Drosophila as a model for age-related impairment in locomotor and other behaviors

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ABSTRACT

Aging is a multifaceted phenomenon that occurs in most species including humans and the fruit fly, *Drosophila melanogaster*. One of the most fundamental features of aging is the progressive decline in functional capacity that occurs with age (i.e. functional senescence). Age-related declines in function undermine many aspects of normal youthful physiology including behavior. Age-related behavioral declines are quite telling because they presumably reflect primary functional defects in the nervous system or musculature. Consequently, a more detailed understanding of behavioral declines that occur with age, including mechanisms that impinge on them, could ultimately lead to improved treatment or diagnosis of age-related defects in physiological processes that depend on normal function of the nervous system or musculature. Such advances in diagnosis or treatment would translate into tremendous gains in quality of life for elderly populations. In this article, we review progress using *Drosophila* to better understand age-related behavioral declines with a focus on age-related locomotor impairment.

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1. Age-related behavioral declines in humans and fruit flies

Aging is a complex process or collection of processes that leads to death. While death in some experimental settings can be a definitive endpoint of aging, age-related declines in function are essential features of aging that likely drive the increased risk of death with age. Furthermore, casual conversations with most individuals beyond the age of ~30–35 years suggest that age-related functional declines, the reduced ability to perform various tasks as we age, is how we define aging in our day to day lives. Model organisms such as *Drosophila* hold tremendous promise for identifying genetic and other mechanisms that influence age-related functional declines. Consequently, studies in the fruit fly and other genetic models can greatly facilitate the identification of interventions that forestall the most troubling features of aging.

Among the functional changes that occur during aging, age-related behavioral deficits are especially distressing. Age-related behavioral changes in humans include a progressive decline in locomotor ability, olfactory sensitivity, memory function, and circadian rhythmicity (Grotewiel et al., 2005). Furthermore, age-related behavioral limitations collectively form the most common single complaint of elderly individuals (Espeland et al., 2007). Since behavior is the result of the combined activity of the nervous system and the musculature, age-related behavioral declines are likely to be manifestations of defects in one or possibly both of these two key organ systems. Improved diagnosis and treatment of age-related behavioral declines could therefore have an enormous positive impact on the quality of life for elderly individuals.

Many of the behavioral changes associated with aging in humans are also observed in *Drosophila* (Grotewiel et al., 2005). Age-related behavioral declines in flies are likely due to dysfunction within specific organ systems as opposed to a generalized deterioration in health (Cook-Wiens and Grotewiel, 2002). Additionally, gene function is generally well conserved in flies and humans (Adams et al., 2000) and numerous assays have been developed to quantitate several different behaviors across age in flies (Grotewiel et al., 2005). These and other features such as a powerful set of genetic tools make flies an excellent model system for investigating the molecular–genetic basis for age-related behavioral declines.

2. *Drosophila* as a model for age-related locomotor impairment (ARLI)

One of the most serious age-related behavioral changes in humans is the progressive decline in locomotor ability including the ability to walk. Good mobility is crucial for maintaining quality of life because daily activities and therefore independence rely on intact locomotor skills (Pahor et al., 2006). Decreased mobility is one of the most common complaints in elderly individuals (Espeland et al., 2007), highlighting the importance of maintaining locomotor ability into late life.

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Age-related locomotor impairment (ARLI) has many negative consequences that transcend effects on mobility alone. An estimated 20% of elderly people living independently have trouble walking, need someone to help them walk, or require the aid of a walking device (Ostchega et al., 2000). ARLI leads to an increase in falls, hospitalizations, and future requirements for a caregiver (Montero-Odasso et al., 2005). An increased fear of falling also leads to further decreases in mobility (Chamberlin et al., 2005). Individuals with locomotor impairment are also at greater risk for depression (Braam et al., 2005) osteoporosis, arthritis, congestive heart failure, muscle pain, stroke, dementia (Duxbury, 2000) and death (Hardy et al., 2007). Furthermore, walking speed may represent a general measure of overall health and can be an indication of risk for future disabilities (Onder et al., 2005). ARLI is therefore a very serious issue with far reaching health consequences.

2.1. ARLI in flies

Flies exhibit several forms of locomotor behavior including negative geotaxis, flying and spontaneous walking. Each of these forms of locomotor behavior can be assessed in the laboratory and, importantly, the robustness of each behavior declines with age. Although all of these behaviors can be used as measures of locomotion across age in *Drosophila*, here we focus on the use of negative geotaxis as an index of locomotor behavior because it has been used most often in mechanistic studies.

Negative geotaxis is startle-induced climbing. In negative geotaxis studies, single flies (Arking and Wells, 1990) or groups of flies (Gargano et al., 2005) are placed in a cylinder, allowed a period of recovery, and then banged to the bottom of the cylinder. This stimulation elicits an escape response (ascending the walls of the cylinder). While young flies have robust negative geotaxis, flies become progressively worse at this task as they age (Arking and Wells, 1990; Gargano et al., 2005).

Negative geotaxis behavior is typically quantitated in one of three ways. In our studies using the rapid iterative negative geotaxis (RING) assay, we measure the distance climbed by flies during a test of defined duration (typically 4 s) (Gargano et al., 2005). Other protocols measure the percentage of flies that meet a performance criterion (the percentage of flies that climb to a defined height during a test of defined duration) (Leffelaar and Grigliatti, 1984) or the time required to climb to a prescribed height (Arking and Wells, 1990). Despite differences in experimental details, all of these approaches show that negative geotaxis becomes progressively impaired with age.

2.2. Mechanisms and manipulations that alter ARLI

Studies using *Drosophila* have identified several mechanisms associated with ARLI (Table 1). Oxidative stress is one potential mechanism that has been directly or indirectly investigated using a variety of approaches. Fly strains selected for late life reproduction have increased resistance to exogenous oxidative stress in conjunction with delayed ARLI (Arking and Wells, 1990). Additionally, overexpression of protein carboxyl methyltransferase (PCMT) or methionine sulfoxide reductase A (MSRA), two enzymes that repair protein oxidative damage, reportedly enhances two simple forms of locomotor behavior in *Drosophila* (Chavous et al., 2001; Ruan et al., 2002). Flies treated with the histone deacetylase inhibitor 4-phenylbutyrate (PBA) are resistant to oxidative stress and exhibit delayed senescence of negative geotaxis (Kang et al., 2002). Furthermore, flies with decreased expression of the major antioxidants Sod1 or Sod2 have accelerated ARLI (Martin et al., 2009a,b). All of these studies are consistent with the hypothesis that oxidative stress or damage is a driving force for ARLI in flies. Interestingly, oxidative damage might also be involved in ARLI in rodents (Lebovitz et al., 1996; Muller et al., 2006) and possibly humans (Nikolic et al.,

Table 1
Manipulations that alter ARLI and/or life span in *Drosophila*. Genetic, pharmacological or environmental manipulations (column 1), the likely or possible mechanism involved (column 2), the effects on ARLI (column 3) and life span (column 4) are shown. GOF, gain of function. LOF, loss of function. Upward and downward arrows indicate increases and decreases, respectively. A single asterisk (*) indicates that conditional overexpression of Sod2 during adult extends life span in an independent study (Sun et al., 2002). A double asterisk (**) indicates 5–10% increases in life span observed in preliminary studies (Jones and Grotewiel, unpublished observations). See main text for citations.

Manipulation	Class	Likely or possible mechanism	Effect on ARLI	Effect on life span
Selection	Positive	↑ ox stress resistance	Delayed	Extended
PCMT	GOF	↑ ox stress resistance	Delayed	Extended
MRSA	GOF	↑ ox stress resistance	Delayed	Extended
PBA	drug	↑ ox stress resistance	Delayed	Extended
HSP22	GOF	↑ ox stress resistance	Delayed	Extended
Sod1	LOF	↓ ox stress resistance	Accelerated	Decreased
Sod2	LOF	↓ ox stress resistance	Accelerated	Decreased
methuselah	LOF	↑ ox stress resistance	No change	Extended
Sod1	GOF	↑ ox stress resistance	No change	Extended
Sod2	GOF	↑ ox stress resistance	No change	No change*
chico	LOF	↓ insulin signaling	Delayed	Extended
PI3K	LOF	↓ insulin signaling	Delayed	Extended**
PDK1	LOF	↓ insulin signaling	Delayed	Extended**
Akt	LOF	↓ insulin signaling	Delayed	Extended**
myospheroid	LOF	↓ integrin expression	Delayed	Extended
OR83b	LOF	↓ nutrient sensing	Delayed	Extended
ecdysone receptor	LOF	Gene expression	Delayed	Extended
Indy	LOF	↓ Metabolite uptake	Delayed	Extended
Exercise training	n/a	Exercise training	Delayed	Not reported
Dietary restriction	n/a	Dietary restriction	No change	Extended

2005), suggesting that conserved mechanisms likely drive ARLI in *Drosophila* and mammals.

The connection between oxidative stress and ARLI in flies is not absolute, however. Flies harboring a loss of function mutation in the *methuselah* gene have greatly increased resistance to exogenous oxidative stress, but ARLI is unabated in these animals (Cook-Wiens and Grotewiel, 2002). Overexpression of the major antioxidants Sod1 or Sod2 does not appear to have positive effects on ARLI (Martin et al., 2009a,b) and not all mutants with delayed ARLI have enhanced resistance to exogenous oxidative stress (Goddeeris et al., 2003; Jones et al., 2009). Thus, although oxidative stress might be an important mechanism influencing ARLI in *Drosophila*, other mechanisms are also likely involved.

Beyond oxidative stress, the best defined mechanism that influences ARLI in flies is insulin/insulin-like signaling (IIS). Blunted IIS is a well known manipulation that extends life span in *C. elegans*, flies, mice (Tatar et al., 2003) and possibly humans (Flachsbart et al., 2009; Suh et al., 2008; Willcox et al., 2008). Flies with a loss of function mutation in *chico*, the gene that encodes a key substrate for the insulin receptor, exhibit delayed ARLI (Gargano et al., 2005). Similarly, forward and reverse genetic approaches showed that ARLI is delayed in flies with partial loss of function mutations in *Dp110*, *PDK1* or *Akt*, genes that encode three core components of the IIS pathway (Jones et al., 2009). *C. elegans daf-2* (insulin receptor) mutants (Collins et al., 2008; Wolkow, 2006) and *insulin receptor substrate 1* knock-out mice (Selman et al., 2008) also have delayed ARLI , suggesting that IIS might be a conserved mechanism that influences locomotor senescence in animals. Given that polymorphisms in IIS genes are associated with longevity in humans (Flachsbart et al., 2009; Suh et al., 2008; Willcox et al., 2008), it would be interesting to determine whether these same polymorphisms are associated with delayed ARLI. Additionally, it would be interesting to determine whether blunted IIS delays ARLI and extends life span via the same downstream mechanisms. Presumably delayed ARLI and extension of life span

occur via changes in IIS-regulated gene expression (Guarente and Kenyon, 2000), but this has not been systematically addressed.

Studies in flies have implicated additional processes in ARLI. Flies treated with PBA (the histone deacetylase inhibitor 4-phenylbuty-rate) have delayed ARLI (Kang et al., 2002). The preservation of locomotor behavior in these flies could be due to enhanced resistance to oxidative stress, altered gene expression via inhibition of histone deacetylase, or both. Partial loss of function mutations in the β integrin gene *myospheroid* delays ARLI (Goddeeris et al., 2003). While the downstream mechanism that drives the delayed ARLI in *myospheroid* mutants has not been identified, altered oxidative stress resistance does not appear to be important in this context. Flies with a mutation in the olfactory receptor gene *OR83b* exhibit a blunted age-related decline of locomotor behavior (Rhodenizer et al., 2008), suggesting that olfactory cues, possibly odorants that signal the presence of nutrients (Libert et al., 2007), influence ARLI. Finally, flies with partial loss of function in *Indy* have delayed ARLI, at least during the first few weeks of life (Gargano et al., 2005), and flies with mutations in the *ecdysone receptor* gene have delayed ARLI (Simon et al., 2006). Identifying the underlying mechanisms associated with the effects of these manipulations would lead to major advances in our understanding of locomotor senescence.

Beyond standard genetic and pharmacological manipulations, recent work indicates that *Drosophila* is an intriguing model for investigating the effects of exercise on ARLI. Exercise training in humans correlates with a reduced incidence of age-related diseases and slower declines in mobility and cardiovascular function (Ascensao et al., 2007; Saraceni and Broderick, 2007). Wessels and co-workers extended this concept by developing a behavioral paradigm for exercising flies called the Power Tower (Piazza et al., 2009). In this paradigm, flies are stimulated to perform negative geotaxis several times each day on a progressive schedule that mimics ramped training for human athletes. Flies trained for several weeks in the Power Tower exhibit significantly preserved negative geotaxis behavior compared to unexercised flies. Thus, exercise training in flies delays ARLI as it does in humans. The Power Tower is an exciting tool that should allow Wessels and colleagues to investigate molecular–genetic mechanisms associated with the positive effects of exercise on ARLI.

2.3. Connections between ARLI and longevity in flies

There is an extensive literature on the regulation of life span in *Drosophila* and, as summarized in the previous sections, there is also an emerging literature on the regulation of ARLI in flies. Interestingly, several studies suggest that there might be mechanistic connections between ARLI and life span. In most studies that assessed locomotor function across age in addition to longevity, flies with extended life span have delayed ARLI. This includes studies on long-lived flies selected for late life reproduction, flies overexpressing HSP22, PCMT and MSRA and flies with mutations in the *myospheroid*, *chico*, *Indy*, *ecdysone receptor*, *OR83b*, *Dp110*, *PDK1* and *Akt* genes (Table 1). Additionally, flies aged at high temperature have shortened life span and accelerated ARLI, while flies aged at low temperature exhibit the opposite changes. Furthermore, treatment with PBA also extends life span and delays ARLI (Table 1). It seems that most manipulations that extend life span also delay ARLI in *Drosophila*.

There are two notable exceptions, however, to the putative connection between life span and ARLI in flies. First, long-lived *methuselah* mutants have extended lifespan (Lin et al., 1998), but ARLI occurs at a normal rate in these animals (Cook-Wiens and Grotewiel, 2002). Second, and more surprisingly, we found that while dietary restriction (achieved via food dilution) extends life span in several different strains as expected, food dilution has no effect on ARLI in these animals (Bhandari et al., 2007). The net effect appears to be that *methuselah* mutant and dietary restricted flies are long lived, but have a greater period of locomotor impairment than do control animals.

There are several implications from the studies on ARLI and life span in flies. First, ARLI and life span appear to be regulated by overlapping mechanisms, but these mechanisms are not identical. Second, while ARLI may contribute to life span determination, other age-related functional changes must also influence life span. Third, it is possible to extend life span without positively impacting all aspects of aging in flies. This final point is particularly important since it suggests that studying several specific functions across age in addition to life span might be a promising approach for identifying interventions that have more global, positive effects on aging.

2.4. Challenges in assessing ARLI

A major challenge in all studies that assess ARLI is to use well defined measures of locomotion. Toward clarifying the use of negative geotaxis as an index of locomotor behavior across age, we used the RING (rapid iterative negative geotaxis) assay to address two inter-related questions. First, we asked whether the ability of the fly to ascend the cylinder wall is truly a climbing behavior or whether it is the result of climbing, jumping and flying combined. Second, we asked whether the age-related decline in negative geotaxis was due to an age-dependent decrease in the speed of locomotion, an age-dependent increase in the latency to initiate locomotion (i.e. time required to start moving), or both. Our studies show that jumping and flying do not contribute significantly to performance of negative geotaxis and that the vast majority of flies ascend the cylinder walls in negative geotaxis assays via legged climbing (Rhodenizer et al., 2008). Additionally, our studies show that while climbing speed decreases and climbing latency increases with age, the decreased speed of climbing is the major determinant of the age-related blunting of negative geotaxis behavior (Rhodenizer et al., 2008). Thus, negative geotaxis is a legged locomotor behavior that declines with age due to an age-dependent decrease in climbing speed. Since one of the behavioral hallmarks of human ARLI is a decrease in walking speed (Espeland et al., 2007), our studies highlight an important parallel between locomotor senescence in fruit flies and man.

Another challenge for studies on ARLI is to distinguish between functional declines that are due to physiological age (strictly dependent on aging) versus those that are due to chronological age (strictly dependent on time). In poikilotherms such as insects, declines that are accelerated or forestalled in animals aged at higher or lower temperature, respectively, are thought to be related to physiological age (Arking, 1998; Helfand and Rogina, 2000). Data from our laboratory show that the age-related decline in negative geotaxis is faster in flies aged at 29 °C and slower in flies aged at 18 °C compared to flies aged at the standard condition of 25 °C (Grotewiel et al., 2005). The age-dependent decline in negative geotaxis, therefore, likely reflects the physiological age of flies.

A further challenge for studies on ARLI is to identify the tissues that become dysfunctional and thereby drive the age-related decline in locomotion. Although in practice this could be very difficult to comprehensively address, it should be possible to identify tissues in which individual genes influence ARLI. We have begun to address this by first identifying neurons that are required for normal negative geotaxis in young animals. Using tissue-specific expression of Shibire[ts], a temperature-sensitive dominant-negative dynamin that blocks neurotransmission, we find that motor neurons, giant fiber neurons (involved in escape behavior), and possibly other neurons within the central brain are important for negative geotaxis (Martin et al., 2009b). Whether genetic manipulations in these or other groups of neurons influence senescence of negative geotaxis is an important question that needs to be addressed. Furthermore, whether genetic manipulations in other organ systems such as the musculature impact senescence of negative geotaxis is a key, open question.

3. *Drosophila* as a model for other age-related behavioral changes

Fruit flies exhibit age-related changes in several behaviors beyond locomotion. Consequently, the *Drosophila* model has been used to probe mechanisms that influence age-related changes in these other behaviors. We briefly summarize below advances in using flies to understand age-related defects in memory and sleep-like behavior. Age-related changes in additional behaviors have been reviewed in detail (Grotewiel et al., 2005).

3.1. Age-related changes in memory performance

Drosophila has a long history as a genetic and neurobiological model for investigating learning and memory. Pioneering studies have extended the use of flies as a model for understanding age-related memory impairment (AMI), a significant cognitive issue for many elderly individuals (Rosenzweig and Barnes, 2003). Olfactory memory is the leading paradigm for assessing associative memory in *Drosophila*. In this paradigm, flies exposed to an odor along with a negative reinforcer (electric shock) will subsequently avoid that shock-paired odor in a T-maze (Connolly and Tully, 1998). While young flies perform well in this paradigm, a reduction in their performance is detectable as early as 10 days of age and their performance continues to decline with age thereafter. This reduced performance in this paradigm as flies age has been termed age-related memory impairment (AMI) (Tamura et al., 2003). Interestingly, AMI in this paradigm might be blunted by mutations in *amnesiac* (Tamura et al., 2003) and *DCO* (Yamazaki et al., 2007), genes thought to encode cAMP-stimulating peptides and the catalytic subunit of protein kinase A, respectively. These studies, the first of their kind in *Drosophila*, implicate cAMP signaling in AMI.

While interesting, some caution regarding interpretation of these studies on AMI is warranted. It is currently unclear whether the apparent delay in AMI in *amnesiac* and *DCO* mutants is due to a change in senescence of memory per se or whether it is due to changes in senescence of some other process required for adequate performance in the olfactory memory assay. For example, flies exhibit pronounced age-related changes in olfactory acuity and it remains possible that *amnesiac* and *DCO* mutants exhibit forestalled AMI as a consequence of delayed senescence of olfaction (Grotewiel et al., 2005). Additionally, while olfactory memory does not change substantially with age in *amnesiac* mutants, memory at a young age in these flies is significantly impaired relative to that of control flies (Tamura et al., 2003). This raises the possibility that the *amnesiac* gene is more important for olfactory memory in young animals than it is for maintaining olfactory memory function across age. While additional studies are required to clarify these issues, the studies on *amnesiac* and *DCO* mutants offer the intriguing possibility that manipulation of cAMP signaling could be a therapeutic avenue for treating AMI or an associated functional change within the nervous system.

Another group recently investigated whether dietary restriction influences age-related changes in olfactory memory. The major finding from this study was that dietary restriction does not alter the age-related decline in memory performance in flies, although as expected it does substantially extend life span (Burger et al., 2010). Additionally, although it was not a focus of this study, dietary restriction delays the age-related decline in olfactory behavior (Burger et al., 2010), consistent with a minor trend in studies from our laboratory (Bhandari et al., 2007). This study indicates that dietary restriction extends life span and might forestall age-related olfactory decline, but does not protect flies from AMI. These findings reinforce previous studies indicating that dietary restriction in *Drosophila* does not positively impact all aspects of aging equally (Bhandari et al., 2007; Burger et al., 2007). The limited effects of dietary restriction on aging in *Drosophila* are in stark contrast to the rather broad positive effects dietary restriction has on aging in rodents (Ingram et al., 1987)

and presumably other mammals. Whether fruit flies truly respond atypically to dietary restriction compared to other animals, whether alternative protocols for dietary restriction in flies would delay age-related functional declines, or whether other age-related functional declines in flies are delayed by dietary restriction are important issues to be resolved.

3.2. Senescence of sleep-like behavior

The fruit fly is emerging as a powerful genetic model to investigate mechanisms that influence sleep. Vertebrate sleep is characterized by reduced responsiveness, increased periods of quiescence after sleep deprivation, and predictable responses to stimulants and hypnotics. Intriguingly, sleep-like behavior in flies exhibits these same hallmarks (Shaw et al., 2000). Furthermore, two well known effects of age on sleep in mammals, decreased sleep duration and increased sleep fragmentation, have been reported to occur in flies with age (Koh et al., 2006; Shaw et al., 2000). Although these age effects are not found in all studies (Bushey et al., 2010), the decreased sleep duration and increased sleep fragmentation in flies suggest that *Drosophila* is a suitable model for senescence of sleep. Understanding the molecular basis of sleep senescence is important because disturbed sleep is associated with several morbidities (Bombois et al., 2010).

The age-related fragmentation of sleep is forestalled in flies aged at lower temperatures (Koh et al., 2006). This temperature-dependence is important because it indicates that sleep fragmentation occurs as a function of physiological age and not chronological age. Interestingly, cAMP signaling might play a role in senescence of sleep in flies. Loss of function mutations in *amnesiac* causes several changes in fly sleep including a blunting of the effect of age on sleep fragmentation (Liu et al., 2008). One note of caution, however, is that mutations in *amnesiac* cause a complex set of changes in fly sleep and it is currently not clear whether the decreased effect of age on sleep in *amnesiac* mutants is secondary to some other change in sleep pattern. Nevertheless, manipulation of cAMP signaling might ultimately be a therapeutic option for treating senescence of sleep as it might be for AMI.

4. Perspectives and major opportunities for further progress

The studies reviewed here highlight the significance of ARLI and other age-related behavioral changes as major consequences of aging. Since behavior principally relies on the coordinated effort of the nervous system and the musculature, investigating age-related behavioral defects will provide fundamental information regarding how the function of these two key organ systems changes with age. The *Drosophila* model has a well developed set of genetic tools and a long track record of providing meaningful molecular information related to behavior and life span. All indications are that the fruit fly model is well positioned to be a major player for investigating mechanisms that influence age-related behavioral declines. Such studies will greatly enhance our understanding of aging, how it occurs, and potentially how to mitigate its effects on critical organ systems.

There are several intriguing opportunities for using the *Drosophila* model to make significant progress toward understanding age-related behavioral declines. For example, flies can be used to develop a comprehensive understanding of the molecular mechanisms that drive various age-related behavioral declines and further to determine whether different age-related behavioral declines are influenced mainly by common or distinct mechanisms. The *Drosophila* model can also be used to determine whether declines in behavior across age are due to age-related functional defects in the nervous system, the musculature, or other tissues. Identification of these mechanisms and tissues will provide a rational framework for investigating whether conserved mechanisms influence multiple aspects of behavioral aging

in mammals. Such studies have the potential to lead to the development of therapeutic options for treating humans affected with age-related behavioral defects.

Flies can also be used to further investigate whether age-related behavioral changes help determine life span and, reciprocally, whether changes in life span typically have similar effects on age-related behavioral changes. We and others have begun to address this issue. In most cases, manipulations that extend life span also delay locomotor aging, but extension of life span can occur without a parallel change in ARLI. Additional studies to investigate mechanisms that influence both life span and ARLI as well as other age-related behavioral declines are critically needed to further delineate the relationship between life span and functionally relevant aspects of aging.

Throughout this review we discuss modeling age-related impairment in locomotor and other behaviors in flies toward identifying mechanisms that influence specific functional consequences of aging. While studying each of these age-related behavioral changes alone has intrinsic merit, evaluating a combination of behaviors or other functions across age in an integrated approach could lead to a much more holistic understanding of how genetic, pharmacological or environmental interventions influence aging. The rationale for this approach is that not all (and possibly few) interventions have uniform or global positive effects on aging. For example, flies selected for late life fecundity have increased life span and delayed senescence of negative geotaxis (Arking and Wells, 1990), but these flies also exhibit impaired olfactory memory at a young age (Burger et al., 2008). Conversely, replicate fly lines selected for delayed age-related memory impairment have decreased life spans (Burger et al., 2008). Furthermore, genetic ablation of median neurosecretory cells (that produce *Drosophila* insulin-like peptides) extends life span, but this manipulation also decreases total spontaneous locomotor activity and sleep-like behavior in a food-dependent manner (Broughton et al., 2010). These and other similar findings (c.f. discussion of dietary restriction above) illustrate several important issues. They suggest that no single behavior is a satisfactory index for all behaviors and that divergent mechanisms could drive age-related declines in different behaviors. Additionally, these studies suggest that there could be important trade-offs between life span and age-related behavioral declines and furthermore that there could even be trade-offs between preserving one behavior while impairing another. Finally, these studies indicate that interventions can have both positive and negative consequences on function in young animals as well as age-related behavioral declines and life span. Given these issues, which are probably intrinsic to aging itself, it seems prudent to assess the effects of anti-aging interventions on life span, several different behavioral outputs and other functions in parallel as a means to comprehensively gauge their effects on health span. We recognize that this approach would likely require the coordinated efforts of groups of laboratories working in concert. Although coordinating across multiple laboratories can have its own challenges, such an approach would more fully exploit *Drosophila* as an integrative model for aging and lead to a greater understanding of how aging can be manipulated.

Acknowledgements

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