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Dopa decarboxylase(Ddc)-GAL4 dramatically reduces life span.

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Abstract

The GAL4 system is extensively used for the directed expression of genes of interest in *Drosophila melanogaster*. One main property of this system is that GAL4, the yeast transcription factor, is believed to be inactive in *Drosophila* and that its expression under the control of *D. melanogaster*-specific promoters will have little effect on its own cells. We have shown that expression of *GAL4* under the control of the *dopa decarboxylase (Ddc)* promoter element does have an effect upon adult viability. Although *Ddc-GAL4* heterozygotes appear normal when raised at 25°C, the homozygotes have a have a greatly reduced life span. While the homozygotes simply do not survive, *Ddc-GAL4* heterozygotes demonstrate normal locomotion over their life span.

Introduction

The GAL4 ectopic expression system (Brand and Perrimon, 1993; reviewed in Phelps and Brand, 1998) has become an extremely useful approach for the study of specific genes in *Drosophila melanogaster*. This expression system relies upon GAL4 to bind the upstream activation sequence (UAS) in order to activate transcription of the target gene. A variety of transgenic *Drosophila* lines are readily available that express *GAL4* in specific tissues or cell types. Our laboratory is interested in models of neurodegenerative diseases and we have initiated work with the *dopa decarboxylase (Ddc)-GAL4* transgenic expression lines to model Parkinson's disease in *Drosophila*.

The *Drosophila melanogaster* model of Parkinson's disease was developed by the generation of transgenic lines bearing wild type and mutant forms of the human α-synuclein gene cloned downstream of the UAS yeast promoter (Feany and Bender, 2000). There is no apparent *Drosophila* homologue of α-synuclein, but expression of the human α-synuclein protein in the *Drosophila* nervous system recapitulated some features of Parkinson's disease. Expression of α-synuclein in the dopaminergic neurons (Feany and Bender, 2000) was driven by a transgene comprised of the *dopa decarboxylase* (*Ddc*) gene promoter cloned upstream of *GAL4*. Originally, this transgene was developed to examine a *Drosophila* model of cocaine addiction (Li *et al*., 2000). The Parkinsonian flies, apparently normal at a young age, demonstrated a premature loss of locomotor (climbing) ability, loss of the dopaminergic neurons and accumulation of α-synuclein-containing inclusions. In addition, *GMR-GAL4* driven expression in the developing eye resulted in age-dependent retinal neurodegeneration. Subsequent treatment of the transgenic α-synuclein expressing flies with a number of pharmacological agents such as the dopamine precursor L-DOPA, dopamine receptor agonists (bromocriptine, pergolide and SK&F38393) and the anticholinergic atropine, all restored or partially restored the age-dependent loss climbing ability (Pendleton *et al*., 2002). Further, this model has been used to examine the suppression

of the α-synuclein toxicity by the molecular chaperone, hsp70 (Auluck *et al.*, 2002). A good understanding of the effects of *Ddc-GAL4* expression in *Drosophila melanogaster* is essential to properly interpreting this model system.

As a prelude to our exploration of models of Parkinson's disease in *Drosophila*, we began to investigate the biological properties of the *Ddc-GAL4* driver lines. We have shown that *Ddc-GAL4* causes reduced viability and is therefore not inactive in *D. melanogaster*.

Materials and Methods

Fly stocks and culture: Ddc-GAL4 $^{4.3D}$ and *Ddc-GAL4* $^{4.36}$ flies (Li *et al.*, 2000) were obtained from Jay Hirsh at the Department of Biology, University of Virginia and w^{1118} was obtained from Dr. Howard Lipshitz at the Hospital for Sick Children in Toronto. To obtain heterozygotes, *Ddc-GAL4* homozygous males were crossed to w^{1118} females. All flies were cultured on standard cornmeal/yeast/molasses/agar media at 25°C.

Aging analysis: Adult males were aged in small groups upon standard media at 25°C and scored for viability every two to three days as described previously (Staveley *et al.*, 1990). The number of individuals aged are as follows: *Ddc-GAL4* $^{4.3D}$ homozygotes n = 102; *Ddc-GAL4* $^{4.36}$ homozygotes n = 133; *Ddc-GAL4* $^{4.3D}$ heterozygotes n = 280; *Ddc-GAL4* $^{4.36}$ heterozygotes n = 119; w^{1118} n = 83).

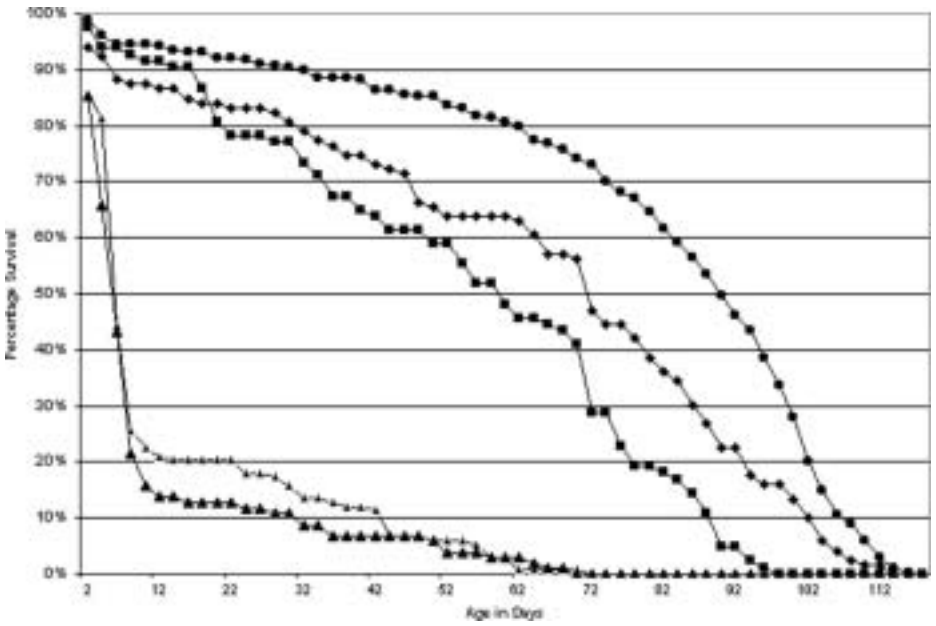

Figure 1. *Ddc-GAL4* reduces life span. Male homozygotes of the independent insertions of the *Ddc-GAL4* transgene, *Ddc-GAL4* $^{4.3D}$ (large triangles) and *Ddc-GAL4* $^{4.36}$ (small triangles) both display a greatly reduced life span. The heterozygotes of *Ddc-GAL4* $^{4.3D}$ (solid circles) and *Ddc-GAL4* $^{4.36}$ (solid diamonds), however display a normal, if not extended life span when compared to the control w^{1118} (solid squares) individuals. The number of males aged are as follows: *Ddc-GAL4* $^{4.3D}$ homozygotes, n = 102; *Ddc-GAL4* $^{4.36}$ homozygotes, n = 133; *Ddc-GAL4*$^{4.3D}$ heterozygotes, n = 280; *Ddc-GAL4* $^{4.36}$ heterozygotes, n = 119; w^{1118}, n = 83.

Locomotion assay: The flies were assayed for their ability to climb in a manner similar to that described by Feany and Bender (2000). Every 4 to 5 days, 10 male flies of a cohort of aged flies were assayed for their ability to climb to the top of the vial within a period of 18 seconds. Twenty trials were carried out for each time point.

Results and Discussion

An investigation of the baseline biological consequences of *GAL4* expression as directed by the *dopa decarboxy-lase(Ddc)* promoter is essential to our studies of Parkinson's disease

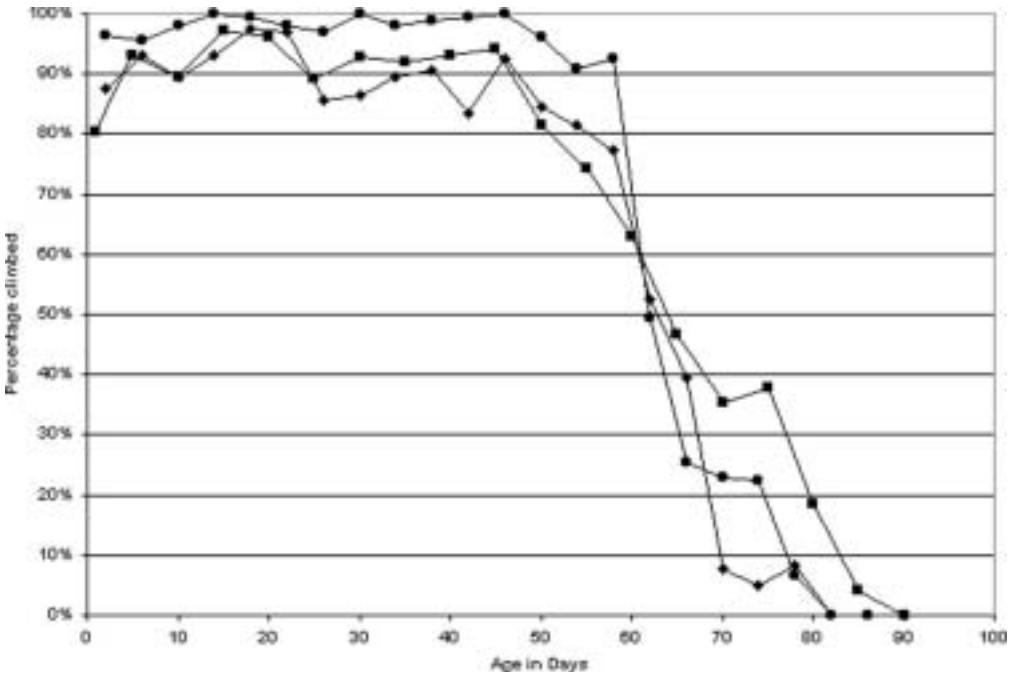

Figure 2. *Ddc-GAL4* heterozygotes have normal locomotor (climbing) activity. The heterozygotes of *Ddc-GAL4* $^{4.3D}$ (solid circles) and *Ddc-GAL4*$^{4.36}$ (solid diamonds) display a normal level of climbing ability when compared to the control w^{1118} (solid squares) flies. The locomotion of homozygotes was not measured due to poor viability.

models in *Drosophila*. We began by crossing the *Ddc-GAL4* $^{4.3D}$ and *Ddc-GAL4*$^{4.36}$ driver lines to w^{1118} to conduct climbing assays. It quickly became apparent that the stocks of both *Ddc-GAL4* insertion lines required extra care to maintain and that the flies were apparently short-lived in both cases. As a result we decided to conduct longevity trials.

Homozygotes of both insertions of the *Ddc-GAL4* trans-gene display a greatly reduced life span (Figure 1). For example, by the 6th day, *Ddc-GAL4*$^{4.3D}$ and *Ddc-GAL4*$^{4.36}$ homozygous males showed only 43% and 44% survival, respectively. While 50% of *Ddc-GAL4*$^{4.3D}$ male het-erozygotes survive past the age of 88 days and *Ddc-GAL4*$^{4.36}$ male heter-ozygotes past 70 days of age. We assigned w^{1118} as the control strain in these experiments, 50% of which survived between 58 and 60 days. The shorter median life span of the control may reflect an insufficiency of the w^{1118} stock rather than an increase in viability the heterozygotes. It is important to note that the very similar longevity profile of the two independent insertions of *Ddc-GAL4* suggest that the reduction in life span is due to the expression of the transgene and not the site of insertion.

The reduced viability of the *Ddc-GAL4* homozygotes forced us to reexamine the locomotor activity of the *Ddc-GAL4* heterozygotes (Figure 2). However, the *Ddc-GAL4* $^{4.3D}$ and *Ddc-GAL4* $^{4.36}$ heterozygotes retain their ability to climb with age in a manner similar to the w^{1118} controls. Due to greatly reduced viability, the locomotion of homozygotes was not measured.

The UAS/GAL4 ectopic expression system has made it possible to express genes and test the effects of overexpression. The *Ddc-GAL4* driver has been used for expression of transgenes in the dopaminergic neurons including expressing the α-synuclein gene to model Parkinson's disease (Feany and Bender, 2000). Although no UAS (cggagtactgtcctcc) promoter sequences are found in *D. melanogaster* (Berkeley Drosophila Genome Project, pers. comm.), our laboratory has demonstrated that expression of *GAL4* in the eye with the *GMR-GAL4* transgene leads to increased levels of apoptosis and morphological defects (Kramer and Staveley, submitted). Although the mechanism by which GAL4 induces cell death is unclear, death of the dopaminergic neurons could certainly result in premature lethality in these flies.

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