Please cite this article in press as: Feng G et al. Down-regulation of K_V4 channel in *Drosophila* mushroom body neurons contributes to Aβ42-induced courtship memory deficits. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.06.008

Neuroscience xxx (2017) xxx–xxx

DOWN-REGULATION OF K_V4 CHANNEL IN *DROSOPHILA* MUSHROOM BODY NEURONS CONTRIBUTES TO Aβ42-INDUCED COURTSHIP MEMORY DEFICITS

GE FENG,[a,b†] JIE PANG,[a,b†] XIN YI,[a] QIAN SONG,[a] JIAXING ZHANG,[a] CAN LI,[a] GUANG HE[a] AND YONG PING[a,b*]

[a] *Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai 200240, China*

[b] *Shanghai Key Laboratory of Psychotic Disorders (No.13dz2260500), Shanghai Mental Health Center, School of Medicine, Shanghai Jiao Tong University, Shanghai 200030, China*

Abstract—Accumulation of amyloid-β (Aβ) is widely believed to be an early event in the pathogenesis of Alzheimer's disease (AD). K_V4 is an A-type K^+ channel, and our previous report shows the degradation of K_V4, induced by the Aβ42 accumulation, may be a critical contributor to the hyperexcitability of neurons in a *Drosophila* AD model. Here, we used well-established courtship memory assay to investigate the contribution of the K_V4 channel to short-term memory (STM) deficits in the Aβ42-expressing AD model. We found that Aβ42 over-expression in *Drosophila* leads to age-dependent courtship STM loss, which can be also induced by driving acute Aβ42 expression post-developmentally. Interestingly, mutants with eliminated K_V4-mediated A-type K^+ currents (I_A) by transgenically expressing dominant-negative subunit (DNK_V4) phenocopied Aβ42 flies in defective courtship STM. K_V4 channels in mushroom body (MB) and projection neurons (PNs) were found to be required for courtship STM. Furthermore, the STM phenotypes can be rescued, at least partially, by restoration of K_V4 expression in Aβ42 flies, indicating the STM deficits could be partially caused by K_V4 degradation. In addition, I_A is significantly decreased in MB neurons (MBNs) but not in PNs, suggesting K_V4 degradation in MBNs, in particular, plays a critical role in courtship STM loss in Aβ42 flies. These data highlight causal relationship between region-specific K_V4 degradation and age-dependent learning decline in the AD model, and provide a mechanism for the disturbed cognitive function in AD.

*Correspondence to, Y. Ping: Bio-X Institutes, 800 Dongchuan Rd, Life Science Buildings #1, Rm 204, Shanghai, 200240, China.
E-mail address: yoping@sjtu.edu.cn (Y. Ping).
† These authors contributed equally to this work.

Abbreviations: Aβ, amyloid-β; AD, Alzheimer's disease; AE, after eclosion; APP, Aβ precursor protein; CI, courtship index; DI, discrimination index; DNK_V4, K_V4 dominant-negative mutant; I_A, A-type K^+ current; LI, learning index; MB, mushroom body; PN, projection neuron; STM, short-term memory.

http://dx.doi.org/10.1016/j.neuroscience.2017.06.008

This article is part of a Special Issue entitled: Molecules & Cognition.

Key words: amyloid-β, courtship memory, K_V4 channel.

INTRODUCTION

Behavior investigations of memory in *Drosophila* often study the ability of flies to retain memory for various times after conditioning (Kamyshev et al., 1999). Among them, the conditioned courtship paradigm (Siegel and Hall, 1979) is a unique one, because it is based on a complex form of learning and applies only natural stimulus (Montague and Baker, 2016). Mature females that have recently mated generally reject the courtship of males. After pairing with unreceptive mated females, the courtship behavior of males will be suppressed (Siegel and Hall, 1979; Siwicki et al., 2005; Keleman et al., 2012). Generally, *cis*-vaccenyl acetate (cVA), the male-specific pheromone, is transferred to female cuticle on mating (Everaerts et al., 2010; Keleman et al., 2012). In courtship conditioning, training can alter male's sensitivity to volatilized cVA from female cuticle, and then the ability to discriminate virgins from mated females will be improved. After training, learning is measured by the courtship memory assay (Kamyshev et al., 1999). Studies have shown that olfactory receptor neurons (ORNs), which mediate the detect of cVA (Kurtovic et al., 2007; Ronderos and Smith, 2010; Fernandez and Kravitz, 2013), projection neurons (PNs), onto which the ORNs' synapse connects (Lebreton et al., 2014), and mushroom body neurons (MBNs), one major memory center in the brain (McBride et al., 1999; Montague and Baker, 2016), are required for the courtship conditioning. These reports indicate excitability changes in these groups of neurons may impair the courtship memory.

K_V4/Shal is an A-type K^+ channel in *Drosophila,* and the homologous protein in mammals is the Shal-type family ($K_V4.x$), comprising $K_V4.1$, $K_V4.2$ and $K_V4.3$. Generally, the $K_V4.x$ family channels are highly expressed in brain, heart and smooth muscles (Birnbaum et al., 2004). A-type K^+ currents (I_A) in dendrites regulate local membrane depolarization at dendritic spines as well as modulate the arrival or/and effects of dendritic backpropagation of action potentials (b-APs)

(Birnbaum et al., 2004; Magee and Johnston, 2005; Zhao et al., 2011; Ping and Tsunoda, 2012; Srinivasan et al., 2012), suggesting changes in K_v4 expression modulate neuronal excitability. Furthermore, I_A may also regulate NMDA receptor-dependent synaptic plasticity in hippocampus (Birnbaum et al., 2004; Jung et al., 2008; Jo and Kim, 2011), suggesting that K_v4 channels may be involved in modulating learning behaviors. Indeed, a few reports on mice support the idea: blockade of K_v4 channels, *$K_v4.2$*-knockout, or phosphorylation modulation of Kv4.2 channels impairs memory behaviors in different learning tasks (Lugo et al., 2012; Truchet et al., 2012; Vernon et al., 2016).

The accumulation of amyloid-β (Aβ) oligomers in the brain likely initiates a cascade of events, which may lead to the onset and progression of Alzheimer's disease (AD) (Glenner and Wong, 1984; Ramsden et al., 2001; Walsh and Selkoe, 2004; Tanzi and Bertram, 2005). Synapse dysfunction, a typical feature in AD, could be the major reason for the early memory loss, which was found to be one of the primary clinical symptoms in AD patients (Selkoe, 2002). Reports also have identified changes in intrinsic excitability in AD models. For example, a reduction in voltage-dependent Na^+ channels in interneurons induced neural hyperexcitability, which could be responsible for cognitive dysfunction in AD (Verret et al., 2012). Moreover, reports suggest Aβ expression leads to neuronal hyperexcitability in cortical and hippocampal neurons in mice AD models (Hartley et al., 1999; Palop et al., 2007; Busche et al., 2008, 2012; Kuchibhotla et al., 2008; Minkeviciene et al., 2009; Brown et al., 2011). Expressing a secreted form of the toxic human Aβ1–42 (Aβ42) using GAL4/UAS system in *Drosophila* (Brand and Perrimon, 1993) can recapitulate AD-like phenotypes *in vivo* (Iijima et al., 2004), including neuronal hyperexcitability (Ping et al., 2015). Two recent studies demonstrated that Aβ- or tau-induced K_v4 loss is partially responsible for the neuronal hyperexcitability in AD models (Hall et al., 2015; Ping et al., 2015). Defects in innate behaviors, including olfactory learning, locomotor, circadian activities and sleep, have been reported in Aβ *Drosophila* models (Iijima et al., 2004; Lang et al., 2013; Chen et al., 2014; Tabuchi et al., 2015; Song et al., 2016) (for review, see (Fernandez-Funez et al., 2015)). These behavior assays would provide the flexibility to investigate the link between neuronal hyperactivity induced by K_v4 depletion and behavior defects.

In this study, courtship memory assay (Kamyshev et al., 1999) was used to measure the short-term memory (STM) in Aβ42 and K_v4 dominant-negative mutant (DNK_v4) flies. Our results show that both Aβ42 and DNK_v4 impaired courtship short-term memory (STM) in males. Our previous report shows the down-regulation of K_v4 channel by Aβ42 expression contributes to neuronal hyperexcitability (Ping et al., 2015). In this work we demonstrated that Aβ42-induced defective STM was rescued by transgenic restoration of K_v4 function in Aβ42 flies, suggesting Aβ42-induced STM deficits could be mediated by down-regulating K_v4 expression. Furthermore, accumulation of Aβ42 down-regulates the K_v4 current in mushroom body neurons (MBNs), but not in projection neurons (PNs). These results indicate that Aβ42-induced K_v4 degradation in fly brains, especially in MBNs, is a major contributory cause of courtship memory loss.

EXPERIMENTAL PROCEDURES

Fly stocks

We used previously generated UAS transgenic lines: *UAS-DNK_v4* (Ping et al., 2011), *UAS-Aβ42/CyO* (Iijima et al., 2004), *UAS-GFP.S65T.T10* (Tanaka et al., 2008). For *UAS-K_v4,* the wild-type *Shal2* isoform was subcloned into the pENTR1A vector (GatewaypENTR vectors, Invitrogen), then recombined in vitro using lambda integrase into the *pTW* destination vector (Drosophila Gateway Vector Collection, available through the Drosophila Genomics Resource Center), generating the *pUAST-Shal2* transformation vector. Microinjection with transposase into *w1118* embryos to generate transgenic lines was performed by Rainbow Transgenics (Camarillo, CA), then mapped and balanced by standard procedures (Ping et al., 2015). *elav-GAL4*(#458); *GH146-GAL4* (#30026); *201y-GAL4* (#4440), *tub-GAL80^{ts}* (#7019) and *UAS-mCD8::GFP* (#5130) were obtained from Bloomington *Drosophila* Stock Center.

Behavioral analysis

Experimental males and females were collected at eclosion, and raised on standard cornmeal and molasses medium. Males were kept individually in culture vials and aged up to 4–7 days (d) after eclosion (AE) or 11–14 AE. Females were collected in groups of 10–15 flies per food vial. Before the behavior experiments, females were pre-mated by pairing 10–15 females together with 15–20 males in one food vial for about 18 h (h) till the test.

For courtship conditioning assay, individual males were placed in vials with (trained) or without (naïve) a mated female for training lasting for 1 h. After training, females were removed from vial, while males were still left in vials for 30 min before test. Before the following 10-min test period, the experimental male was moved from the vial to the test chamber (15 mm diameter × 7 mm deep) paired with a virgin or mated female, which is at the same age as the male. All 10 min of testing period was videotaped. Courtship index (CI) was defined as the percentage of the total 10 min that was spent in courtship behaviors. Discrimination index (DI) and learning index (LI) were calculated using the mean CIs: $DI = [CI_{virgin} - CI_{mated}]/CI_{virgin}$ (Keleman et al., 2012); $LI = [CI_{naive} - CI_{trained}]/CI_{naive}$ (Kamyshev et al., 1999; Keleman et al., 2012).

As the distributions of DIs and LIs differ between groups, DIs and LIs were compared using the permutation test with 100,000 random permutations (Kamyshev et al., 1999; Keleman et al., 2012), and performed with R software. One-tail testing against the null hypothesis was used in the comparison.

Please cite this article in press as: Feng G et al. Down-regulation of K_v4 channel in *Drosophila* mushroom body neurons contributes to Aβ42-induced courtship memory deficits. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.06.008