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"Lights-Off" phenotype in *Drosophila* containing an UAS-Aβ42 transgene.

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Summary

The UAS-Aβ42$^{H29.3}$ transgenic *Drosophila* line used to model Alzheimer type neurodegeneration and other phenotypes falls down when transitioned from a light to a dark environment. This unusual phenotype is not dependent on the presence of a Gal4 driver and is thus unrelated to Aβ42 induced proteotoxicity or neurodegeneration. We propose that it may instead be the result of disruption in another gene function caused by the P element insertion site. Since this transgenic line is used by several groups to analyze neurological phenotypes associated with Aβ42 neuronal expression, the results of these studies must be interpreted with caution.

Introduction

Drosophila has been used to model neurodegenerative aspects of Alzheimer disease (AD) by expressing the neurotoxic human Aβ42 peptide in neurons (Crowther, *et al*., 2005; Crowther, *et al*., 2004; Crowther, *et al*., 2006; Finelli, *et al*., 2004; Iijima, *et al*., 2008; Iijima, *et al*., 2004; Ling, *et al*., 2009). As part of the usual phenotypic characterization of these flies, they are often examined for neurological phenotypes, such as locomotor functions or learning and memory tasks. We recently characterized phenotypes in one of the *Drosophila* AD models that has a UAS-Aβ42 transgene (UAS-Aβ42$^{H29.3}$) inserted on the 2nd chromosome. When Aβ42 expression was driven in either cholinergic, GABAergic, or glutaminergic motor neurons, flies show a variety of phenotypes. This includes reduced locomotor function, increased autophagy activity, and shortened lifespan. All of these phenotypes are dependent on the presence of a Gal4 neuronal driver as well as the UAS-Aβ42 responder. Since neurological phenotypes are an excellent way to analyze the consequences of transgene expression before extensive neurodegeneration takes place, we have been interested in defining additional phenotypes. We noticed that when light-adapted UAS-Aβ42$^{H29.3}$ flies are challenged with a sudden transition to darkness (*i.e*. we turn off the lights), they become disoriented and fall to the bottom of their vials. Further analysis of this phenotype, however, revealed that it was not dependent upon the presence of a Gal4 driver.

Materials and Methods

Light to Dark Sensitivity Assay

Flies containing the UAS-Aβ42$^{H29.3}$ transgene were obtained from Novartis (Finelli, *et al*., 2004) and stocks were carried over *CyO*. Expression was driven in cholinergic neurons using a 7.4 kb Cha-Gal4 transgene, and stocks also contained a UAS-GFP(S65T) reporter gene ([Cha-Gal4], UAS-GFP(S65T)/*CyO*; line 19B). Adult flies (one to four weeks old) of mixed gender were housed in groups of 100-200 in glass milk bottles at room temperature on standard *Drosophila* medium. In a darkened room, groups of 10 flies were placed in a 100 ml glass cylinder and conditioned to light by placing them directly below a fluorescent desk lamp equipped with 2 model F15T8 daylight balanced fluorescent bulbs (18 inches long, 15 watts) for 10 minutes. The light was then turned off for 10 seconds and back on for 30 seconds, and this cycle was repeated 5 times. Flies that dropped to the bottom of the cylinder each time the light was turned off were counted.

Western Blot

Expression of Aβ42 only in flies containing the Gal4 driver was confirmed by Western blotting using a protocol adapted from Ijima *et al*. (2004). Proteins were electroblotted to a PVDF membrane, which was boiled for 5 minutes in PBS and stained with anti-Aβ mouse monoclonal antibody 6E10 (Covance, 1:500 dilution). This was visualized using a horseradish peroxidase coupled goat anti-mouse second antibody (Bio-Rad, 1:40,000 dilution) followed by luminol detection (Pierce, Picoquant) using X-ray film.

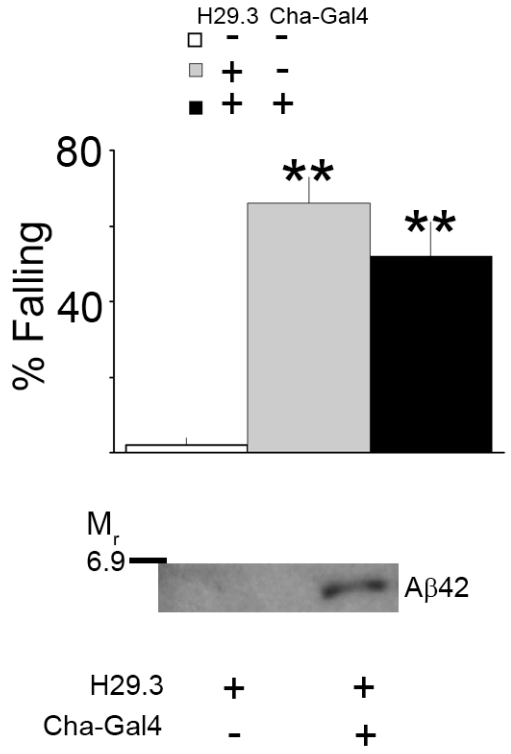

Figure 1. "Lights-Off" phenotype depends on the presence of the H29.3 UAS-Aβ42 transgene. Top: Control flies with a Cha-Gal4 driver, but no responder, rarely fall when lights are turned off. The presence of either the H29.3 responder alone or in combination with the Cha-Gal4 driver results in more than 50% of flies with the "Lights-Off" phenotype. Values are the means (± SEM) for 5 trials of groups of 10 individuals. Statistical significance was determined by individual t tests (**, $p < .01$). Bottom: Western blot showing Aβ42 expression only when the responder and driver are present together. Adult fly heads were homogenized in RIPA/SDS extraction buffer, and soluble proteins were immunoblotted with an anti-Aβ42 monoclonal antibody (Iijima, *et al*., 2004).

Results

All transgenic fly stocks were constructed by P-element transformation using a w^{118} genetic background. UAS-Aβ42$^{H29.3}$/*CyO* flies show no obvious abnormal locomotor or behavioral phenotypes and have a similar lifespan to CantonS or w^{118} flies. When stocks containing the UAS-Aβ42$^{H29.3}$ transgene are recombined with the 7.4kb Cha-Gal4

cholinergic neuron driver, significant phenotypes have been observed, including decreased lifespan and locomotor function.

As shown in Figure 1 (Top) 52-66% of light-adapted UAS-Aβ42$^{H29.3}$/*CyO* or UAS-Aβ42$^{H29.3}$ stocks recombined with the Cha-Gal4 driver fall to the bottom of the cylinder when the lights are turned off. In contrast only an occasional control fly (19B) shows this unusual behavior in response to lights off. Otherwise wild type stocks containing the *CyO* marked balancer chromosome also do not fall down in response to lights off (data not shown). This unusual "Lights-Off" behavior thus appears to be a result of the UAS-Aβ42$^{H29.3}$ insertion and does not depend on the presence of the Gal4 driver.

Western blot analysis confirms that only stocks where the UAS-Aβ42$^{H29.3}$ responder has been recombined with the Cha-Gal4 driver express detectable levels of Aβ42 protein (Figure 1 (Bottom)). The "Lights Off" phenotype is thus also unrelated to Aβ42 protein accumulation in neurons.

Discussion

H29.3 flies display a "Lights-Off" phenotype characterized by spontaneous falling to the bottom of a cylinder in response to a sudden light to dark transition. This unusual behavior is not dependent on the presence of a Gal4 driver, occurs in flies with no detectable Aβ42 expression, and is not seen in control flies. The phenotype thus appears to be independent of Aβ42 expression and depends instead on the presence of the H29.3 transgene insertion. We propose that this phenotype is related to disruption of an unknown genetic function by the P-element mediated insertion site of the H29.3 transgene. Caution should be used when interpreting neurological phenotypes of stocks containing the H29.3 transgene, especially for visually mediated phenotypes.

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Analysis of morphometric traits among few species of *Drosophila*.

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Understanding the microevolutionary basis of macroevolutionary change has been challenging evolutionary biologist for decades. Recently fresh attention is given to the origin and control of morphological variation (Hallgrimsson and Hall, 2005), since accumulation of small variation for several generations provides the raw material for natural selection and understanding of