory mechanism is more powerful than the effect of GSK3.

Increased production of $A\beta_{42}$ and higher activation of GSK3β are also in a vicious circle [2]. 15-day-old flies that expressed Arctic $A\beta_{42}$ only in adult neurons revealed a reduction in the levels of inhibitory ser9 phosphorylation of shaggy, leading to an increase in its activity [39]. As we did not observe any changes in the mRNA levels of shaggy in $A\beta_{42}$ expressing flies, it seems that $A\beta_{42}$ probably dysregulates GSK3β

activity without affecting its transcriptional levels. Further investigation is required to verify the mechanisms involved in GSK3 dysregulation in AD.

To sum up, here we have shown, for the first time, that Tau^{WT}, $A\beta_{42}$, and shaggy differentially and independently alter the expression of synaptic genes (*syt1* and *SNAP25*) and transcription factor of *CREB* in transgenic flies at an early time point of their life cycle. While both Tau^{WT} and $A\beta_{42}$ had similar effects on synaptic gene expression, shaggy had a different impact on the expression of those genes. It seems that dysregulation of synaptic genes occurs as a consequence of changes in *CREB* expression and is likely independent of GSK3/CREB pathway. While Tau^{WT} increased the levels of *shaggy*, no change was observed in $A\beta_{42}$ expressing flies.

In addition, shaggy has a more significant impact on ethanol sensitivity and motor dysfunction in flies. This could be due to more dramatic effect of shaggy on synaptic dysfunction compared to Tau^{WT} and $A\beta_{42}$, as we observed in the current research.

With regard to the result of this study, it appears that GSK3 has more adverse effect on Alzheimer's disease's symptoms such as locomotor and synaptic dysfunction. It seems that using GSK3 inhibitors in treating Alzheimer disease or improving patient's quality of life, accompanied by drugs that target tau and amyloid beta, would be essential.

Declarations

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Authors Contributions

Raheleh Masoudi, ORCID ID: 0000-0002-2124-6451, conceived the presented idea, designed the experiments, contributed to the interpretation of the results and writing the manuscript.

Fatemeh Ataellahi, ORCID ID: 0000-0003-3928-0014, performed the molecular and behavioral experiments and their statistical analysis and interpretation, wrote the manuscript and contributed to the conception of the idea.

Mohammad Haddadi, ORCID ID: 0000-0002-3374-0879, guided and helped to design the experiments related to flies and provided the Drosophila stocks.

Ethics Approval

Drosophila experiments do not require ethics committee approval. However, all expreriments were conducted ethically and the number of animals used as kept to a minimum.

Consent to Participate

Not applicable

Consent to Publication

Not applicable

Competing Interests

The authors of this article declare that they have no conflict of interest.

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Figures

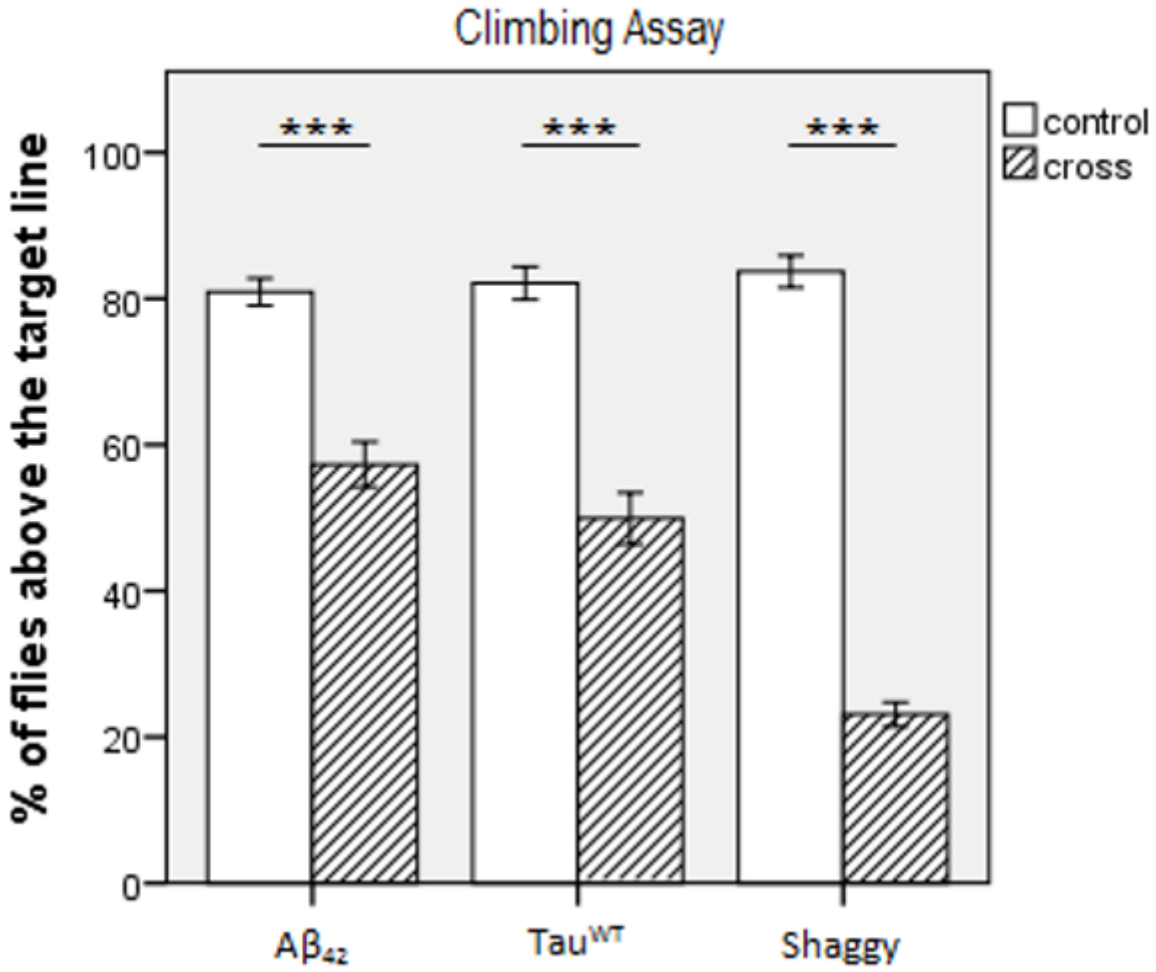

Figure 1

Lower climbing performance in AD transgenic flies. Flies expressing $A\beta_{42}$, Tau^{WT}, and shaggy indicated a significant decline in their locomotor activity compared to their counterpart control flies (***P < 0.001, n=10 groups of ten flies). Error bars indicate standard error.

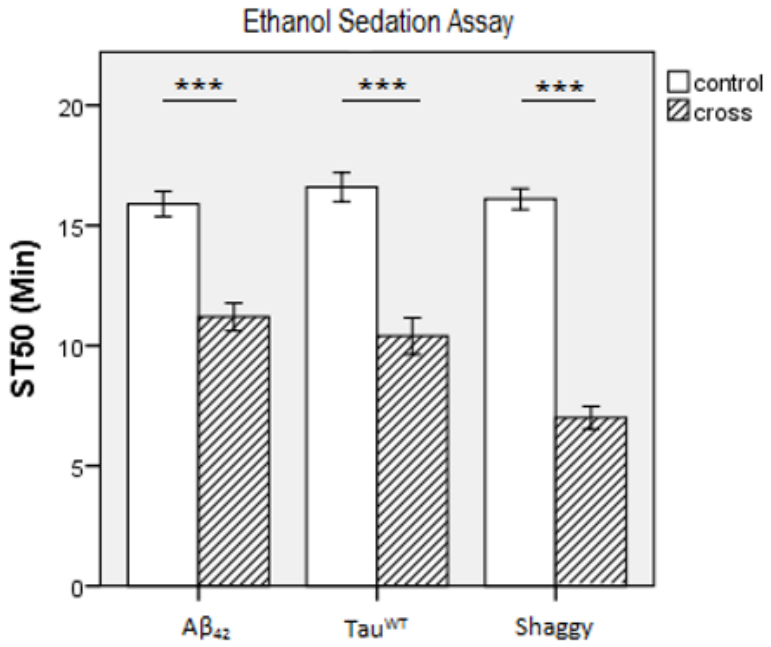

Fig 2a

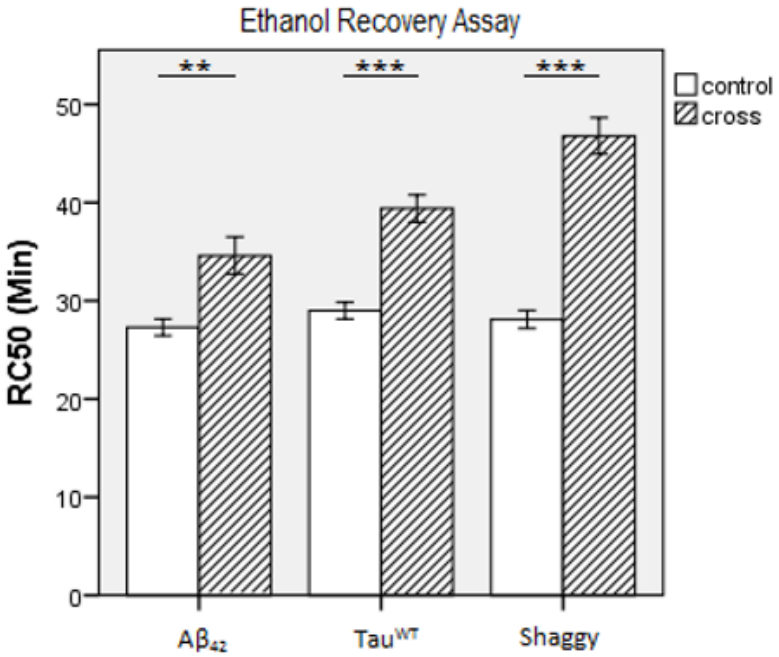

Fig 2b

Figure 2

Increase in sensitivity to ethanol in AD transgenic flies. a) ST50 (the time needed for half of the flies to become sedated) was reduced significantly in Tau^{WT}, $A\beta_{42}$, and shaggy expressing flies versus their controls. b) Tau^{WT}, $A\beta_{42}$, and shaggy expressing flies showed a significantly greater RC50 (the time taken

for half of the sedated flies to become completely revived) compared to their counterpart controls. (**P < 0.01, ***P < 0.001, n=10). Error bars indicate standard error.

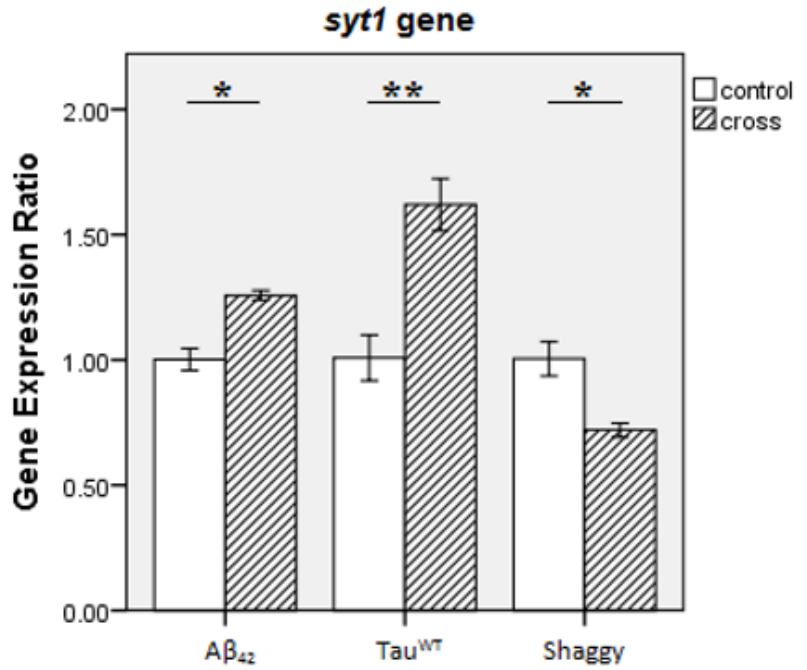

Fig 3a

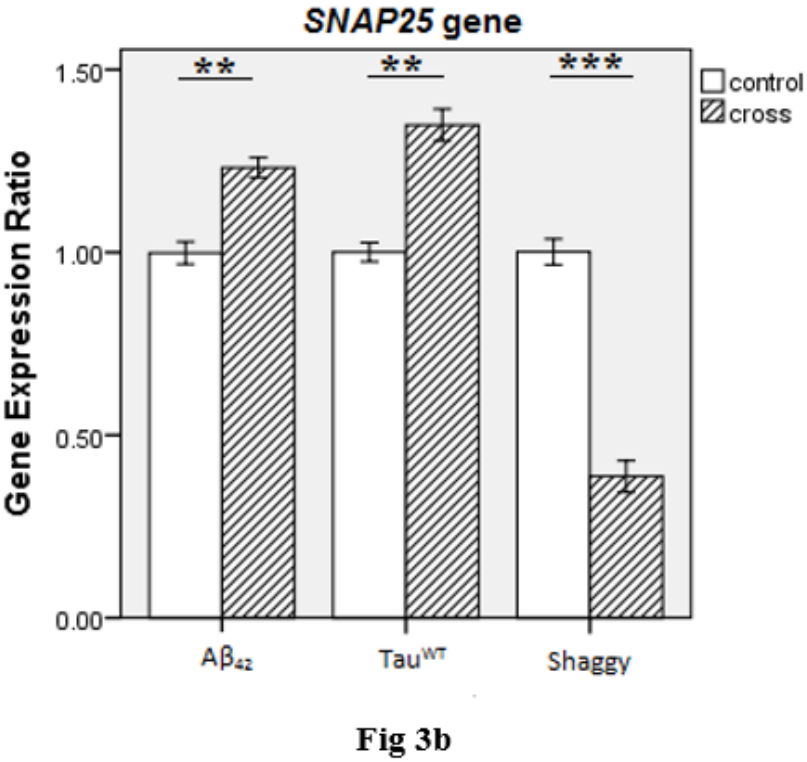

Fig 3b

Figure 3

Tau^{WT} or $A\beta_{42}$ altered *syt1 and SNAP25* expression differently from shaggy. a) *Syt1* mRNA level was significantly upregulated in Tau^{WT} or $A\beta_{42}$ transgenic flies, whereas it was significantly downregulated in

shaggy overexpressing flies. b) There was an increase in *SNAP25* transcription in Tau^{WT} or $A\beta_{42}$ transgenic flies. shaggy transgenic flies showed a reduction in this regard. (*P < 0.05, **P < 0.01, ***P < 0.001, n= 3). Error bars represent S.E.M.

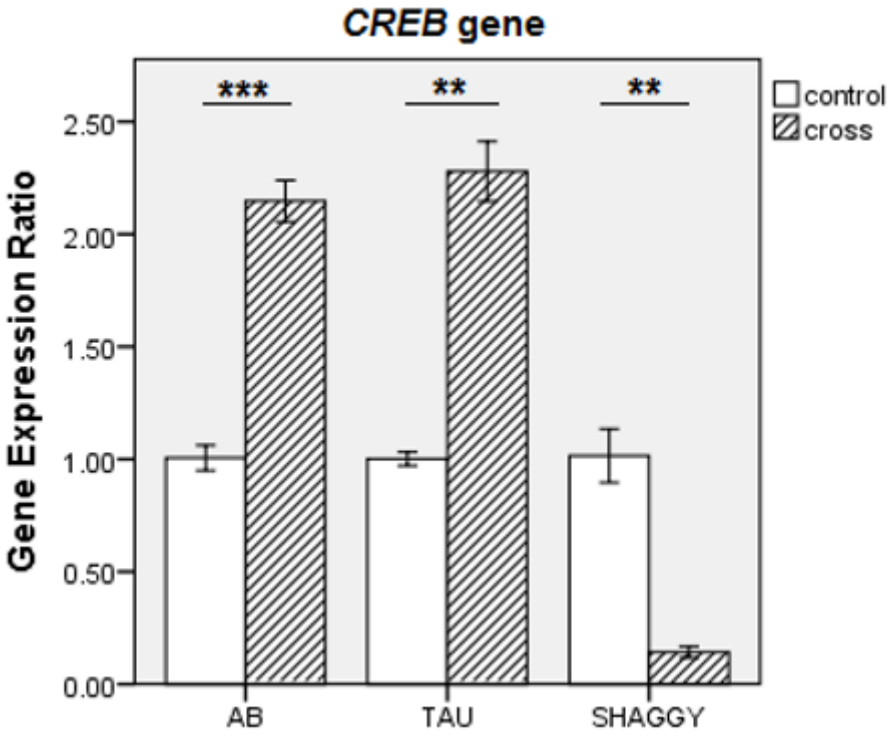

Figure 4

***CREB* was dysregulated diversely in Tau^{WT} or $A\beta_{42}$ expressing flies compared to shaggy transgenic flies.** *CREB* mRNA level witnessed a significant elevation in Tau^{WT} or $A\beta_{42}$ expressing flies. In contrast, in shaggy overexpressing flies a significant decline was observed (**P < 0.01, ***P < 0.001, n= 3). Error bars represent S.E.M.

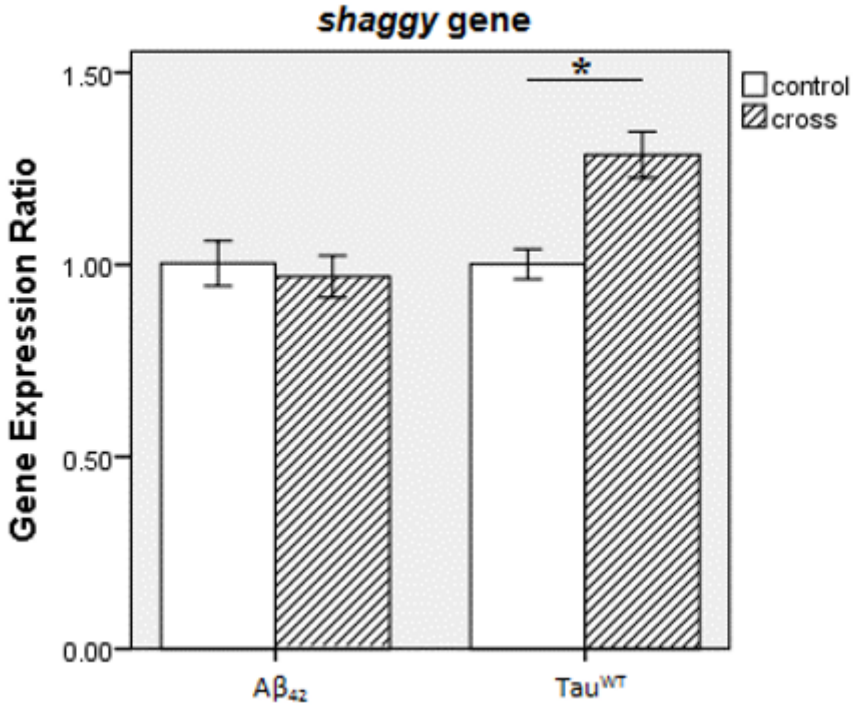

Figure 5

Tau^{WT} affected *shaggy* expression, whereas $A\beta_{42}$ had no impact on it. *shaggy* indicated a significant upregulation in Tau^{WT} transgenic flies, while there was not any significant difference in terms of *shaggy* in $A\beta_{42}$ expressing flies. (*P < 0.05, n= 3). Error bars represent S.E.M.

Supplementary Files

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