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Research Article

Enhanced tethered-flight duration and locomotor activity by overexpression of the human gene *SOD1* in *Drosophila* motorneurons

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Abstract

Mutation of the human gene superoxide dismutase (*hSOD1*) is associated with the fatal neurodegenerative disease familial amyotrophic lateral sclerosis (Lou Gehrig's disease). Selective overexpression of *hSOD1* in *Drosophila* motorneurons increases lifespan to 140% of normal. The current study was designed to determine resistance to lifespan decline and failure of sensorimotor functions by overexpressing *hSOD1* in *Drosophila*'s motorneurons. First, we measured the ability to maintain continuous flight and wingbeat frequency (WBF) as a function of age (5 to 50 days). Flies overexpressing *hSOD1* under the D42-GAL4 activator were able to sustain flight significantly longer than controls, with the largest effect observed in the middle stages of life. The *hSOD1*-expressed line also had, on average, slower wingbeat frequencies in late, but not early life relative to age-matched controls. Second, we examined locomotor (exploratory walking) behavior in late life when flies had lost the ability to fly (age ≥ 60 d). *hSOD1*-expressed flies showed significantly more robust walking activity relative to controls. Findings show patterns of functional decline dissimilar to those reported for other life-extended lines, and suggest that the *hSOD1* gene not only delays death but enhances sensorimotor abilities critical to survival even in late life.

Keywords: behavioral genetics, superoxide dismutase, *Drosophila*, motorneuron, longevity.

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Introduction

Drosophila melanogaster is an important animal model in the study of aging, life-extension, and functional decline, partly because of its short lifespan of approximately two months, which facilitates population-level studies of changes in behavior and physiology at various stages of life, and partly because of the high homology of its genes to other species including mammals (Rose *et al.*, 1992; Curtsinger *et al.*, 1995; Osiewacz, 1997; Parkes *et al.*, 1998; Phillips *et al.*, 2000). Comparative genomic studies, for example, have reported that of the hundreds of human disease genes examined, between 65 to 87% are conserved in *Drosophila* (Fortini *et al.*, 2000; Reiter *et al.*, 2001; Inlow and Restifo, 2004). *Drosophila* also displays a large repertoire of natural behaviors (visual, locomotor, olfactory, and auditory) that facilitate the study of sensorimotor decline as a function of aging.

The current study investigated age-dependent decline and failure of behavioral functions in transgenic *Drosophila* carrying the human gene superoxide dismutase (*hSOD1*). A gain-of-function mutation of the *hSOD1* gene in humans is associated with the life-shortening paralytic disease Familial Amyotrophic Lateral Sclerosis (*i.e.*, Lou Gehrig's disease; Rosen *et al.*, 1993) which is characterized by gradual loss of motor functions, muscle fasciculation and atrophy, difficulty in speaking, breathing, and early death. Broad systemwide expression of human or endogenous *SOD1* in *Drosophila* has no effect on longevity (Seto *et al.*, 1990; Kirby *et al.*, 2008). However, selective overexpression of *hSOD1* in *Drosophila*'s motorneurons increases longevity of healthy flies by 40% and rescues short-lived *SOD1*-null mutants to near-normal lifespan (Parkes *et al.*, 1998). Increased longevity by *hSOD1* overexpression is thought to occur by antioxidant intervention that mitigates cumulative DNA and cell damage caused by reactive oxygen species (Harman, 1956, 2003; Martin *et al.*, 1996). This process is thought to be further mediated by *hSOD1*-triggered changes in signal transduction pathways, possibly through the neuroendocrine system, that regulate patterns of gene expression in a variety of aerobic cell types other than motorneurons (Phillips *et al.*, 2000).

We describe here experiments on flight functions and locomotor activity of *hSOD1*-expressed *Drosophila*. This is an important question for two reasons. First, an extended life devoid of healthy sensorimotor functions may not ex-

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perience a quality of life worth extending, and hence, determining the extent to which functional abilities are enhanced in life-extended lines is of value. Second, different life-extending genes (*e.g.*, *hSOD1, Methuselah; INDY:* I'm not dead yet) will likely yield different patterns of gerontological decline in sensory, motor, memory, and other behavioral functions. Findings from our earlier work on the age-dependent decline of functions in the *Methuselah* mutant differ markedly from those observed in the current study, which may be due to each gene's action on mechanisms subserving behavior, or to the patterns of a gene's expression in different tissues as we will describe later. These differing patterns of functional decline, nonetheless, suggest that multiple strategies to life extension may be useful in preserving distinct behavioral functions within an organism, strategies that may include different genetic as well as environmental approaches, *e.g.*, drug consumption or calorie restriction (Sohal and Weindruch, 1996; Kang *et al.*, 2002).

In the first part of the current study we examined the ability of *hSOD1*-expressed and control flies to maintain continuous flight in a tethered-flight paradigm. We measured flight duration and wingbeat frequency (WBF) using an infrared laser coupled with a photodiode sensor system that recorded wingbeat time-series during phototaxic flight. We found that *hSOD1*-expressed flies sustained flight longer than controls throughout much of their lifespan and had slower WBFs in late, but not early stages of life. In part two, we measured locomotor activity during late life in darkness and in a well-lit arena. *hSOD1* flies showed significantly more robust locomotor ability relative to controls. In addition, females outperformed males, but only for the *hSOD1* line, suggesting an interaction between genotype and sex in locomotor ability.

Materials and Methods

Fly stocks

hSOD1-expressed and control lines were generated at the University of Guelph as described fully in Parkes *et al.* (1998). Briefly, expression of a human *SOD1* transgene in *Drosophila* motorneurons was achieved using the yeast GAL4/UAS system (Brand and Perrimon, 1993; Yeh *et al.*, 1995; Gustafson and Boulianne, 1996). The D42-GAL4 activator used here is expressed broadly during embryogenesis, becomes restricted to motorneurons and interneurons during larval stages, and with the exception of a small number of unidentified neurons in the central brain, is restricted to motorneurons within the ventral ganglia in the adult fly. The *hSOD1* transgene consisted of a human *SOD1* cDNA coupled to a yeast UAS element within a *Drosophila* P-transformation vector. Because both lifespan and behavior are affected by variation in genetic background, a number of genetic measures were taken in introducing the D42-GAL4 and UAS-*hSOD1* transgenes into a uniform *Sod*+/+ genetic background, and to construct expressing and non-expressing lines that were co-isogenic for most of the genome, with minimal differences in the genetic background between strains (see Parkes *et al.*, 1998; Kirby *et al.*, 2008). This allows tracing of behavioral phenotypes specifically to GAL4-activated *hSOD1* expression.

Confirmation of life extension

Virgin flies were sex-segregated within 4 h of eclosion and maintained in small laboratory vials (Genesee Scientific Corp.; polystyrene, O.D. x H 25 x 95 mm) containing fresh food media in a low-temperature incubator at 25 °C and 40% humidity on a 12/12 h dark/light cycle (VWR Scientific, Model 2015). They were transferred to fresh food vials every 3 to 4 days. We confirmed extension of the lifespan of *hSOD1*-expressed flies relative to controls by approximately 30% in virgin females (68 *vs.* 52 days) and 48% in virgin males (65 *vs.* 44 days), measured at 50% mortality levels of a population of approximately 400 flies. Kaplan-Meier survival analyses (log rank Mantel-Cox test) showed that *hSOD1*-expressed males significantly outlived control males ($\chi^2 = 119.1$, $p < 0.001$) and *hSOD1*-expressed females significantly outlived control females ($\chi^2 = 123.0$, $p < 0.001$).

Tethered-flight experiment

Flight duration and wingbeat frequency were measured in a tethered-flight paradigm at ~5 h after onset of subjective day. The tethering process involved several steps. First, an individual fly was lightly CO_2 anesthetized and transferred to a custom-made aluminum block in a Peltier cooler (Boekel Scientific, Model 260014) on which a small opening (2 x 1 x 1 mm^3) had been drilled to allow accurate positioning of an anesthetized fly (Figure 1). The fly remained under cold anesthesia at 4 °C. Individual flies were gently handled either with a small brush or a jeweler's vacuum tweezers. The tip of a tungsten wire (130 μ in diameter) was dipped in glass glue (Loctite, New York, NY, USA), and under a stereomicroscope (Olympus SZ40) lowered using a micropositioner (Stoelting Co./Prior, England) onto the anesthetized fly's thorax. The glue was cured with a UV gun (Electro-Lite Corp., Model ELC-403) for 20 s and the fly was removed from the Peltier cooler using the micropositioner. Flies usually recovered from cold anesthesia and began flight within 3 to 4 min. Tethered flies were moved to the experimental chamber, fed with a small piece of filter paper dipped in sucrose-water, and allowed to rest and become acclimated to the experimental environment for an additional 30-60 min prior to data collection. Prior work has shown that this is a sufficient period of time for full recovery from anesthesia (Lehmann and Dickinson, 1997; Petrosyan *et al.*, 2007, 2013, 2014).

The tethered fly was positioned under a solid-state infrared (IR) laser (808 nm; Lasermate Group Inc., Pomona, Ca, USA, Model PLC8082AE) with an adjustable focus

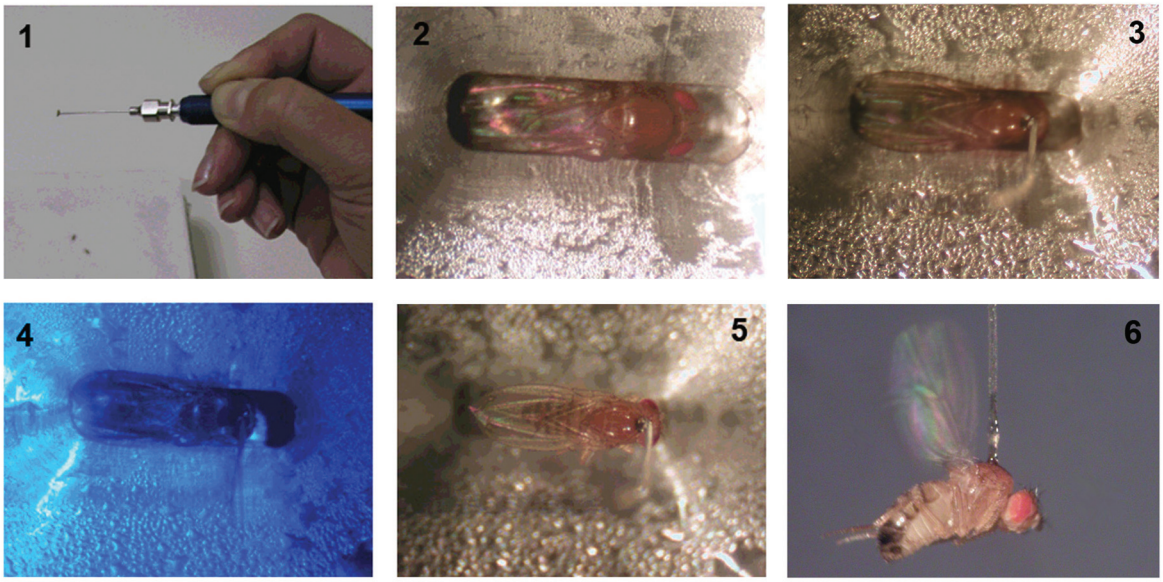

Figure 1 - Tethering a *Drosophila* for flight: 1) A CO_2 anesthetized fly was selected using vacuum tweezers, 2) positioned in a Peltier cooler at 4 °C which kept it under cold anesthesia, 3) a micropositioner was used to lower a tungsten wire dipped in glass glue onto its thorax, 4) cured with UV for approximately 20 s, 5) removed from the Peltier cooler using the micropositioner, 6) The fly recovered from anesthesia in 3 to 4 min and began flight.

that cast shadows of the wing beats onto fast-response IR photodiode sensors (Photonic Detectors Inc., Simi Valley, CA, USA, Part no. PDB-C615-2). This setup is shown in Figure 2. The sensors were placed in a small plastic box covered with an IR bandpass filter (Edmund Industrial Optics, Barrington, NJ, USA, Part. no. NT32769). The experiment was run in complete darkness in a steel chamber (2 x 2 x 2 m^3; IAC) with only a single green LED (555 nm) positioned directly in the fly's line of sight at a distance of 15 cm to provide a visual target for phototaxis (Hadler, 1964; Miller *et al.*, 1981).

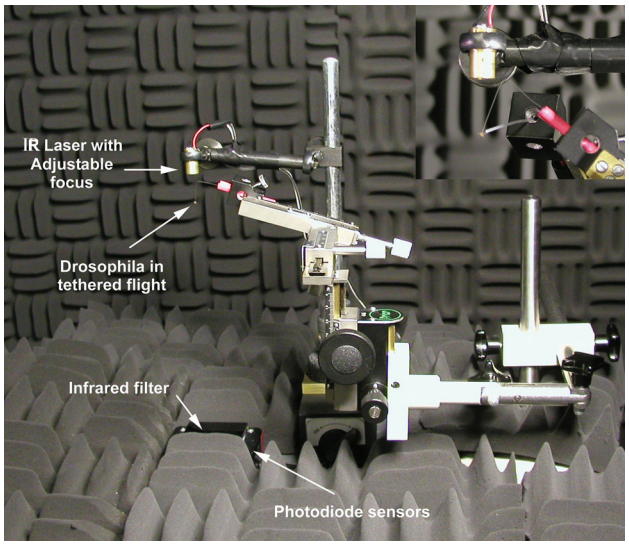

Figure 2 - Apparatus used for measurement of wingbeat frequency. A tethered fly was positioned under a solid-state infrared (IR) laser with an adjustable focus. Wingbeat shadows were cast onto IR photodiode sensors. The experiment was run in complete darkness in a steel chamber with only a single green LED (555 nm) positioned directly in the fly's line of sight to stimulate phototaxic flight. Inset shows the laser and acoustic microphone assembly (see text).

The output of the photodiode sensors were sent to an amplifier and fed into an analog-to-digital converter (Sound Blaster Live, -120 dB noise floor) positioned outside the chamber, and recorded at a sampling rate of 10 kHz. WBF was measured during the first minute of flight after the rest period. Each fly's wing-beat data were saved in a digital file for later processing. An individual fly's WBF was determined as the average of five to ten one-second samples of flight. This sampling scheme has been shown to be sufficiently representative of the average WBF in phototaxic flight (Petrosyan *et al.*, 2007). For each one-second sample, the wing-beat waveform was Fast-Fourier transformed and the frequency corresponding to the peak of this function was determined as the WBF for that sample (*i.e.*, 1-Hz frequency resolution).

To verify that WBF measured using the laser system is accurate, wing beats of one individual fly were recorded using the laser-system and, simultaneously, with a silicon probe-tube acoustic microphone (Figure 2 inset; Etymotic Research, Elk Grove Village, IL, USA ER-7C), fed to a digital-to-analog convertor (Sound Blaster Live) and digitized at a sampling rate of 10 kHz. The outputs of the two measurement systems were led to two separate computers. We found a near-perfect correlation between the acoustic measure of wing-beat waveform and laser measurements ($r > 0.99$).

Flight performance was measured at four age categories of 5, 10, 30, and 50 days for both genotypes, and additionally at the ages of 20 and 40 d for the *hSOD1* line. The

age categories of 10 and 50 days represent groups of flies that were between 9 to 10 days of age, and 50 to 51 days of age respectively. After each experimental run, flies were CO_2 anesthetized, removed from tether, and discarded in citrus oil.

Locomotor (walking) behavior

The distance walked in a fixed period of time has previously been used as a measure of exploratory behavior and locomotor ability in *Drosophila* (Strauss *et al.*, 1992; Hayward *et al.*, 1993; Roberts, 1998). The current experiment measured average walking distance for both genotypes in a large arena (60x45 cm). Since the main goal of the experiments was to examine sensorimotor senescence at old age, the minimum age tested was 60 days. At this age, flies cannot sustain their weight in free flight and therefore nearly always walk to explore their environment. On the day of the experiment, none of the flies were anesthetized. A total of 161 flies were tested (100 females and 61 males), with an average of approximately 12 flies per age group for each genotype. Flies were gently positioned in a 2.54 cm^2 (1-inch) square grid drawn in the center of the arena and the distance that they moved away from the center square in a 5-min period was used as a measure of locomotor activity. The experiment was run either in a well-lit arena or in complete darkness, recorded by an infrared camera, and walking distance was determined after the session from the video recordings. If a fly did not move out of the center square, the distance traveled was recorded as zero. Male and female flies were run in separate sessions.

Results

Tethered-flight experiment

Figure 3 shows duration of the longest segment of sustained flight measured during the first minute of flight after rest for *hSOD1* and its parental strain. The *hSOD1* data are based on 28 individually tethered flies and the data from the parental control group are based on 25 flies. Flight duration generally declines as a function of age for both genotypes. Female *hSOD1* flies maintain flight significantly longer than female controls throughout most of their lifespan, but not at the oldest age measured (50 days). The convergence of sustained flight durations at late life may possibly represent a floor effect, where all flies were near the end of their ability to maintain flight. No significant differences were observed in the duration of sustained flight between male *hSOD1* and male control flies. Female *hSOD1* flies also sustained flight longer than male *hSOD1* flies, but no differences in flight duration were observed as a function of age between male and female control flies (two red-dashed curves). A 2x2x4 independent-groups ANOVA showed a significant effect of genotype ($F_{(1,37)}$ = 6.49, $p < 0.05$), a significant effect of sex ($F_{(1,37)}$ = 4.43, $p < 0.05$), a significant effect of age ($F_{(3,37)}$ = 7.67, $p < 0.001$), and significant interaction between genotype and sex ($F_{(1,37)} = 4.98$, $p < 0.05$). No other interaction terms were significant.

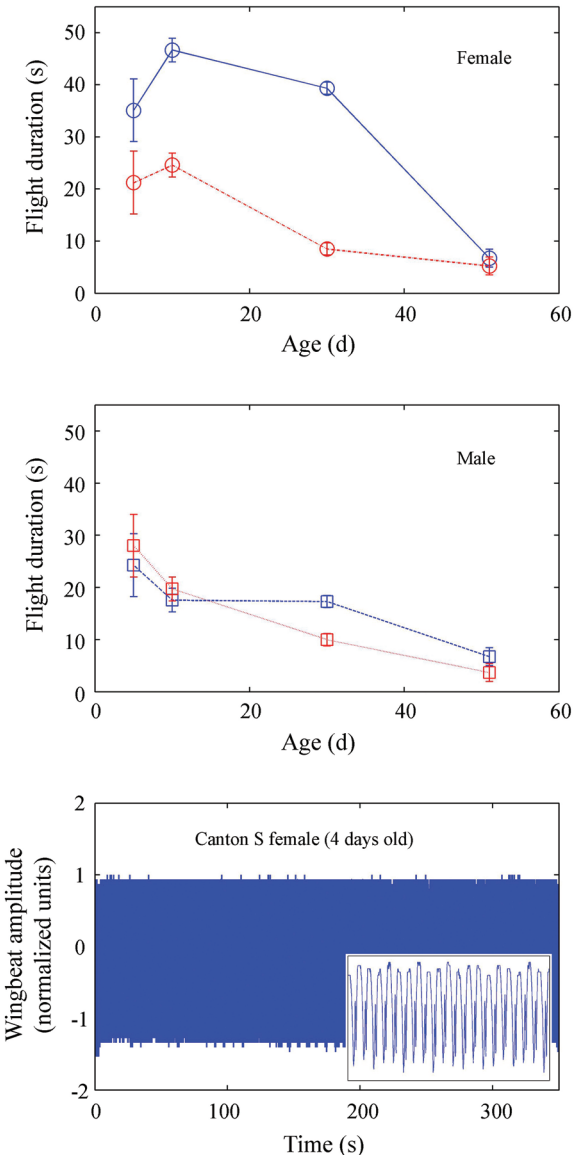

Figure 3 - Duration of the longest segment of sustained flight as a function of age and genotype. Top panel shows data for females and middle panel for males. Error bars represent one standard error. Bottom panel shows an extended uninterrupted flight of 5 min (approximately 50,000 wingbeats) by a 4-day old female Canton-S wild-type fly measured using the same apparatus and procedures employed in the current study. *hSOD1* and their parental controls maintain flight at considerably shorter durations than wild types. Inset shows a 120 millisecond segment (20 wingbeats) of the waveform in the bottom panel.

Figure 4 shows averaged WBFs as a function of age for the same flies as those shown in Figure 3. WBF for both genotypes declined monotonically as a function of age, but was lower for *hSOD1* flies at older ages. A 2x2x4 independent-groups ANOVA showed a significant effect of genotype: $F_{(1,42)} = 6.34$, $p < 0.05$, a significant effect of age:

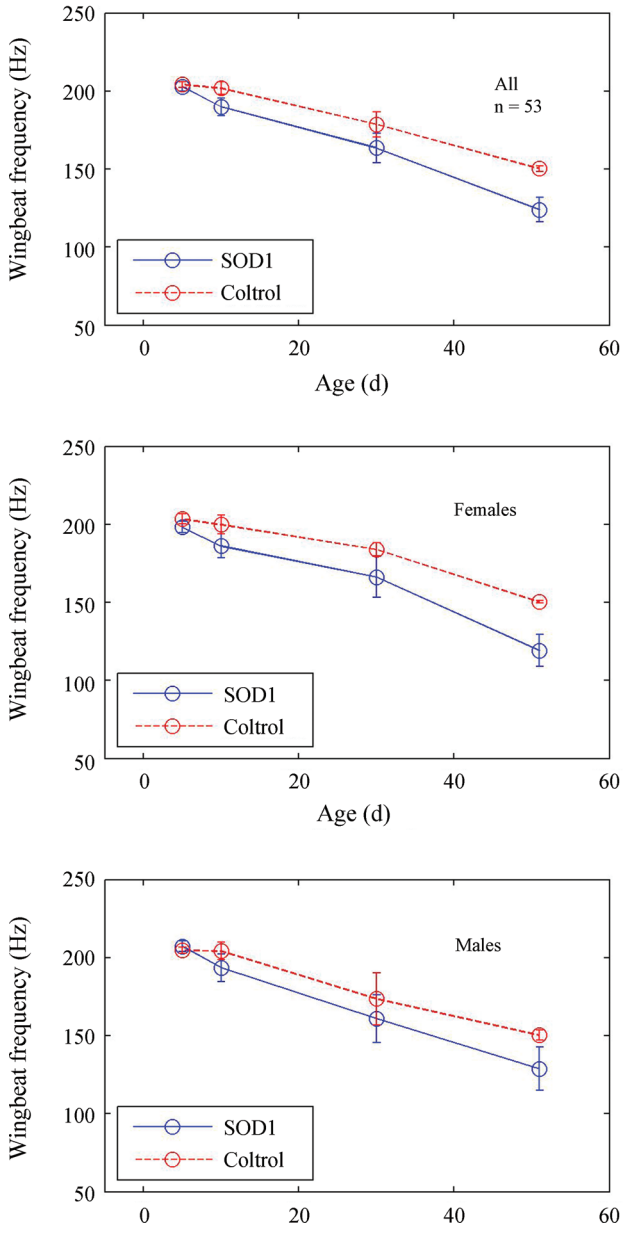

Figure 4 - Wingbeat frequency as a function of age for the *hSOD1* and control groups (top panel). Middle and bottom panels show data for female and male flies respectively. Error bars represent one standard error.

$F_{(3,42)} = 26.68$, $p < 0.001$, but no significant effect of sex: $F_{(1,42)} = 0.135$, n.s., and no significant interaction effects. The effect of genotype on WBF appears to result largely from differences in later stages of life. *Post-hoc* t-test analysis showed no significant genotype differences in WBFs at the earliest ages tested ($t_{(10)} = 0.395$, n.s. and $t_{(17)} = 1.46$, n.s. for 5 and 10 day olds respectively), whereas a statistically significant genotype difference was observed for the oldest group ($t_{(9)} = 2.90$, $p < 0.05$).

Locomotion (walking) experiment

Figure 5 shows results of the late-life locomotor behavior experiment. The average distance that *hSOD1* and control groups had moved away from the center square in a 5-min period is shown as a function of age. This distance was measured from video recordings in both the well-lit and dark (via infrared) conditions. The left panels are data for males and the right panels for females. Black and white bars, respectively represent data collected in complete darkness and in the well-lit condition. A number of trends are evident. First, as expected, the level of locomotor activity declines with age. Second, there is generally a greater level of activity in the well-lit condition, though this advantage is less evident for the very oldest age groups. The *hSOD1* flies are clearly more active compared to controls (compare activity at age 63 d). The *hSOD1* females were the most active of all groups at all age categories. Data were not collected for the control group at ages 67 and higher because none had survived till that age.

We conducted a 2x2x2 mixed-design ANOVA on the locomotor data of one age group (63 d) at which we had data across genotype, sex, and lighting condition. Genotype and sex served as the between-subjects variables and "lighting condition" as the repeated measures variable because locomotor activity of the same flies were measured in both dark and light conditions. We found a significant effect of lighting condition: $F_{(1,52)} = 8.43$, $p < 0.01$, a significant effect of genotype: $F_{(1,52)} = 15.79$, $p < 0.001$, but no significant effect of sex: $F_{(1,52)} = 3.64$ (though this effect did approach significance at $p = 0.062$). Several interaction terms were also significant, including the interaction between genotype and sex: $F_{(1,52)} = 7.07$, $p < 0.05$), between lighting condition and sex: $F_{(1,52)} = 5.94$, $p < 0.05$, and the 3-way interaction between genotype, sex, and lighting: $F_{(1,52)} = 5.52$, $p < 0.05$.

Discussion

The experiments described here provide data on functional abilities of a transgenic line of *Drosophila melanogaster* whose lifespan has been extended by overexpression of the human gene *hSOD1* in motorneurons. We found that female *hSOD1* flies were able to sustain continuous flight longer than control females. The effect was largest during middle stages of life between 10 and 30 days of age (Figure 3). No significant difference in the ability to maintain continuous flight was observed between the male *hSOD1* and male control flies. Both genotypes (*hSOD1* and controls) generally sustained flight for shorter durations compared to wild types previously tested using similar (*e.g.*, tethered; Lehmann and Dickinson, 1997) or different experimental paradigms (*e.g.*, free-flight box experiments; Miller *et al.*, 2008). For comparison, we measured the duration of sustained tethered flight for a 4-day old female Canton-S wild-type fly using the exact same apparatus and preparation protocol employed in our study of *hSOD1* flies. Bottom panel of Figure 3 shows that this genotype maintains flight significantly longer than *hSOD1* and parental controls. The figure shows an extended uninterrupted flight

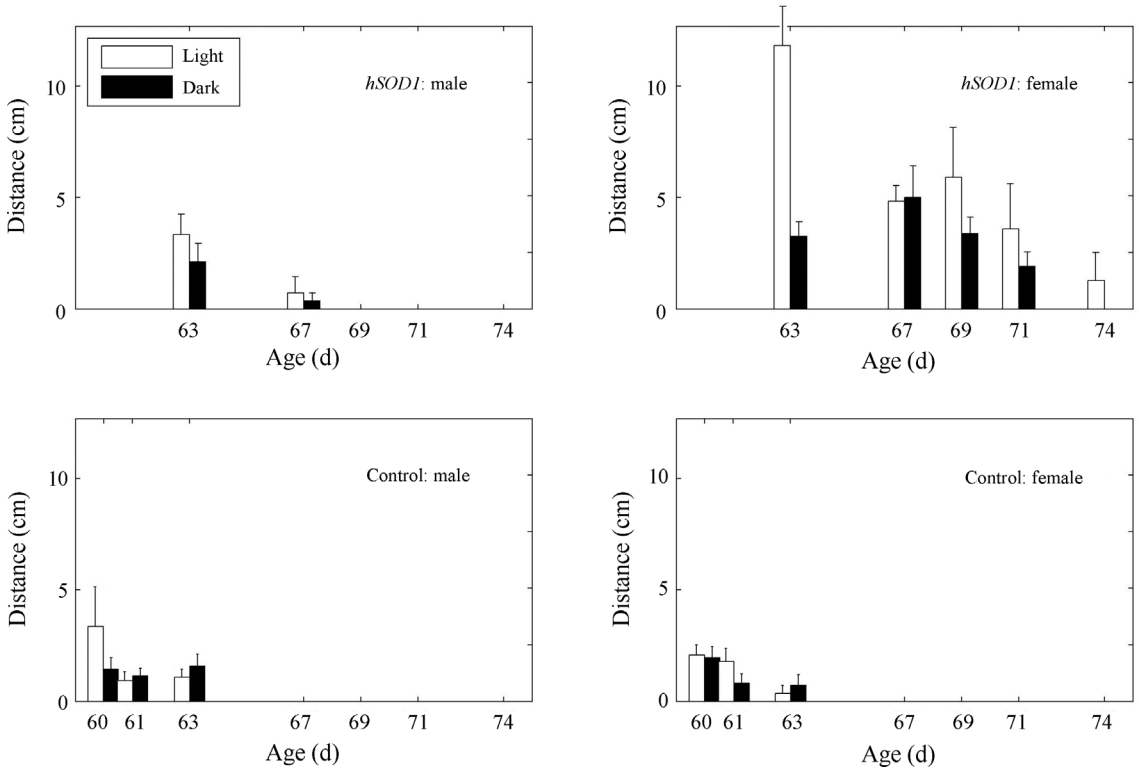

Figure 5 - Distance walked as a measure of locomotor activity for the *hSOD1* (top panels) and control groups (bottom) as a function of age. Left panels show data from males and right panels for females. Black bars represent activity measured in complete darkness, and white bars under well-lit conditions. Error bars represent one standard error.

of 5 min (approximately 50,000 wingbeats). Inset shows a 120 millisecond segment (20 wingbeats) of the 5-min waveform. The reason for this difference in flight duration ability between wild types and *hSOD1* flies is not clear and may be due to differences in motor output efficiency, levels of metabolic activity, or other genetic and physiological factors.

An additional finding was a small but statistically significant lower wingbeat frequency for *hSOD1* flies in middle and late, but not early life relative to controls. It is unlikely, however, that this lower wingbeat frequency signifies a reduced level of metabolic activity since levels of locomotor (walking) activity at old age were significantly higher for *hSOD1* than control flies. In addition, measures of respiration rate in a prior study suggest equivalent levels of oxygen consumption for *hSOD1* and control flies, even in late life (Parkes *et al.*, 1998). A lower wingbeat frequency does not necessarily signal a disadvantage in flight ability, but may potentially signify improved motor output efficiency, especially in light of the observed longer sustained flight in these same flies. Flight velocity for some species (*e.g.*, bats and birds) has been shown to be negatively correlated with wingbeat frequency for a wide range of velocities (Bullen and McKenzie, 2002; Tobalske *et al.*, 2003), supporting the idea that a slower wingbeat frequency may allow use of less energy in sustaining higher-velocity and longer distance/duration flights. While we have not measured wing-stroke amplitude to determine if total aerodynamic power (a nonlinear product of wingbeat frequency and stroke amplitude) has increased or decreased as a result of overexpressing *hSOD1*, the increase in flight duration itself suggests that at least this aspect of sustained-flight has been enhanced, likely providing an adaptive advantage for survival. Furthermore, we have also noticed that the wingbeat frequencies of some *Drosophila* genotypes that maintain flight duration considerably longer than the *hSOD1* (and control) flies are lower than the latter genotypes. For example, the average wingbeat frequency of the Canton-S fly shown in the bottom panel of Figure 3 measured in identical conditions (temperature, humidity, lighting, diet, etc.) to those used in the current study is approximately 160 Hz as compared to over 200 Hz for *hSOD1* and controls at a similar age category (4 *vs.* 5 day olds).

The observed improvements in flight duration and locomotor ability are likely due to enhanced antioxidant defense and resistance of cells, especially motorneurons, to cumulative damage caused by reactive oxygen species. These improvements may also partly be related to secondary mechanisms, for example, through *hSOD1*-triggered changes in signal transduction pathways that regulate gene expression in other cell types. Our results, however, also suggest that flight and locomotor systems are affected differently by *hSOD1* overexpression, at least in late life. We observed no differences in flight duration and a signifi-

cantly lower wingbeat frequency for *hSOD1* flies relative to controls at late life. Conversely, we observed significantly higher levels of locomotor activity in late life by *hSOD1* overexpression.

Female flies in our study showed a significant effect of *hSOD1* overexpression on flight duration, while no effect was observed for males. Locomotor abilities, however, were improved for both, though still significantly more for females. Why there may be a larger effect of *hSOD1* overexpression on functional abilities of female *Drosophila* compared to males is not clear. Female *Drosophila* of a number of genotypes have previously been shown to have more robust functional abilities, better survival rates, and more resistance to toxicity than males, likely, at least in part, due to their significantly larger bodies. Perhaps the more robust abilities of female *Drosophila* translates into more effective motor function improvements in response to *hSOD1* overexpression. There is, in addition, evidence from the mouse ALS model that female *SOD1* mice respond more effectively to drug treatment (methionine sulfoximine; MSO) than do males in mitigating the effects of motorneuron degeneration as measured by a significant improvement in maintaining muscle grip on a wire grid (Bame *et al.*, 2012).

A comparison of age-dependent changes in behavioral functions reported here to that reported for a different life-extended mutant, the *Methuselah* (Lin *et al.*, 1998), reveals markedly different patterns of functional decline. Petrosyan *et al.* (2007) have shown that the *mth* mutant has a higher wingbeat frequency relative to its parental control group throughout most of its lifespan, but especially during early and middle stages of life. This finding is opposite to that observed for the *hSOD1* line which displays a lower wingbeat frequency than its parental line throughout most of its lifespan, and equal WBF during early life. Locomotor abilities of the *mth* flies also decline in a different pattern compared to that of *hSOD1*-expressed flies. *mth* flies show a lower level of locomotor activity relative to controls in late life. *hSOD1*-expressed flies, however, show higher levels of locomotion relative to controls at old age. Interestingly, the *INDY* life-extended mutant has been reported to display even a third pattern, with no difference observed in locomotor activity (negative geotaxis) relative to control flies in late life (Martin and Grotewiel, 2006).

One should, however, be cautious in interpreting patterns of behavioral change across genotypes. Such differences may be due to the consequence of each gene's action on functional mechanisms subserving a specific behavior, or they may be due to selectivity of expression within different tissues, or due to other complex factors. In *hSOD1* flies, for example, expression is restricted primarily to motorneurons and a small number of other cell types, whereas the *mth* gene is expressed broadly, and *INDY* is primarily expressed on cell membranes of the midgut and the plasma membranes of fat body and oenocytes (Knauf *et al.*, 2002). Furthermore, the *hSOD1* gene is not expressed in all motorneurons and may itself mediate changes in signal transduction pathways that regulate patterns of gene expression in cell types other than motorneurons (Phillips *et al.*, 2000). Nonetheless, such differences in enhancement of behavioral abilities do exist across life-extended genotypes, and are both informative and significant.

We should also caution that the enhanced functions observed are a result of overexpressing the normal human *SOD1* gene, whereas familial ALS is associated with a mutation of *SOD1*. There is evidence that the most common type of ALS-related *SOD1* mutation results in a gain (not loss) of function that leads to induced motorneuron toxicity and eventual death (Bruijn *et al.*, 1998). Overexpressing the normal *hSOD1*, however, is likely to enhance antioxidant resistance to free radicals (and toxicity) whose accumulative effects may cause motorneuron dysfunction and death (Parkes *et al.*, 1998). We should also caution that extension of the current findings to therapeutic strategies for human ALS is complex and challenging, as prior attempts to enhance antioxidant defenses in humans afflicted with ALS have had mixed results due to a number of factors such as dosage, safety, and different pharmacokinetic effects across species (Lanka and Cudkowicz, 2008).

In summary, we have found that for a simple organism, expression of the human gene *hSOD1* in motorneurons enhances sensorimotor functions at different ages. These include simple motor functions such as flight duration at early and middle life, and locomotor activity in late life. A comparison of the current results to our earlier studies (Petrosyan *et al.*, 2007, 2013, 2014) suggests that different life-extending genes may yield different patterns of functional stability at various stages of life. Whether these different genetic strategies may be used in concert to broadly promote robust motor, sensory, memory, and other functions in late life, merits further investigation.

Acknowledgments

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