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On the role of v-ATPase V0a1-dependent degradation in Alzheimer disease

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Defective autophagy and lysosomal degradation are hallmarks of numerous neurodegenerative disorders. Vesicular ATPases are intracellular proton pumps that acidify autophagosomes and lysosomes. V0a1 is a key component of the v-ATPase that is only required in neurons in *Drosophila melanogaster*. We have recently shown that loss of V0a1 in Drosophila photoreceptor neurons leads to slow, adult-onset degeneration.[1] Concurrently, Lee et al.[2] reported that V0a1 fails to localize to lysosomal compartments in cells from Presenilin 1 knock-out cells. Together these two reports suggest that a neuronal V0a1-dependent degradation mechanism may be causally linked to Alzheimer pathology. Indeed, we now show that loss of V0a1 makes Drosophila neurons more susceptible to insult with human Alzheimer-related neurotoxic Aβ and tau proteins. Furthermore, we discuss the potential significance of the discovery of the neuron-specific degradation mechanism in Drosophila for intracellular degradation defects in Alzheimer Disease.

Key words: Neurodegeneration, Autophagy, degradation, acidification, vesicular ATPase, Alzheimer

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Until recently, the degradation machinery implicated in neuronal degeneration was thought to be ubiquitous and there was little evidence for a dedicated neuronal pathway. In addition, the causal relationship between adult-onset neurodegeneration and accumulations of undegraded proteins in Alzheimer Disease as well as other neurodegenerative disorders is unclear.[3,4] We have recently shown that loss of the v-ATPase component V0a1 leads to a ~50% reduction of strongly acidified compartments in neurons, an increase of undegraded endosomal and autophagosomal compartments, and ultimately to slow, adult-onset degeneration.[1] Furthermore, loss of *v0a1* also causes severe neurotransmission defects and thereby embryonic lethality. The recent Drosophila studies were made possible by a genetic method that only renders photoreceptor neurons homozygous mutant in otherwise heterozygous animals.[5] Since photoreceptors are not required for viability, this system allows one to overcome early neuronal death due to embryonic lethality and follow the maintenance and degeneration of homozygous mutant neurons in an otherwise healthy fly. *v0a1* photoreceptors exhibit loss of neurotransmission immediately after eclosion,[6] whereas significant functional and morphological neurodegeneration occurs only after 1–2 weeks.[1] Importantly, *v0a1* is only required in neurons in Drosophila.[6] The discovery of a neuron-specific degradation mechanism in Drosophila establishes a causal link between intracellular degradation and adult-onset degeneration. Similarly, disruption of basal autophagy in mice through either loss of *atg5* or *atg7* leads to neurodegeneration within weeks,[7,8] and induction of autophagy can alleviate neurodegenerative effects.[9,10] Lysosomal storage disorders show how mutants in endolysosomal and autophagic pathways lead to late onset neurodegeneration.[11] Finally, neurons in mouse models of Down Syndrome and Alzheimer Disease exhibit early accumulations of Rab5-marked early endosomes as well as late endosomes.[3] All these findings are consistent with the idea that loss of degradation capacity sensitizes neurons

to 'cargo overload' as a cause for slow, adult-onset degeneration.

Lee et al.[2] recently reported that mislocalization of V0a1 is at least partially responsible for causing degenerative phenotypes in Presenilin 1 (PS1) knock-out cells. This finding is consistent with our discovery of the V0a1-dependent neuronal degradation mechanism in Drosophila and the degenerative phenotypes caused by loss of *v0a1*. The Drosophila, mouse and human V0a1 genes are precise orthologs within the V0a gene families in each of these different species that comprise exactly four V0a1-4 genes. Since loss of *v0a1* in Drosophila causes embryonic lethality due to neurotransmission failure, and neurotransmission has been shown to be preserved in PS1 KO neurons,[12] we conclude that the trafficking defect of V0a1 reported in PS1 KO cells should not represent a full *v0a1* loss-of-function. Instead, we propose that in PS1 KO cells only the trafficking to a subset of degradative compartments is affected. Furthermore, since lethality due to loss of neurotransmission precedes and masks slow degeneration, it may be unlikely to find disease-related *v0a1* mutations in humans. In contrast, loss of Presenilin 1 may cause a restricted loss of V0a1 in a specialized degradation pathway.

We were intrigued by the possibility that loss of *v0a1*-dependent degradation renders neurons more susceptible to neurotoxic insults. To directly test the idea that neurons lacking the neuron-specific degradation pathway are more susceptible to neurotoxic proteins, we designed challenge experiments with human Aβ[13] and tau[14] variants. Aβ peptides are the neurotoxic cleavage products of Amyloid Precursor Proteins (APP) that are causally associated with Alzheimer Disease and may have neurotoxic effects by disrupting lysosomal function.[15] Of the two possible Aβ cleavage products of APP, Aβ42 is more toxic and its expression in Drosophila photoreceptors leads to early cell death, whereas expression of Aβ40 has much milder phenotypes.[13] We established a protocol using Aβ40 expression and *v0a1* RNAi expression in addition to two days intense light stimulation; under these conditions, neither Aβ40 nor *v100* RNAi cause significant degeneration as measured by ERG response amplitudes (**Fig. 1A and B**). Knock-down of *v0a1* using RNAi causes the same phenotypes as loss of *v100* in photoreceptors (**Fig. 2**), and it provides a sensitized system for degradative challenges, as shown below. Indeed, when we co-express *v0a1* RNAi and Aβ40 under these conditions, ERG response amplitudes are significantly reduced compared to both Aβ40 or *v100* RNAi alone (**Fig. 1A and B**). Next, we expressed the human microtubule-binding protein tau as well as a mutant version of tau (R406W) that has been implicated in the pathogenesis of Alzheimer's disease and related disorders.[14] Similar to Aβ40, expression of tau and tau^{R406W} lead to increased functional degeneration when co-expressed with *v0a1* RNAi (**Fig. 1A and B**). Morphologically, two day intense light stimulation already leads to a few minor disruptions of the photoreceptor arrangement in the eye that are apparent as 'holes', i.e., unlabeled spaces between the rhabdomeric structures (**Fig. 1C**). These aberrations are increased by *v100* RNAi expression and indicative of early stages of photoreceptor degeneration. Co-expression of either Aβ40 or tau leads to a dramatic acceleration of this degenerative phenotype (**Fig. 1C**). These results support the idea that loss of *v0a1*-dependent degradation increases neuronal sensitivity to neurotoxic insults and accelerates neurodegeneration. Taken together with the mouse studies, loss of Presenilin 1 may cause a double burden by decreasing the degradative capacity through mislocalization of V0a1 and increasing the degradative load through faulty APP cleavage, Aβ accumulations or tau tangles.

Does V0a1 define a neuron-specific degradation pathway? In Drosophila, V0a1 is only required in neurons which lead to the idea of a neuron-specific mechanism.[1] In contrast, Lee et al.[2] characterize a V0a1-dependent acidification defect in PS1 KO mouse blastocysts. Degradative compartments require acidification for the maturation of Cathepsin proteases. PS1 KO blastocysts have a defect in Cathepsin D maturation, although a similar defect has not yet been demonstrated for neurons.[2,16] Furthermore, Presenilin double knock-out hippocampal neurons have significant ER calcium signaling abnormalities which may also potentially be linked to defects in V0a1 trafficking and impaired acidification of intracellular compartments.[17] Overall, the data from Lee et al.[2] suggest that a degradation mechanism that is neuron-specific in Drosophila may also operate in non-neuronal cells in mammals. Indeed, expression data inferred from Expressed Sequence Tag sources available from the public database of the National Center for Biotechnology Information (NCBI) indicates that *v0a1* expression is not restricted to neurons in mouse and human. Interestingly, the observation of neuronal specificity for a protein that is more widely expressed in mammals has also been made for APPL, the Drosophila ortholog of the Aβ-precursor protein APP. While expression of Drosophila APPL is a restricted to the nervous system,[18,19] two of the three APP homologs in the mouse are ubiquitously expressed.[20] However, the recent findings in *v0a1* Drosophila neurons and PS1 mouse KO cells suggest that *v0a1* is not required for all intracellular degradation. Instead, V0a1 increases degradative capacity and its loss alone is sufficient to cause adult-onset degeneration in Drosophila neurons. In addition, loss of V0a1-dependent degradation makes cells more vulnerable to neurotoxic insults including Aβ and tau proteins. Further experiments will be needed to validate the contribution of the *v0a1*-dependent degradation mechanism to neurodegenerative pathology.

Materials and Methods

Experiments were conducted using flies of the genotype UAS-Dicer2;GMR-Gal4;UAS-*v0a1*-RNAi and additionally expressing either UAS-Aβ40, UAS-tau or UAS-tau^{406}. The *v0a1* RNAi line was obtained from the VDRC.[21] Electroretinogram recordings, immunohistochemistry and quantification were all conducted as described in Williamson et al.[1] Dissections were performed as described in Williamson and Hiesinger.[22] For immunohistochemistry, the following antibodies and dilutions were used: Chaoptin (mAb 24B10) at 1:50, Avalanche/Syx7 at 1:1,000 and Sunglasses at 1:1,000.

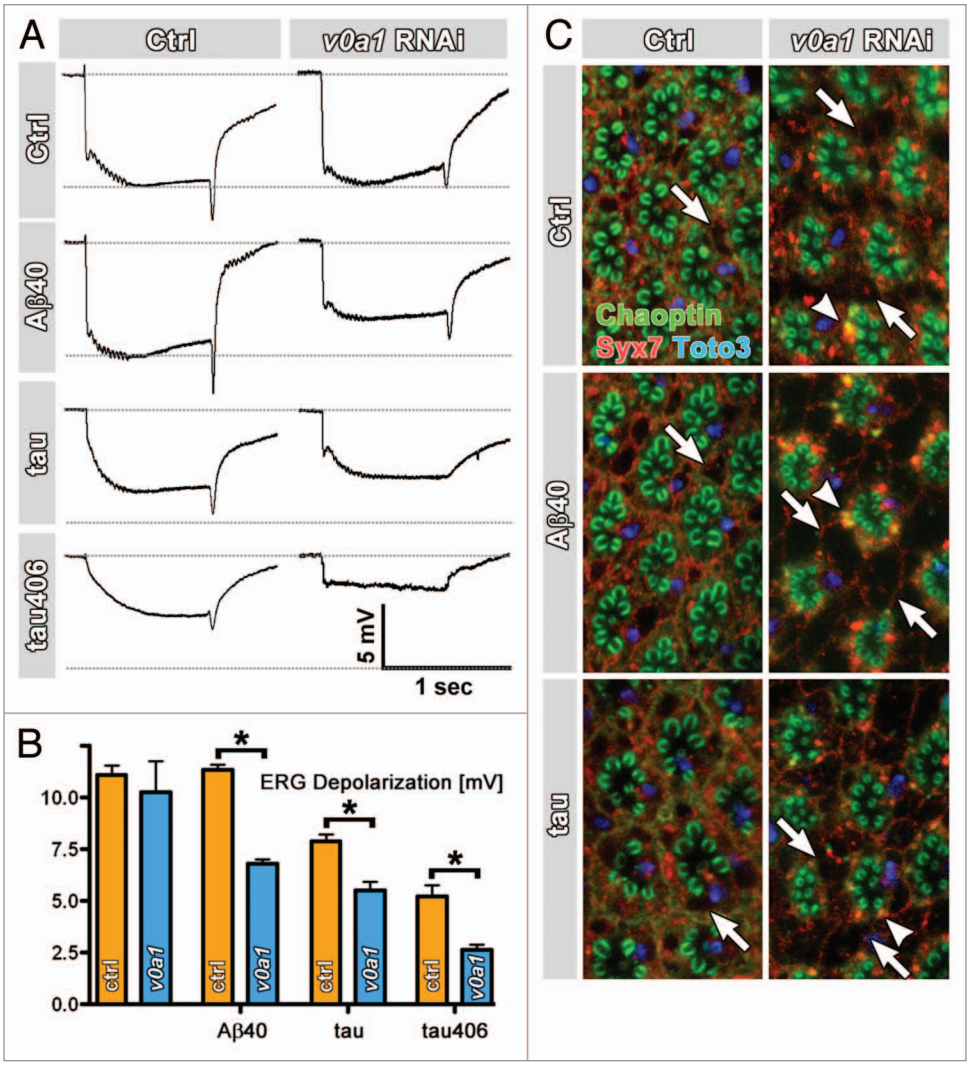

Figure 1. Loss of the *v0a1*-dependent degradation pathway increases susceptibility to neurotoxic insults by human Aβ and tau proteins. (A) Representative ERG recording for at least n = 20 three day-old flies of the genotypes indicated and raised at room temperature under constant light stimulation. (B) Quantification of ERG depolarizations. Orange bar shows ERG depolarization for control or overexpressed proteins in wild type photoreceptors; blue bars are corresponding experiments with dicer2-enhanced *v0a1* RNAi. Asterisks show statistical significance in pairwise comparisons ($p < 0.005$). (C) Immunolabeled adult eyes from experimental flies used for ERG recordings shown in (A). Chaoptin in green and nuclear labeling with Toto-3 in blue; Syx7/Avl labeling in red. Arrows indicate degenerative 'holes' in the immunolabeling. Arrowheads indicate early endosomal Syx7-positive accumulations with Chaoptin that are typical for loss of *v0a1* function. Error bars are S.E.M.

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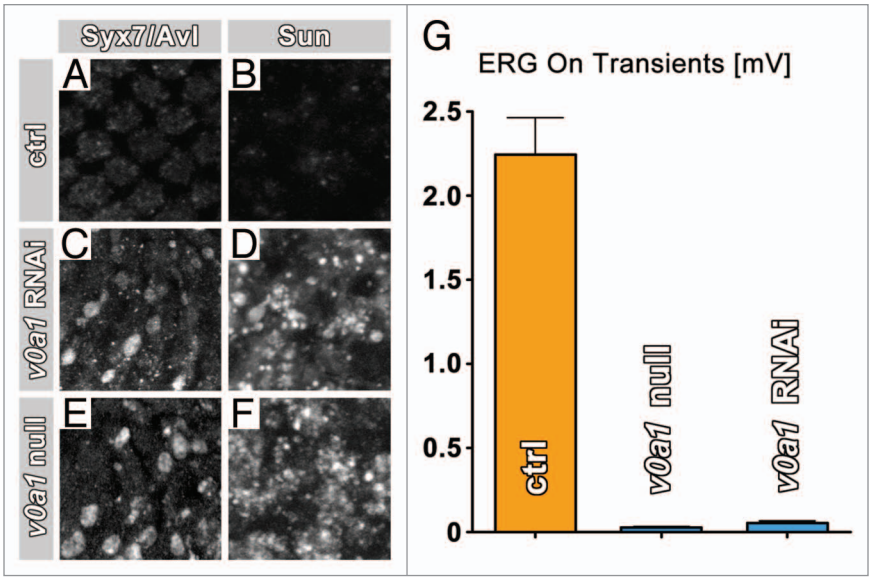

Figure 2. *v0a1* RNAi in photoreceptors causes phenotypes identical to loss of *v0a1*. (A–F) Antibody-labeling of Syx7/Avl and Sunglasses reveals similar accumulations of both markers in photoreceptors expressing dicer2-enhanced *v0a1* RNAi (C and D) as in *v0a1*null mutant photoreceptors in *ey35*FLP *v0a1* flies (E and F). (G) Both *v0a1* RNAi as well as *ey35*FLP *v0a1* lead to a complete loss of neurotransmission, as indicated by the loss of electroretinogram (ERG) 'On' transients.

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