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SPECIAL ISSUE

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Disrupted cellular calcium homeostasis is responsible for Aβ-induced learning and memory damage and lifespan shortening in a model of Aβ transgenic fly

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Abstract

Accumulated Aβ is one of the hallmarks of Alzheimer's disease. Although accumulated results from in vivo and in vitro studies have shown that accumulated Aβ causes learning and memory deficit, cell death, and lifespan reduction, the underlying mechanism remains elusive. In neurons, calcium dynamics is regulated by voltage-gated calcium channel (VGCC) and endoplasmic reticulum and is important for neuron survival and formation of learning and memory. The current study employs in vivo genetics to reveal the role of calcium regulation systems in Aβ-induced behavioral damage. Our data shows that although increased VGCC improves learning and memory in Aβ42 flies, reduction of VGCC and Inositol trisphosphate receptors extends Aβ42 flies' lifespan and improves cell viability. The complex role of calcium regulation systems in Aβ-induced damage suggests that the imbalance of calcium dynamic is one of the main factors to trigger learning and memory deficit and cell death in the disease.

KEYWORDS

Alzheimer's disease, Aβ, calcium, learning and memory

1 | INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia and is characterized by age-dependent memory loss, severe neuronal death, and lifespan shortening. Genetic studies provide a causative link between Aβ peptides and disease.[1–3] Accumulated evidence from different AD animal models and cell culture experiments has

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; A*β*, beta-amyloid; dae, day after eclosion; GAL4, yeast transcriptional activato; LTD, long-term depression; MB, mushroom body; MCH, 4-methylcyclohexanol; OCT, 3-octanol; PI, performance index; UAS, upstream activating sequences.

Kuan-Chung Cheng and Chih-Yuan Huang contributed equally.

shown that many cellular signals are altered by Aβ peptides.[4,5] However, it remains to be determined how learning and memory is damaged during disease progression and what signaling is affected by Aβ to cause cell death and reduce the lifespan.

The imbalanced activity of intracellular signaling, overactivation or hypoactivation, dysregulates many cellular functions and eventually triggers cell death. In neurons, it is important to keep intracellular calcium concentration at a constant level to maintain proper cellular functions. Intracellular calcium imbalance in neurons has been shown to affect long-term potentiation and long-term depression, which may damage learning and memory formation and trigger apoptosis.[6–8] Transient intracellular calcium dynamics can be achieved by two different regulations, voltage-gated calcium channel (VGCC) and endoplasmic reticulum (ER)-stored calcium release. Membrane depolarization triggers VGCC opening, which allows extracellular calcium influx across the plasma membrane to regulate exocytosis and endocytosis. Activated VGCC transiently elevates local calcium concentration in the axon terminals is important to regulate synaptic plasticity, a cellular basis of learning and memory, and cell–cell communication.[9,10] The activation of phospholipase hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP2) to diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) to stimulate ER to release sored calcium to the cytosol to activate downstream signals. Increased intracellular free calcium is rapidly stabilized by calcium-buffering proteins to prevent calcium-dependent signals overactivation.[11,12] Dysregulated VGCC and ER functions in neurons cause intracellular calcium imbalance and have been reported in many neurodegenerative diseases, includes AD.[13] Application of Aβ40 promotes voltage-dependent L- and N-type calcium channels activity in cortical neurons.[14] Two hundred nanomolar of synthetically generated Aβ oligomer facilitates the P/Q calcium channel current in the *Xenopus* oocytes.[15] In the Tg2576 transgenic mice, overexpression of the Swedish mutant (K670N, M671L) human amyloid precursor protein gene, the L-type Ca^{2+} current is increased in Neuropeptide Y (NPY) neurons.[16] On the other hand, intracellular stored calcium is released upon Aβ stimulation to increase cytosol calcium concentration. Activated ryanodine receptor (RyR) causes intracellular stored calcium releasing and increases $[Ca^{2+}]_{cyt}$ in the 3xTg-AD hippocampus neurons.[17] Aβ can promote IP3 production to trigger calcium release from ER to increase cytosol calcium level.[18,19] Although a body of evidence has shown that calcium homeostasis is disrupted in the brain of AD and many different AD animal models, the current consensus on the role of calcium in mediating Aβ toxicity is not conclusive. It remains unclear how dysregulated calcium regulation systems contribute to the disease progression and pathological phenotypes.

The current study showed that disrupted calcium regulation systems affect learning and memory performance and lifespan in Aβ42 transgenic flies, a *Drosophila* model of AD. Overexpression of *cacophony* (*cac*), VGCC α1 subunit homologous to vertebrate α1 subunits, improves learning and memory performance of Aβ flies. However, knocked-down *cac* and IP3R extended the lifespan of Aβ flies. Our results suggest that imbalanced calcium homeostasis is the major factor to mediate Aβ toxicity.

2 | MATERIAL AND METHODS

2.1 | Fly stocks

All experiments were used elav–Gal4 as the pan-neuronal driver. Following stocks were obtained from the Bloomington Drosophila Stock Center (BDSC): Tublin-Gal80^{TS} (#7017), UAS-cac1-EGFP (#8581), UAS-IP3R RNAi (#51795). UAS-Cac RNAi (#77174) was obtained from Vienna Drosophila Resource Center (VDRC). Elav-gal4, UAS-Aβ42 were gifts from Dr. Yi Zhong. Cac^{TS} was gift from Dr. Richard W. Ordway.

2.2 | Western blot

Fly heads were collected and homogenized in sample buffer containing 1% sodium dodecyl sulfate (Thermo Fisher Scientific, Waltham, MA, USA). After lysates were centrifuged, the supernatants were collected and separated on Tris-Tricine gels, and then transferred to nitrocellulose membranes. The membranes will be blocked with 5% non-fat dry milk and blotted with anti-β-Amyloid (1:2000, #8243 CST) antibodies at 4°C overnight. The appropriate horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch) was applied, and the signal was detected by chemiluminescence (Thermo Fisher Scientific). The signal intensity was quantified by Image J software (National Institutes of Health).

2.3 | Pavlovian olfactory associative learning

This assay was first described by Drs. Tim Tully and Quinn.[20] Briefly, approximately 80–100 flies were trained by exposing them to electrical shock paired with one odor (octanol or methylcyclohexanol), CS+, for 60 s and subsequent exposure to the other odor, CS−, without

electroshock for 60 s. Odor concentration was modified every day before the experiment to avoid potential bias. Immediately after training, learning or so-called immediate short-term memory was measured by allowing flies to choose between the two odors for 120 s. For 2-hr memory, trained flies were transferred to food vials and placed in the dark until testing. The performance index was calculated by measuring the number of flies that stayed in between CS+ odor and CS− odor side.

2.4 | Survival assay

The lifespan studies were measured on regular food and performed at least twice. The flies were transferred to fresh food vials every 3–4 days and the number of dead flies was counted. The experiments were placed at 30°C, with 70% humidity, on a 12-h light/dark cycle. All results were analyzed from females.

2.5 | Drug feeding

Adopted from the previous study.[21] Briefly, chemicals (Dantrolene) were stored at −20°C after being dissolved in dimethyl sulfoxide and diluted in 4% sucrose (chemical concentrations are vol/vol for liquid and wt/vol for solid) for using. Flies were maintained at 29°C after eclosion to induce targeted protein. Before feeding, all the flies were placed in empty vials and starved for 3 hr, and then a paper rinsed with sucrose with/without the drug is placed in the vial for 4 hr. Flies were transferred to normal food after treatment.

2.6 | Quantitative RT-PCR

Total RNA was isolated from 20 fly heads in Trizol, DNA traces were eliminated with RevertAid RT Reverse Transcription Kit, and cDNAs were prepared with One-Step RT-PCR. For detection of *Cac*, we used primers Cac-F 5′-GGGAGACCATCACGAACATC-3′ and Cac-R 5′-GAGAGCCATTGTGCCTCAAG-3′ to amplify the 312 bp fragment. For quantification of *RyR* in *Drosophila*, we used primers RyR-F 5′-CAGCAATGTGGAGCTGATTCTTA-3′ and RyR-R 5′-AATCCATTTCTTCCGGTGTGT-3′ to amplify 173 bp fragment in the 5′ exon. For detection of *NorpA*, we used primers NorpA-F 5′-ACCTTGAAG ACCAAGAACGAG-3′ and NorpA-R 5′-CTGCTTAACTC CGATTTGCTTCT-3′ to amplify 102 bp fragments. For quantification of *Itpr* in *Drosophila*, we used primers Itpr-F 5′-TTTGATAATACTGTGCCAGAGC-3′ and Itpr-R 5′-AATATGAGACCGCAGAAGCA-3′ to amplify 332 bp fragments. For quantification of *Plc21c* in *Drosophila*, we used primers Plc21c-F 5′-GCTGATCAGAATGAAACG CGAG-3′ and Plc21c-R 5′-TTATTGCCTGAAAGGG CTGAC-3′ to amplify 285 bp fragments. As an internal control, we used the primers for *dRpl32*: dRpl32-F 5′-AATCCTCGTTGGCACTCACC-3′ and dRpl32-R 5′-TGTTGTGTCCTTCCAGCTTCA-3′ that amplified a fragment of 135 bp. For qPCR with the SYBR Select Master Mix and StepOnePlus™ Real-Time PCR System, we used 10 ng of purified RNA. Cycling conditions were set holding state at 50°C for 2 min, 95°C for 2 min and 40 cycles of 95°C for 15 s, 56°C for 1 min and 72°C for 1 min. The levels of RNA were calculated using the StepOne Software v2.3, which relied on the comparative Ct method of quantification. All mRNA levels were normalized to *dRpl32*.

2.7 | Propidium iodide staining

Fly brains were quickly dissected in phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde in PBS overnight. The next day, after being vacuumed 3 times for 20 minutes each, brains were permeabilized with 2% TritonX-100 in PBS overnight. Before confocal exposure, fly brains were incubated with 1:200 propidium iodide (PI) in PBS at 4°C overnight. The next day, after three times for 20 min each washing in PBS, brains were mounted between two glass coverslips in focusclear™, and imaged on a FluoView FV1000 confocal microscope. For cell loss measurement, we used Image J software to quantify the area of empty holes and then divided the area of cell bodies around calyx. The final data we presented was the percentage of cell loss. To prevent bias, we used double-blind to analyzed data.

2.8 | Statistics

All data were analyzed using the Graphpad Prism 6.0 software. Comparisons between two groups used a two-tailed *t*-test. Comparisons of multiple groups used one-way analysis of variance. Statistical significance was shown with $*p < .05$; $**p < .01$; $***p < .001$; n.s., non-significance ($p > .05$). Statistical results are presented as means ± standard error of the mean.

3 | RESULTS

3.1 | Overexpression of VGCC improves learning and memory performance in the Aβ flies

A conditional expression system, Gal80^{ts}, was introduced to temporarily regulate targeted protein expression.[22]

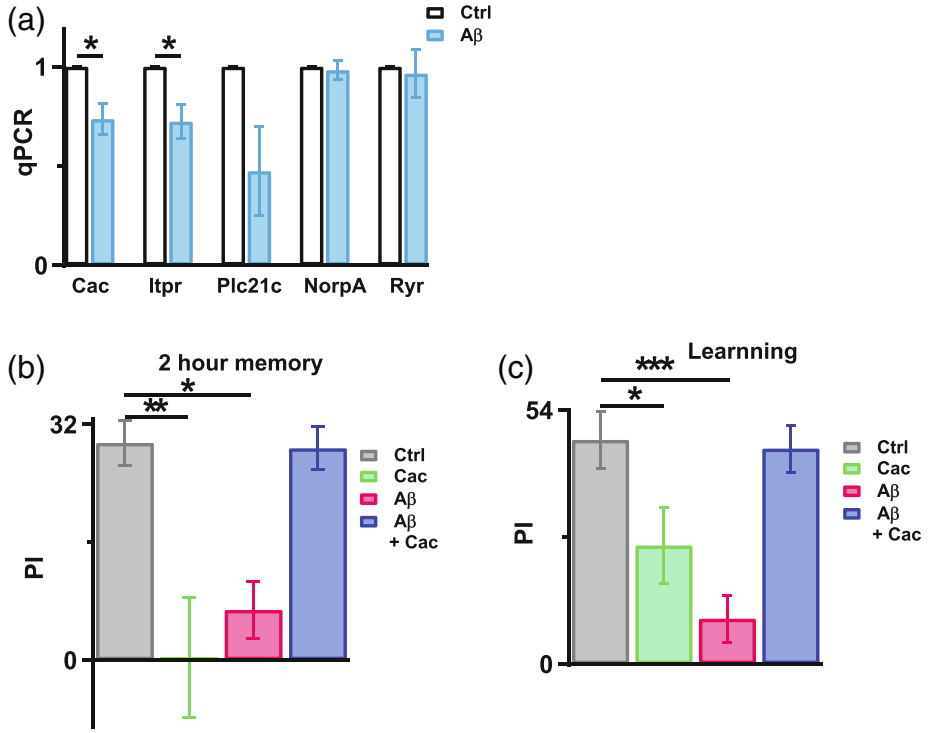

FIGURE 1 Overexpression of cac improves 2 hr memory and learning in Aβ42 flies. (a) Heads were collected from 8 dae Aβ flies to perform quantitative PCR analysis. (*Cac*: Two-tailed *t*-test, $p = .0142$; *Itpr*: Two-tailed *t*-test, $p = .0168$; *Plc21c*: Two-tailed *t*-test, $p = .113$; *NorpA*: Two-tailed *t*-test, $p = .7697$; *Ryr*: Two-tailed *t*-test, $p = .7835$, $n = 3$–4). *$p < .05$. (b, c) Overexpression of Cac reversed 2 hr memory deficit in 4 days old Aβ42 flies (b) and learning deficit in 8 days old Aβ42 flies (c). In both experiments, overexpression cac alone also induced memory and learning deficit (Memory: $F(3, 22) = 9.001$, $p = .0004$; Ctrl vs. Aβ42: $p = .0155$; Ctrl vs. Cac: $p = .0016$; Aβ42 vs. Aβ42 + Cac: $p = .0353$, $n = 5$–7; Learning: $F(3, 22) = 9.002$, $p = .0004$; Ctrl vs. Aβ42: $p = .0009$; Ctrl vs. Cac: $p = .0487$; Aβ42 vs. Aβ42 + Cac: $p = .0022$, $n = 6$–7). *$p < .05$, **$p < .01$, ***$p < .001$. dae, days after eclosion

Gal80^{ts} is the temperature-sensitive version of Gal80. Unless mentioned otherwise all the experiments were done with *Elav-Gal4* promoter to induce Aβ42 expression in neurons.

Intracellular calcium concentration can be regulated by (a) extracellular calcium influx via VGCC and (b) IP3 receptor (IP3R) and RyR dependent intracellular calcium store release. We sought to first understand the expression level of these genes in Aβ42 flies. Heads of 8 days old Aβ42 flies were collected to perform quantitative PCR analysis. We examined the expression level of *cac*, *Inositol 1,4,5,-trisphosphate receptor* (*Itpr*, *IP3R*), *Phospholipase C at 21C* (*Plc21c*, encodes a Drosophila Phospholipase Cβ [PLCβ] homolog), *no receptor potential A* (*NorpA*, encodes a phosphoinositide-specific phospholipase C in *Drosophila*), and *Ryr* in Aβ42 flies. Figure 1a showed that there was decreased expression of *cac*, *Itpr* and *Plc21c* in Aβ flies. As VGCC is responsible for the extracellular calcium influx during membrane depolarization and affecting synaptic plasticity, the role of *cac* in Aβ42 flies in learning and memory was evaluated. Overexpression of *cac*, ~7.93 ± 0.32 times increased, improved 2-hr memory and learning performance in 3 days old and 8 days old Aβ42 flies, respectively. (Figure 1b,c). This data suggests that reduced cac expression in Aβ42 flies causes learning and memory deficit. As overexpression of cac alone also induced learning and memory deficit, our results further suggest that the level of cac in a cell is critical, too much and too little affects the formation of learning and memory.

3.2 | Knocked-down VGCC and IP3R extend Aβ42 flies' lifespan but the only reduction of IP3R improves cell viability

To gain more insight on the role of cac in Aβ toxicity, cac RNAi was introduced in Aβ42 flies to reduce endogenous cac levels. Overexpression of cac RNAi did not affect 2-hr memory and learning performance in 3 days old and 8 days old Aβ42 flies, respectively (Figure 2a,b). However, reduction of lifespan in Aβ42 flies was improved after the *cac* was knocked down (Figure 2c), as well as in the Cac^{TS2} mutant, a temperature-sensitive paralytic allele of

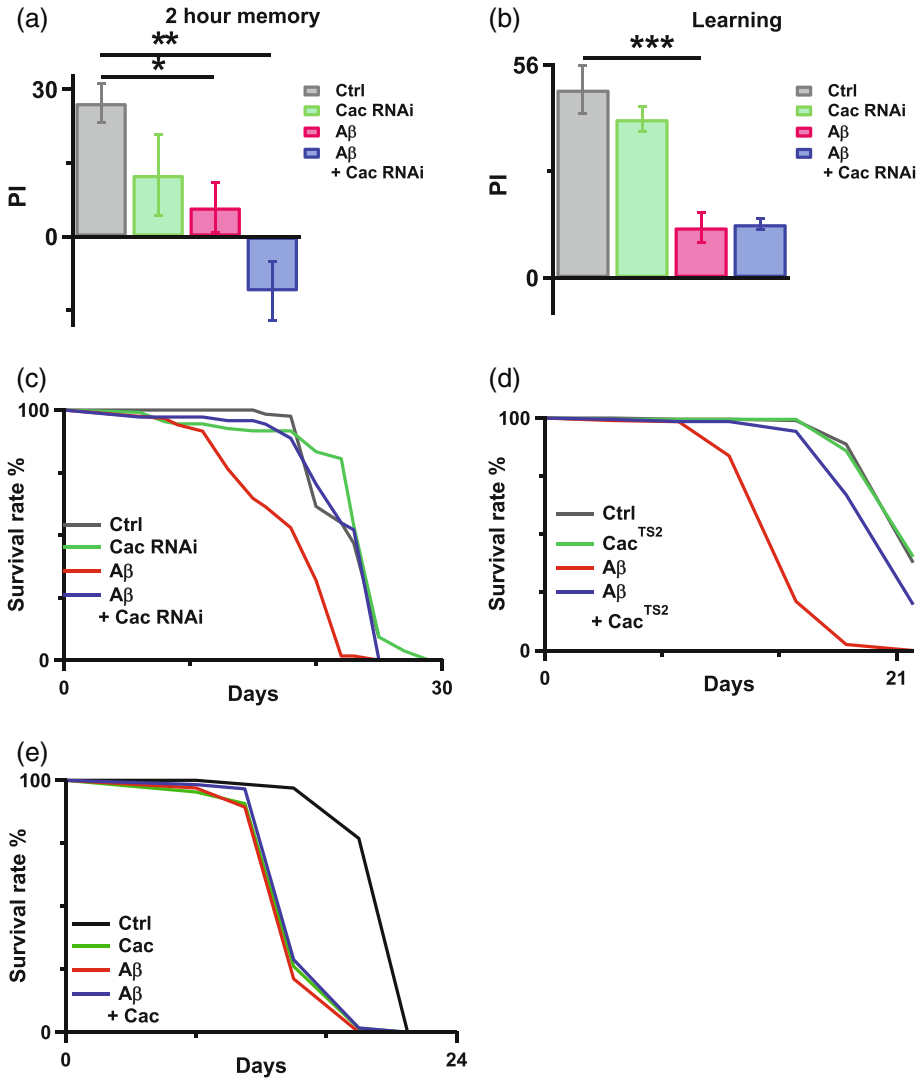

FIGURE 2 Reduced cac in Aβ flies improves the lifespan. (a, b) Overexpression of cac RNAi in Aβ flies did not affect 2 hr memory performance in 4 days old Aβ42 flies (a) and learning performance in 8 days old Aβ42 flies (b) (Memory: $F(3, 16) = 6.756$, $p = .0037$; Ctrl vs. Aβ42: $p = .0474$; Aβ42 vs. Aβ42 + Cac RNAi: $p = .2335$, $n = 5$; Learning: $F(3, 16) = 17.15$, $p < .0001$; Ctrl vs. Aβ42: $p = .0002$; Aβ42 vs. Aβ42 + Cac RNAi: $p = .9993$, $n = 5$). $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$. (c, d) Overexpression of cac RNAi (c) and cac^{TS2} (d) in Aβ flies extended lifespan. $n = 100–120$ flies for each genotype. Log-rank test. (Cac RNAi: Ctrl vs. Aβ42: $p < .0001$; Aβ42 vs. Aβ42 + Cac RNAi: $p < .0001$; Cac^{TS2}: Ctrl vs. Aβ42: $p < .0001$; Aβ42 vs. Aβ42 + Cac RNAi: $p < .0001$). (e) Overexpression of cac did not affect lifespan of Aβ flies. $n = 110–120$ flies for each genotype. Log-rank test. (Ctrl vs. Cac: $p < .0001$; Ctrl vs. Aβ42: $p < .0001$; Aβ42 vs. Aβ42 + Cac: $p = .1159$)

cac[23] (Figure 2d). Overexpression of cac, however, did not affect Aβ42 flies' lifespan (Figure 2e). This result is consistent with our learning experiment, overexpression of cac damages animal behaviors (Figure 2e).

To extend our understanding of the role of intracellular calcium regulation systems in Aβ42 flies, the involvement of IP3R and RyR in Aβ toxicity was examined. Knocked-down IP3R and pharmacological treatment, Dantrolene 50 μM, to reduce RyR activity did not affect 2 hr memory and learning in Aβ42 flies (Figure 3a–c). Consistent with the result of cac knocking-down, reduced IP3R also extended the lifespan of Aβ42 flies (Figure 3d).

Accumulated evidence has shown that increased calcium accumulation triggers cell death.[6,7,24] As massive cell death has been documented in the brain of AD and also the brain of Aβ flies, we were wondering if manipulation of cac and IP3R can improve cell viability. PI staining was used to stain the morphology of cells in the cell body region of the mushroom body, center for learning and memory in fruit fly.[25] There was more vacuolated area in the cell body region in 14 days after eclosion Aβ42 flies than in age-matched control flies (Figure 4), which indicated there was cell loss in the brain of Aβ42 flies. Interestingly, the reduction of the vacuolated area was only found in Aβ42 flies with a reduction of IP3R but not cac, (Figure 4a,b).

3.3 | Knocked-down IP3R reduces Aβ42 accumulation

Accumulated Aβ in the brain is considered one of the hallmarks of AD. To understand whether manipulation of cac and IP3R can change the Aβ accumulation, heads of Aβ flies with different manipulation were collected for western analysis. Our analysis showed that there was a reduction of Aβ monomer accumulation in Aβ flies after IP3R was knocked-down, the accumulation of higher aggregates were not included due to the resolution of Western blot resolution. Overexpression of cac and

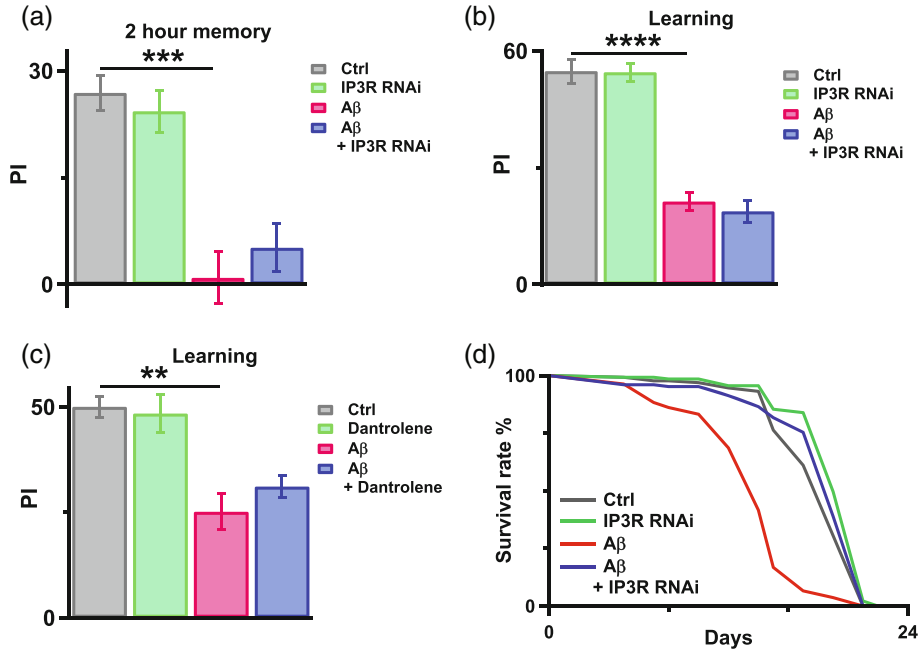

FIGURE 3 Reduced IP3R in Aβ flies improves lifespan. (a, b) Overexpression of IP3R RNAi did not affect 2 hr memory performance in 4 days old Aβ42 flies (a) and learning performance in 8 days old Aβ42 flies (b) (Memory: $F(3, 16) = 14.57$, $p < .0001$; Ctrl vs. Aβ42: $p = .0004$; Aβ42 vs. Aβ42 + IP3R RNAi: $p = .8294$, $n = 5$; Learning: $F(3, 16) = 47.62$, $p < .0001$; Ctrl vs. Aβ42: $p < .0001$; Aβ42 vs. Aβ42 + IP3R RNAi: $p = .9188$, respectively, $n = 5$). ***$p < .001$, ****$p < .0001$. (c) Dantrolene 50 μM treatment for 8 days did not affect learning performance in 8 days old Aβ42 flies ($F(3, 16) = 10.23$, $p = .0005$; Ctrl vs. Aβ42: $p = .0018$; Aβ42 vs. Aβ42 + Dantrolene: $p = .7403$, $n = 5$). **$p < .01$. (d) Overexpression of IP3R RNAi in Aβ flies extended lifespan. $n = 100$–120 flies for each genotype. Log-rank test. (Ctrl vs. Aβ42: $p < .0001$; Aβ42 vs. Aβ42 + IP3R RNAi: $p < .0001$)

knocked down cac did not affect Aβ accumulation (Figure 5).

4 | DISCUSSION

The current study demonstrated that calcium regulation systems play an important role in Aβ-induced pathologies. Overexpression of VGCC improves the learning and memory in Aβ flies, while reduced VGCC and IP3R extend lifespan in Aβ flies. However, the only reduction of IP3R improves cell viability and reduces Aβ accumulation. The current finding shows the complex role of calcium regulation systems in Aβ-induced pathologies and raises a caution of using drugs to manipulate intracellular calcium levels to treat the disease.

Although increased calcium accumulation is observed in different AD animal models and the brain of AD,[13,26,27] the role of calcium in Aβ toxicity remains elusive. Our study suggests a rather complicated role of calcium regulation systems in Aβ toxicity. We hypothesize that there are temporal and spatial effects of dysfunctional calcium regulation systems in Aβ toxicity. It is well known that calcium is important in neurons for regulating synaptic plasticity. Most of VGCCs are accumulated in the nerve terminals around synapses. Membrane depolarization reaches the threshold for VGCC activation to allow calcium influx to trigger a series of calcium-dependent downstream signals to regulate the synaptic plasticity, which eventually affects learning and memory formation. Although results of our behavioral studies, overexpression of cac improved learning and memory performance in Aβ flies, seem to contradict the observation that Aβ accumulation increases intracellular calcium accumulation, it is consistent with our qPCR results in Aβ flies. We hypothesize that, in the nerve terminal, reduction of VGCC, in Aβ flies, decreases cells' ability to locally regulate synaptic activity and manipulate synaptic plasticity. As the result, the formation of learning and memory is difficult. Therefore, overexpression of cac brings up the number of cac in the synapse, which enable neuron locally to regulate the dynamic of calcium level in the nerve terminal after membrane depolarization and to strengthen the synaptic plasticity accordingly in Aβ flies. In addition, our finding that overexpression of cac also induces memory and learning deficit suggests that the expression of cac is tightly regulated, too much and too little would affect the formation of learning and memory. On the other hand, chronic dysregulated calcium accumulation in the cytosol affects many cellular signaling and organelles, for

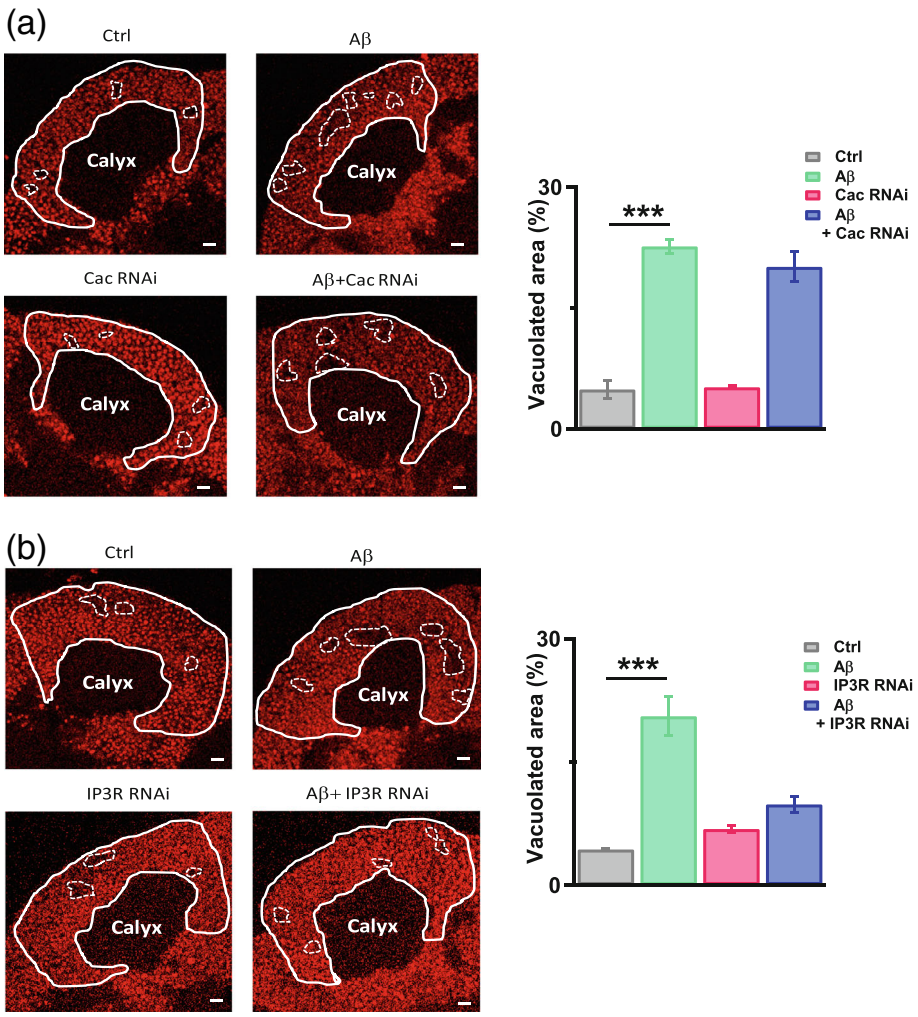

FIGURE 4 Reduced IP3R but not cac in Aβ flies promotes cells viability. (a, b) Propidium iodide staining in the cell body region of mushroom body showed that, compared to the overexpression of cac RNAi (a), overexpression of IP3R RNAi in Aβ flies improved cells viability, less vacuolated area in Aβ flies with reduced IP3R (b) (Cac RNAi: $F(3, 13) = 53.54$, $p < .0001$; Ctrl vs. Aβ42: $p < .0001$; Aβ42 vs. Aβ42 + Cac RNAi: $p = .5044$, $n = 4–5$; IP3R RNAi: $F(3, 13) = 25.32$, $p < .0001$; Ctrl vs. Aβ42: $p < .0001$; Aβ42 vs. Aβ42 + IP3R RNAi: $p = .0004$, $n = 4–5$). ***$p < .001$. Dish circle indicated quantified vacuolated area and white circle indicated whole quantified cell body region. White scale bar indicated 4 μm

example, mitochondria. It has been shown in different models and systems that accumulated calcium in cytosol triggers apoptotic pathways.[6,8,28] Our findings that VGCC and IP3R expression was reduced in Aβ flies, suggesting that the reduction of VGCC and IP3R could be the result of compensation trying to bring down the increased intracellular calcium induced by Aβ. Therefore, further decreased VGCC or IP3R in Aβ flies is able to extend the lifespan and improve cell viability. A recent study in fruit flies also demonstrated that reduction of IP3R is able to improve the lifespan in the tau transgenic flies,[29] which is also consistent with our findings. Furthermore, the exact function of VGCC and IP3R could be different or partially overlapped. It is possible that the reduction of lifespan in Aβ flies could be the combination of different affected cellular functions. One particular function recovered by VGCC or IP3R can improve lifespan but not cell viability. Secondly, we only examined the cell viability in the mushroom body area, as this area is the primary region for learning and memory in the fly. The possibility of regional specificity on the animal function affected by Aβ could not be totally excluded.

It is also worth noting that there was a reduction of Aβ accumulation in Aβ flies after IP3R was knocked down. Although the exact involved mechanism is not clear, we do not think the level of IP3R is directly involving in Aβ clearance. Previous of our studies and others have shown that ER stress regulates the function of ubiquitin-proteasome system (UPS) and autophagy. Aβ accumulation affects ER stress and down-regulates the activity of UPS and autophagy.[26,30–32] As dysregulated calcium also affects the function of ER, we hypothesize that reduced IP3R improves the ER function and thus the cellular degradation systems, UPS and autophagy. Therefore, the degradation of Aβ is observed. This hypothesis is supported by the finding that reduction of IP3R improves cell viability.

Calcium has long been suggested to play an important role during AD progression. Different strategies to balance the calcium level intracellularly have also been proposed,[27,33–35] however, until now, it remains unclear how dysregulated calcium affects Aβ-induced learning and memory deficit, cell death, and lifespan shortening. The current study not only reveals the opposite roles of

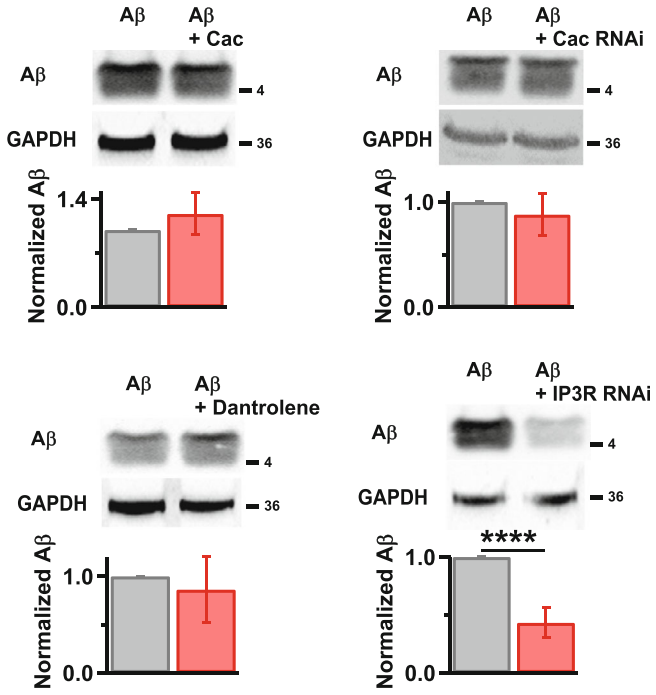

FIGURE 5 Reduced IP3R but not cac in Aβ flies decreases Aβ accumulation. Five heads of 8 days old Aβ flies were collected to assay the Aβ accumulation in the brain. Reduced IP3R significantly reduced Aβ accumulation (Cac: Two-tailed *t*-test, $p = .6405$, $n = 8$; Cac RNAi: Two-tailed *t*-test, $p = .3271$, $n = 8$; Dentrolene: Two-tailed *t*-test, $p = .2077$, $n = 8$; IP3R RNAi: Two-tailed *t*-test, $p < .0001$, $n = 8$). ****$p < .0001$

calcium dynamics in Aβ-induced pathologies but also raises a caution of using calcium regulators for future pharmacology treatment.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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