Circadian Rhythms, Light, and Social Perception: Sensory Regulation of Lifespan in *Drosophila*

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Molecular and Integrative Physiology)
in the University of Michigan
2021

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Dedication

I would like to dedicate this dissertation to Ted Crossman.

Acknowledgements

The undertaking that is finishing a PhD would not have been have been possible without the help, support, guidance, and mentorship from many friends, family, and colleagues. First and foremost, I want to thank my mentor, Scott Pletcher. Scott's fastidious scientific nature has inspired me since my first day in his lab. When I joined, I was told to take a whole week to read and come up with the best, and most well controlled experiments before doing any work. This level of insight and rational thinking were always present in our meetings. I appreciate your willingness to help me chase moonshot questions, and then to help me figure things out when they failed. I can only strive to see the larger picture as you do. Thank you also for your flexibility and understanding of work life balance. I would also like to thank my past scientific mentors and good friends Chris Barney and Leah Chase for encouraging my development as an independent scientist. Thanks to the current and past members of my dissertation committee; though the committee has changed over the years I was lucky to receive valuable scientific and career advice from all members: Daniel Michele, Carol Elias, Matthias Truttmann, Swathi Yadlapalli, Orie Schafer, and Ao-Lin Hsu.

I also have much gratitude for the many members of the Pletcher Lab who have helped me over the years. First, I want to thank Zach Harvanek, for it was his mentorship and encouragement that drew me to join the lab. Special thanks go to Brian Chung, who was responsible for teaching me numerous molecular biology techniques, the history of circadian research, and the intricacies of *Drosophila* genetics. Brian was the go-to man for science and culture. With Brian and Zach around there was always a rousing debate to participate in. Jenny Ro, you really deserve a whole section to

yourself. I’m ever grateful we biked to the Dexter Cider Mill together. You were an excellent scientific mentor but more importantly I was fortunate to grow and learn much about life with you. Allyson Munneke, Haley Richardson, and Marta Plumhoff, thanks for being friends and for many pie nights. Haley faithfully counted thousands of dead flies in complete darkness with hardly a complaint and always the proper amount of sass. David Paris, thanks for keeping everything running smoothly and helping create numerous behavioral devices. Christi Gendron, you deserve much credit for organizing everything from social events to lab meetings, but perhaps most importantly for helping us spot Scott even in the absence of a proper application. I also want to thank the many Pletcher lab members who provided input on experimental design, presentations, and just made time in lab more pleasurable: Kristy Holme, Yang Lyu, Tuhin Subhra Chakraborty, Evie Henry, Lindy Jensen, Yuan Luo, Tatyana Fedina, Kevin Yi, and Jung-Eun Lee. It has been a pleasure to call you colleagues.

Many thanks to the people of the Molecular Integrative Physiology (MIP) department as a whole. I was fortunate to have two exceptional graduate chairs, Sue Moenter and Dan Michele, who were committed to both the scientific success and personal happiness of the graduate students. Thank you to Michele Boggs who was responsible for most behind the scene goings on, from reserving rooms, to making sure all forms are submitted correctly. This department felt like a second family.

I want to express my appreciation for the friends I made through the Program in Biomedical Sciences (PiBS). Allison Ho Kowalsky, I was fortunate to have you just a few bays down as my giggly lab buddy and ever supportive friend. Your made many crises melt away and the daily tedium infinitely more fun. Andrew Schwartz, you are an excellent role model and friend. Thank you also to Owen Funk, Bree Doering, Paloma Garcia and Daniel Kowalsky for many gourmet dinners, outlandish movie nights, and easy travel. You’ve all been there for me over the last six years; I look forward too many more years of friendship.

I've been fortunate to have many housemates turned close friends during my time in Ann Arbor. Coulter Stone, thank you for all the wacky adventures and turning me on to the joys to be had in the mountains. Greg Tucker, and Mark Painter, I cherished the time we had together. Boone Marois, thanks for being good to both me and my family. Tim Everhart and Mr. Andrew Bernard, thank you for being constants in my graduate school journey. You helped me maintain sanity over many GP mugs, rousing conversations and too many puzzles. I'm glad to have you as friends.

I also need to recognize my college friends, Joel Brogan and Parker Bussies. I'm thankful we were able to stay close throughout our doctoral degrees despite being spread across the country. Thanks also to many climbing and acro yoga friends who kept me fit and slightly limber: Alex Linsalata, Alex Rand, Sam Bear, Sara Wong, Gus Schissler, Sydney Forester, Yining Zhang, Tejas Navaratna. Christi Courtney, Ben Juliar, Jessie Lipkowitz, Lucia Li, and of course, Dale Rocks. Carl and Tea Egelke, thank you for keeping me sane through climbing and conversation during quarantine. Nick Wilkinson, thanks for numerous motorcycle rides, ice cream cones, and help with many house projects. Dana Felker, you have been instrumental in helping me during the last chapter of my PhD. I will always look fondly on our late-night colloquies in The Red. I'm impressed by your unique ability to make people feel appreciated, and positive try-hard attitude. Cheers all.

I've been extraordinarily lucky to have an extended family that not only gets along, but genuinely enjoys spending time together. Thank you to the Johnson family for all your love, support, and many shenanigans: Grandpa, Grandma, Kris, Dave, Mike, Shar, Harper, Matt, Amy, Kristen, Charlie, and Paul, thank you all. Thanks to the Crossman family: Poppa, Grosi, Pete, Chrissy, Mel, Emily, and Mark, your love and kindness inspire me. I have tremendous gratitude to Poppa (Theodore Crossman) for sparking a love for the natural world in me when I was young.

Finally, I want to thank my family. Thanks to my brother, Benjamin Johnson, who has been supportive and encouraging all throughout my life. I'm lucky to have you. Lastly want to thank my parents Mark Johnson and Heidi Crossman-Johnson. I would not be where I am today without your unconditional love and support. Thanks for listening me to talk about my science, and all aspects of life. You are my greatest advocates and bring me much joy. I love you all.

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Abstract

Sensory perception modulates aging across taxa in response to important ecological cues, such as food, sex, and danger. The biological mechanisms underlying these effects and the range of sensory cues involved are largely unknown. Two important ecological cues whose effects on aging have yet to be carefully explored are light and social environment. I sought to determine whether light and social perception modulates physiology, behavior, and lifespan in *Drosophila*.

First, I discovered flies lived significantly longer in constant darkness. I found this extended lifespan was not accompanied by behavioral changes that might indirectly slow aging such as activity, feeding, or fecundity, nor were circadian rhythms necessary for the effect. The lifespans of flies lacking eyes and photoreceptor neurons were unaffected by environmental light, and transgenic activation of these same neurons was sufficient to phenocopy the effects of environmental light on lifespan when flies were reared in darkness. The relationship between light and lifespan was not correlated with its intensity or duration or the frequency of light-dark transitions, and high-intensity light, particularly of shorter wavelengths, reduced lifespan in eyeless flies, indicating that the effects we observed are largely independent of known non-specific damaging effects of bright light. My results suggest that, much like other sensory systems, light perception through visual photoreceptors deserves attention as a longevity intervention. Future studies may be directed toward determining whether this is due to adverse systemic effects of light on visual neurons or to the activation of signaling pathways that directly modulate aging.

Second, I show that social perception is capable of modulating both behavior and starvation resistance. I begin by demonstrating social perception can be targeted for longevity intervention in an established context, that of pheromone exposure. Under the context of perceiving the opposite sex without an opportunity to mate, corazonin neurons are both necessary and sufficient for the mating dependent lifespan rescue. I then document how density and social perception leads to behavioral and physiological consequences. Isolated flies displayed behavioral markers of a depressive-like state, and these behaviors could be rescued by providing the olfactory sensory environment from a group of flies. Further, I showed there is also a physiological impact of isolation, in that starvation survival is reduced. The reduction in starvation resistance can also be rescued through olfactory, but not visual cues of nearby conspecifics. In addition, I devised a simple and high-throughput assay by which survival can be measured autonomously through activity recordings.

Chapter 1

Introduction

Environmental Sensing and Light

Organisms from archea, plants and fungi to humans live in an ever-changing world, and the ability to monitor the environment and adapt is key to employ optimal survival strategies (Renard, Vacelet et al. 2009). As such, most all organisms, even the most primitive, have evolved sense organs to monitor and relay information about the external environment in order to generate a biological response. More complex organisms have evolved processing centers where multisensory information can be integrated to produce a further curated biological response. Often these biological responses result in changes in physiology to optimize survival. In fact sensory information alone can be sufficient to modulate lifespan as discovered by Apfeld and Kenyon who showed ablation of the *Caenorhabditis elegans* sensory cilia neurons extended lifespan (Apfeld and Kenyon 1999). This concept that sensory information influences organismal aging serves as the focus of my thesis work.

The field of sensory biology has boomed since the Kenyon lab's landmark discovery that sensory manipulation influences lifespan (Apfeld and Kenyon 1999). Our laboratory was the first to show the phenomena works across species when it demonstrated olfactory food cues modulate *Drosophila melanogaster* lifespan in a sensory dependent manner (Libert, Zwiener et al. 2007). This is important as it indicates the mechanisms are likely conserved, and work done in this field may be translatable to higher organisms. Indeed, while most work has been performed with invertebrate

model systems, mammalian lifespan is also capable of being modulated by environmental sensing. Mice deficient in the TRPV1 pain receptor are long lived and metabolically resemble younger animals at an old age (Riera, Huising et al. 2014). Later work showed even long held lifespan manipulations such as dietary restriction and temperature have a sensory component (Libert and Pletcher 2007, Lee and Kenyon 2009). There is no evidence one unified mechanism exists by which sensory environment is integrated to control lifespan. So, work has instead focused on identifying which sensory modalities can be targeted, and the manner in which they can be manipulated to control health and lifespan. Thus far many sensory modalities have been implicated including: smell, taste, water sensation, and nutrient perception, and these correspond to ecological cues indicating food, mates, danger, all of which are crucial for optimizing organismal fitness (Poon, Kuo et al. 2010, Gendron, Kuo et al. 2014, Ro, Pak et al. 2016, Harvanek, Lyu et al. 2017, Chakraborty, Gendron et al. 2019). Light is an environmental cue that may be used to sense most ecological cues and thus it deserves exploration in the context of sensory cues capable of modulating aging.

Of all sensory inputs organisms receive one of the most important is light. Light detection has been utilized for millions of years, and is thought to have originated during the Cambrian explosion, when the *Planerian* flatworm evolved light sensitive discs, termed ocelli (Strother 2017). Despite being energetically costly, most all organisms rely heavily on vision (McArthur, Lewy et al. 1996). Light information is used in a multitude of ways, from standard vision used to navigate, forage, and detect predators, to more intricate uses of light, such as navigation based on light polarization (Krapp 2007). Moreover, light is also the main input into the circadian timekeeping system, which coordinates many physiological responses (Yoshii, Hermann-Luibl et al. 2015, Yoshii, Hermann-Luibl et al. 2016). Although light has such an important role in

organismal survival, there are few studies investigating how light and visual information influences longevity.

Light has impact on a broad range of physiological processes and behaviors. Due to its biological importance organisms have evolved numerous ways of perceiving light. The fruit fly (*Drosophila melanogaster*) has four main pathways by which light is detected (starting from the most complex): eyes, ocelli, Hofbauer-Buchner eyelets, and lastly through the nonvisual photoreceptor Cryptochrome and Rh7. Eyes are obvious and well known as the main organ of the visual system used for just that, vision. They utilize a layer of photosensitive neurons, retina, that can sense both still objects and motion, and the electrochemical information from the retina is used to create an image of the environment. In addition retinal information contributes to the entrainment of the circadian clock (Helfrich-Forster 2020). Ocelli, or eyespots, are photosensitive discs on insects' heads that provide no image forming information. *Drosophila* have three of these eyespots located on top of their head between the eyes. Ocelli are used to both measure overall light levels and determine the optimal sensitivity or gain for the compound eye, in addition to having input on stabilization through input into the motor system (Taylor, G.M., and Krapp, H.G. 2007)(Krapp, H.G. and Wicklein, M. 2008). Hofbauer-Buchner eyelets are a small four neuron structure located directly behind the retina. Much like the ocelli they convey no visual information, rather they provide direct input to circadian clock neurons (Li, Cao et al. 2018). Last, the least complex light detection method utilized by flies is done by the non-retinal photopigments Cryptochrome and Rh7 which serve as light sensors directly in circadian clock neurons, and other cells across the body (Stanewsky, Kaneko et al. 1998, Ni, Baik et al. 2017).

There are several mechanisms by which visible light perception could influence longevity. First light may interact with the circadian timing system which has numerous outputs on physiological state. Second, light may induce oxidative, photochemical damage, or act through perceptual systems as has been demonstrated in other sensory modalities. Lastly, light may cause stress based upon the visual information conveyed, be it sightings of a predator or social environmental cues. In this thesis, I will explore those three pathways by which light and visual perception may modulate longevity using the model organism *Drosophila melanogaster.*

***Drosophila* as a Model Organism and Aging**

The fruit fly is an ideal model system to investigate links between neurosensory systems, circadian biology, behavior, and aging for several reasons. The flies' relatively simple nervous system, consisting of only about 100,000 neurons, make *Drosophila* an ideal model to establish functional links between neurons and identify causal relationships with behavioral and physiological outcomes. Further, genetic manipulations are commonplace in these animals, and there is a diverse genetic toolkit for modulating the activity of individual genes or neurons. Lastly, due to the short generation time and lifespan, performing an aging experiment takes but a few months. Taken together, the combination of great genetic control, short generation time, and simple nervous system make *Drosophila* an ideal model to study how light perception may affect aging through either circadian rhythms, damage, or perception.

The goal of aging research is not to simply prolong life; understanding how and why organisms age is an age old question that has garnered much speculation (Flatt and Partridge 2018). Recently, there has been a shift in understanding. While aging was once considered a process that animals experience passively, through mutation

accumulation, molecular damage or exhausting limited biological resources, it is now clear that aging is capable of being modulated by specific genetic and neuronal pathways.

In understanding the details of how and why we age, strides can be made toward slowing the onset of age-related diseases such as cancer, cardiovascular disease, and many neurological disorders. Several lines of evidence support that slowing aging can do this. First, mice that live longer as a result of caloric restriction (Weindruch and Walford 1982) or knock-out of the Growth Hormone Receptor/binding protein (Ikeno, Hubbard et al. 2009) experience lower cancer incidence. Further, *Macaques* given a reduced calorie diet show a decreased morbidity that is accompanied with decreased incidences of cancer and diabetes (Colman, Anderson et al. 2009, Mattison, Colman et al. 2017). Studies of this nature have uncovered evolutionarily conserved molecular targets and neuronal pathways that have strong modulatory effects on aging across species. Some of the most well understood pathways include insulin signaling, target of rapamycin signaling (mTOR), and the unfolded protein response (UPR) (Lopez-Otin, Blasco et al. 2013). The fact that discrete molecular, cellular, and neuronal pathways can be specifically modulated in a manner to slow the rate of aging and extend lifespan demonstrates the utility of studying aging in model organisms. My work aims to uncover potential neuronal and environmental interventions that will be beneficial to slowing the aging process.

Circadian Rhythms, Time Perception, and Lifespan[1]

An organism's ability to predict daily events, such as sunrise and sunset, is important for the employment of optimal survival strategies. The mechanism through which this occurs is called the circadian clock, a transcriptional-translational negative feedback loop that is similar across species (Mohawk, Green et al. 2012, Tataroglu and Emery 2015). It can be set and reset by the perception of specific environmental cues, or zeitgebers, such as light and temperature, through a process known as entrainment. Molecular rhythms are coordinated between circadian neurons and communicated to control downstream physiological rhythms. Circadian clocks exhibit a period of approximately 24 hours that persists even in the absence of time cues, which is thought to ensure that behavioral, physiologic and metabolic processes align with environmental conditions. Much like food odors induce physiological changes in anticipation of nutrient intake, circadian rhythms may be considered a sensory modality that allows organisms to perceive time and to anticipate meal times, periods of predator activity, and even seasonal changes.

Disruptions of the circadian system are associated with an increased risk of age-related disease such as cancer, diabetes, and neurodegeneration (Scheer, Hilton et al. 2009, Evans and Davidson 2013, James, Honn et al. 2017). Night-time shift work is correlated with these poorer health outcomes, potentially because the body incorrectly anticipates the timing of feeding or other time-dependent behaviors such as sleep (Karlsson, Knutsson et al. 2003). On the other hand, well-timed manipulation of sensory inputs or

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[1] This section was written by J.C. Johnson with significant intellectual contributions from S.D. Pletcher and editorial work from C.M. Gendron. It was published as a component to the review: Gendron, C. M., T. S. Chakraborty, B. Y. Chung, Z. M. Harvanek, K. J. Holme, J. C. Johnson, Y. Lyu, A. S. Munneke and S. D. Pletcher (2020). "Neuronal Mechanisms that Drive Organismal Aging Through the Lens of Perception." Annu Rev Physiol **82**: 227-249.

environmental cues can increase the strength of organismal rhythms and improve health-related outcomes. For example, temperature cycles reinforced rhythms in *Drosophila* and allow arrhythmic flies to become rhythmic again (Yoshii, Heshiki et al. 2005). In mice, time-restricted feeding has been used to prevent obesity in mouse models of chronic shift work (Oike, Sakurai et al. 2015). In humans, bright light exposure at specific times of the day has been used effectively to improve sleep and cognitive performance, particularly during the winter months and in patients with neurodegenerative diseases (Yamadera, Ito et al. 2000, Skjerve, Holsten et al. 2004, Videnovic, Klerman et al. 2017). These manipulations indicate improving circadian function, and thus restoring proper perception of time, may be capable of improving age related disorders.

It is well-established that aging affects the function of the circadian clock. Established sleep-wake cycles breakdown as animals age, which is detected as weakened, fragmented sleep cycles as well as changes in circadian-affected behavioral outputs. Such changes have been documented in many different species, including *Drosophila* (Koh, Evans et al. 2006), rodents (Nakamura, Nakamura et al. 2011), and humans (Buysse, Monk et al. 2005). In *Drosophila*, transcriptional oscillations of clock genes in peripheral tissues have been reported to be reduced with age (Luo, Chen et al. 2012). The effect of age on clock gene oscillations in the brain is not as clear; one study reported an age-related decline in the strength of neuronal oscillations (Rakshit, Krishnan et al. 2012), while another reported strong rhythms that were largely unchanged throughout life in brains, but declines in peripheral clocks (Luo, Chen et al. 2012). Aging has also been reported to reduce the strength of circadian clock gene cycling in peripheral clocks, and in the suprachiasmatic nucleus (SCN), the region of the mammalian in which the master pacemaker resides (Kolker, Fukuyama et al. 2003, Bonaconsa, Malpeli et al. 2014, Nakamura, Nakamura et al. 2015). Generally speaking,

neural activity rhythms in the SCN are also degraded with age (Nakamura, Nakamura et al. 2011). Combating circadian declines accompanying aging via a SCN transplant derived from fetal tissue was shown to restore rhythms in rats and increase longevity in the golden hamster (Hurd and Ralph 1998, Li and Satinoff 1998). Thus, there is ample evidence to support the general observation that tissue-specific clock protein expression changes with age, such as the brain (Xu, Zheng et al. 2008, Rakshit and Giebultowicz 2013, Long and Giebultowicz 2017).

Altering the way in which organisms perceive time through genetic and environmental manipulation of clock molecules influences lifespan. In *Drosophila*, mutations in the *period* (*per*), which abolished endogenous rhythms, reduced lifespan (Krishnan, Kretzschmar et al. 2009). *Per* mutant flies also exhibited increased neurodegeneration and decreased oxidative stress resistance (Krishnan, Davis et al. 2008). Conversely, *per* overexpression has been reported to increase fly lifespan, although this observation was dependent on both sex and diet (Solovev, Shegoleva et al. 2019). *Timeless* (*tim*), the binding partner of *per* that forms the repressive limb of the feedback loop, was reported to be required for extended lifespan through diet restriction (Katewa, Akagi et al. 2016), although this result has been strongly contested (Ulgherait, Chen et al. 2016). Peripheral *tim* overexpression specifically in the fly fat body increased lifespan (Solovev, Shegoleva et al. 2019). *Cycle* is a transcription factor target of the PER/TIM complex, and *cyc*-deficiency in male flies significantly shortened lifespan (Hendricks, Lu et al. 2003). Increasing *cyc* expression in the fly fat body significantly increased lifespan when flies were aged on a protein-rich diet (Solovev, Shegoleva et al. 2019). *Bmal1* is the mammalian ortholog of *cyc*, and *Bmal1* deficiency in mice caused significant increases in physiologies associated with aging, such as sarcopenia, cataracts, decreased hair growth, and reduced lifespan (Kondratov, Kondratova et al. 2006). Brain-specific *Bmal1*

rescue of circadian clock function was sufficient to restore peripheral transcriptional rhythms in the liver and increase survival (McDearmon, Patel et al. 2006, Hughes, Hong et al. 2012). Finally, overexpressing the *Drosophila cryptochrome-1* gene (*cry)*, which is a blue light photoreceptor that modulates TIM degradation, was shown to increase rhythmicity with age, increase oxidative stress and extend lifespan (Rakshit and Giebultowicz 2013).). These studies further suggest that manipulation of circadian systems holds potential for improving health and longevity.

<u>Light Damage and Perception</u>

It is well established that light is capable of causing damage to living cells, however, not all wavelengths of light have the same effect. Light can be divided broadly into three categories based on wavelength. Starting with the shortest, ultraviolet light (UV), is categorized as having a wavelength less than 400nm, then comes visible light from blue to red with wavelengths ranging between 400 and 700nm respectively, and lastly infrared light with wavelengths over 700nm. Ultra violet (UV) light has drastic consequences to organismal health and lifespan. UV light reduces lifespan in invertebrates, and increasing incidences of skin diseases and the rate of skin aging in mammals (Beard 1972, Sanches Silveira and Myaki Pedroso 2014). To cope with this, many invertebrates have a mechanism by which they sense ionizing UV radiation and then trigger an escape response (Ward, Liu et al. 2008). Despite knowing much about the UV light detriments and mechanism, we had previously assumed visible light was largely innocuous. Recently there has been a surge in research into the effects of visible light on health and lifespan, and it has been shown visible light may be deleterious to lifespan.

The most thorough investigation of visible lights' influence on longevity has been in the nematode worm *Caenorhabditis elegans*. In *C. elegans* visible light reduced longevity, and the effect was dependent on the wavelength of light, with shorter wavelengths (blue light) of light being more detrimental (De Magalhaes Filho, Henriquez et al. 2018). This light induced lifespan shortening effect has been observed in other invertebrate models as well. Perhaps the first time this was shown in *Drosophila melanogaster* was back in 1972 when Colin Pittendrigh demonstrated flies aged under a 24 hour light regime (LL) were significantly shorter lived than those on a standard 12hr:12hr LD cycle (Pittendrigh and Minis 1972). Pittendrigh attributed this to a byproduct of circadian asynchrony caused by constant light inducing arrhythmicity, and not due to potentially damaging effects of light. Blue light has also been shown to shorten lifespan in adult *Drosophila*, though by means of neurodegeneration in the fly brain (Nash, Chow et al. 2019). This was the second study showing stronger effects of shorter wavelength visible light, providing support for the hypothesis that visible light can be damaging and reduces lifespan in a wavelength dependent manner. Blue light also has profound effects on pupal mortality in several insect species, with shorter wavelengths again being more damaging (Hori, Shibuya et al. 2014, Shibuya, Onodera et al. 2018). Other recent work in *Drosophila* has demonstrated higher levels of visible light shorten lifespan in both sexes, and the lifespan shortening is dependent on dietary protein content (Shen, Zhu et al. 2019). However, although higher protein content ameliorated the effect of brighter lights, the overall lifespans were significantly shorter even in the control diet, high light exposure flies (Shen et al. 2019). This comes as no surprise since dietary protein content is known to be a potent modulator of aging (Partridge, Piper et al. 2005, Smith, Kaeberlein et al. 2008). This was not the first study to investigate how dietary components may interact with light to influence aging. Massie et al. showed constant light to detrimental to lifespan, but that there could be a partial rescue by feeding flies riboflavin(Massie, Aiello et al. 1993). One consistency across all these

studies is that visible light is detrimental to longevity in invertebrates, largely in a dose and wavelength dependent manner. It will be beneficial to study how perception is integrated with these phenotypes and explore if damage alone is causal in the lifespan shortening effects of visible light.

Few studies have been performed in mammalian models investigating the effects of visible light on health and aging, however bright light exposure has been shown to induce neurodegeneration in the mouse and rat substantia nigra (Romeo, Viaggi et al. 2013, Romeo, Vitale et al. 2017). One potential issue both the mammalian and invertebrate studies of this nature have is that few control for other environmental factors that may also influence lifespan, such as temperature and humidity, and the studies that subject animals to different lighting conditions were done across incubators without controlling for any incubator or animal room effects. This is something we will improve upon in our studies.

Contrary to the negative effects of shorter wavelength light, there seems to be beneficial effects of longer wavelength light. Longer wavelength light has been shown to be advantageous for both health and aging in *Drosophila,* where exposure to a red 670nm light increased ATP levels, reduced inflammation, increased mobility, and increased average lifespan (Begum, Calaza et al. 2015). Though no aging studies have been done exploring effects of long wavelength light on mammals, there is evidence near infrared light also improves mitochondrial function, at least in limited tissues. In the mouse 670nm light was able to increase ATP levels in retinal tissues and reduce markers of retinal oxidative stress (Gkotsi, Begum et al. 2014). Both of these results are likely due to increased mitochondrial function. In line with this, old flies exposed to 670nm light had increased mitochondrial function as measured by cytochrome c oxidase activity, increased mitochondrial DNA content, and reduced reactive oxygen species, all

indicators of healthier mitochondria (Weinrich, Coyne et al. 2017). Further, in aged animals retinal function and cognitive measures were also improved relative to that of a 2-week old fly(Weinrich, Coyne et al. 2017). As will be detailed later in the thesis, preliminary work from our lab shows beneficial effects of red light exposure on longevity.

While the studies detailed above have shown light in the visible spectrum to be damaging in a wavelength dependent manner, none have explored how perception may mediate this damage. It is possible light sensing neuron activation itself is sufficient to mediate some of the negative light effects, and that the physical damage only occurs at super physiologic doses of light. There is some limited evidence perception may play a role in lights effects on lifespan. *Drosophila* kept under constant light conditions where photoreceptors are continuously activated experienced decreased synaptic function accompanied by rhabdomere degradation and activation of endoplasmic reticulum (ER) stress reporters (Moehlman, Casey et al. 2018). This is a reversible phenomenon, and neurons recover after removing flies from the constant light environment and can be ameliorated by AMPylation to induce the unfolded protein response. Although these were unnatural lighting conditions neuronal activation was required for this phenotype, and shorter activation bouts allowed recovery. It is also possible vision is like other sensory modalities where activation results in a physiological change and that change needs to be in concordance with the environment to be beneficial. Or perhaps the light information conveys differential responses depending how the visual information is processed and interpreted.

Visual Information and Links to Social Perception

In *Drosophila* visual perception, well reviewed by Zuh, begins when light enters the compound eye subunit, the ommatidia, and initiates a visual transduction cascade (Zhu 2013). Briefly, there are 8 photoreceptor cells in the retina, and each expresses a different combination of the 5 rhodopsins which excite at a specific wavelength to give the fly vision from 345-508nm(Montell 2012). There is work that suggests flies can sense and will change behaviors based on red light, however it is likely not used in vision (Hanai, Hamasaka et al. 2008). From the retina visual information is transduced downstream for visual processing in the lamina, medulla, and lobula complex (Zhu 2013). This is where objects are identified and behaviors specified based on the environment (Wu, Nern et al. 2016). From here information is transduced to higher centers where the information can be used for complex tasks such as navigation (Weir and Dickinson 2012) and predator avoidance (de Vries and Clandinin 2012). It is the processed visual information that will be explored in this section.

Vision is tightly linked to many organismal decision making processes, therefore there are likely evolutionarily conserved links between vision and other neural pathways. The classic psychological demonstration of this phenomenon is with a matching task where participants had to match the name “Bouba” and “Kiki” with a bulbous or sharply angled shape (Hanson-Vaux, Crisinel et al. 2013). Humans, regardless of culture have similar visual : auditory associations with spoken language and geometric shapes. Links such as this may manifest in other aspects of life and have profound effects on organismal fitness. Vision can also have strong associations with other objects, such as food. Much like Bouba and Kiki are associated with sounds, there is limited evidence there may also be visual links to food (Gallace, Boschin et al. 2011). The visual : food link indicates vision is closely linked to items or behaviors that may have control over

longevity. Studies of this nature serve to indicate the strong links between vision and sociality, something I hope to demonstrate in *Drosophila*.

Vision is strongly linked with quality of life, and numerous studies show people with greater visual acuity are more likely to have a higher overall level of health and related quality of life (Varma, Wu et al. 2006, Park, Shin et al. 2015, Taipale, Mikhailova et al. 2019). This introduces yet another mechanism by which visible light could have an effect on organismal aging, through the interpretation of visual information, particularly social cues. When you think of an acquaintance, friend, or loved one the thing you'll likely think of first is their appearance. Vision is strongly integrated with sociality, even in *Drosophila* where vision is used for social interactions at life stages from larvae to adults. For example, *Drosophila* larvae exhibit a social feeding behavior where larvae aggregate for more efficient feeding (Dombrovski, Kuhar et al. 2020). Vision is the critical sensory modality for learning the morphology and movement of other larvae, which is required to enable group feeding behavior (Slepian, Sundby et al. 2015, Dombrovski, Kim et al. 2019). In adults vision is used for sexual selection and mating, and lack of vision negatively impacts mating success (Roy and Gleason 2019). All of these studies demonstrate how vision and social perception are linked to have beneficial effects on physiology and behavior.

Recent work done in our lab demonstrates social perception, particularly perception of the opposite sex, as having significant impact on aging (Gendron, Kuo et al. 2014). This has also been shown in hermaphroditic *C. elegans* where exposure to male pheromones lead to shortened lifespan (Maures, Booth et al. 2014). While both of these results are linked with olfactory cues there is limited evidence vision is required for several social perception mediated behaviors. In particular, socially impoverished flies sleep less than flies kept in a cohort, and the detection of the social cohort is at least partially mediated

by vision. Blind flies did not respond to group housing with increased sleep, indicating vision is required for detecting socially enriched environments (Ganguly-Fitzgerald, Donlea et al. 2006). These results taken together make visual social interactions worth investigating.

In addition to the positive effects of vision it can also serve as a stressor. Our lab has shown the sight of dead flies induces a strong avoidance response, one that is dependent on vision to perceive the dead conspecifics and olfaction to warn nearby flies of the presumed noxious environment which caused flies to perish (Chakraborty, Gendron et al. 2019). Another visual stress shown to modulate behavior is predators in the environment. When flies see parasitoid wasps in their environment they respond by changing their egg laying behavior and choose a substrate that is likely to ensure larval safety, or by depressing egg laying behavior till a safer environment exists (Lefevre, de Roode et al. 2012, Kacsoh, Lynch et al. 2013). This behavior is persistent, and is maintained even after the parasites are removed. Further, the flies exposed to wasps change physiology by decreasing egg production, and communicate the danger to nearby flies who also show a depression in egg laying behavior based on the potentially dangerous environment (Kacsoh, Bozler et al. 2015). This is a good example of the power visual information holds and how it may be implicated in social situations.

Outstanding Questions

The preceding research has left several unanswered questions that became the motivation for my dissertation. Though much is known about the molecular nature and neural circuitry of the circadian clock, fewer studies have been done investigating how the clock interacts with environmental entrainment cues, or zeitgebers, to influence physiology and lifespan. This leads to the first question I address: Can an animal's

lifespan be extended by ensuring endogenous rhythms are synchronous with zeitgebers, and what zeitgebers could be modulated? This is a thorough test of the circadian resonance hypothesis. It was actually in testing environmental lighting conditions that we began wondering if social cues were zeitgebers, and whether perceived density would induce asynchrony and thus decrease lifespan. It had been previously shown social synchronization of locomotor rhythms occurs in flies of similar endogenous periods, and that social synchronization can be strong enough to override photic entrainment (Lone and Sharma 2011, Fuchikawa, Eban-Rothschild et al. 2016). This lead us to test mirrored vials with the hypothesis that a being housed in a mirrored environment would in effect double the perceived density, and given flies have slight variances in their circadian periods that this would magnify the asynchrony in the population. In order to control for the mirror effects we also aged flies in total darkness. In testing this hypothesis, we serendipitously discovered flies were significantly longer lived in the dark. This would become the foundation of a paper, and my main project exploring effects of visible light on longevity. With the main question being: what lighting parameters can be used to maximize lifespan, and by what mechanism is visible light deleterious to lifespan? The last chapter goes back to social interactions and density, and explores how social environments are perceived, and what effects they have on behavior and physiology. During this exploration I saw the need for a high throughput starvation assay that is relatively autonomous and does not require experimenter monitoring around the clock. This lead to me coopting the Trikinetics *Drosophila* Activity Monitor system in conjunction with an R script that can be used to run a "set it and forget it" starvation assay with automated analysis. In sum, my thesis brings insight to how vision and light interact with both the circadian systems and social parameters to modulate organismal health and longevity. Moreover, my studies suggest that vision is a potentially targetable pathway for aging interventions.
(Araujo, Poetini et al. 2018)

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Chapter 2

Visual Light Impacts Longevity Through Perceptual Pathways

Abstract

Sensory perception modulates aging across taxa in response to important ecological cues, such as food, sex, and danger. The mechanisms underlying these effects and range of sensory cues involved are largely unknown. One important ecological cue whose effects on aging have yet to be carefully explored is light. We therefore sought to determine whether light perception modulates lifespan in *Drosophila*. We discovered flies lived significantly longer in constant darkness. Extended lifespan was not accompanied by behavioral changes that might indirectly slow aging such as activity, feeding, or fecundity, nor were circadian rhythms necessary for the effect. The lifespans of flies lacking eyes and photoreceptor neurons were unaffected by environmental light, and transgenic activation of these same neurons was sufficient to phenocopy the effects of environmental light on lifespan when flies were reared in darkness. The relationship between light and lifespan was not correlated with its intensity or duration or the frequency of light-dark transitions, and high-intensity light, particularly of shorter wavelengths, reduced lifespan in eyeless flies, indicating that the effects we observed are largely independent of known non-specific damaging effects of bright light. Our results suggest that, much like other sensory systems, light perception through visual photoreceptors deserves attention as a longevity intervention. Future studies may be directed toward determining whether this is due to adverse systemic effects of light on visual neurons or to the activation of signaling pathways that directly modulate aging.

Introduction

Sensory perception influences energy homeostasis, tissue physiology, and organismal aging through neuronal circuits that emanate from sensory tissues and interface with deeper regions of the central nervous system (Gendron, Chakraborty et al. 2020). The molecular study of these relationships is often traced back to the work of Apfeld and Kenyon in the nematode, *Caenorhabditis elegans* (Apfeld and Kenyon 1999), and in the years since, sensory effects on aging have been observed across the phylogeny of vertebrate and invertebrate animals (Libert, Zwiener et al. 2007, Smedal, Brynem et al. 2009, Linford, Kuo et al. 2011, Waterson, Chan et al. 2015, Riera and Dillin 2016, Fletcher and Kim 2017). Several sensory modalities have been implicated in this relationship including smell, taste, sight, and pain (Linford, Kuo et al. 2011), and the ecological cues most often involved are those of food, mates, or danger, detection of which is critical to organism fitness (Gendron, Chakraborty et al. 2020).

One ecological cue whose effects on aging have yet to be carefully explored is light. Most animals are exposed to light regularly, and its perception influences nearly all aspects of life from foraging to navigation, and from reproduction to survival. Depending on ecological context, light may serve as an attractive or repulsive stimulus. For example, long wavelength light is attractive to planarians, while short wavelength light produces a strong photophobic response (Paskin, Jellies et al. 2014). In vertebrate models light can also produce a range of responses, being attractive to larval zebrafish and repulsive to neonatal mice (Johnson, Wu et al. 2010, Wolf, Dubreuil et al. 2017). Diverse characteristics of light are perceived by different species, and in humans, different light intensities and wavelengths influence depression scores, cognitive performance, mood, and energy levels. Light cues are also the most powerful known entrainment stimulus for circadian rhythms, and therefore they work in tandem with the molecular circadian clock to define daily time perception. Much like the cephalic

phase response, in which the smell of food prepares the body in anticipation of consumption, circadian time perception directs changes in physiology and behavior in anticipation of night-time or day-time transitions.

Exposure to light has been shown to influence physiology and lifespan in several model systems. In *C. elegans*, lifespan is inversely correlated with the time that worms are exposed to visible light, with effects attributed to photooxidative stress (De Magalhaes Filho, Henriquez et al. 2018). Short-wavelength visible light increases pupal mortality in several insect species including the vinegar fly (*Drosophila melanogaster*), the mosquito (*Culex pipiens molestus*), and the flour beetle (*Tribolium confusum*) (Hori, Shibuya et al. 2014, Shibuya, Onodera et al. 2018), and it reduces adult lifespan and increases markers of neurodegeneration in adult *Drosophila* (Nash, Chow et al. 2019). Again, oxidative and other physical stresses are thought to be the cause (Chen, Hall et al. 2017, De Magalhaes Filho, Henriquez et al. 2018). Bright light exposure has been shown to induce neurodegeneration and reduce dopamine levels in the mouse and rat *substantia nigra* (Romeo, Viaggi et al. 2013, Romeo, Vitale et al. 2017) potentially by oxidation and generation of cytotoxic byproducts (Graham 1978). The damaging effects of UV light on many aspects of biology are well known (Beard 1972, Sinha and Hader 2002). The ability of light energy to compromise healthy aging through physical damage is, therefore generally accepted, although it is important to note many of these studies used unnaturally bright light or exposed animals to a light intensity or wavelength outside their normal ecological conditions.

It remains largely unknown whether there are subtler effects of natural light on aging that do not involve cell-autonomous physical damage but are instead modulated non-autonomously by the sensory systems designed to detect it. There are several indications that this may be the case. First, the pattern of light exposure can influence

health independent of duration or intensity. Short pulses of dim light effectively entrain circadian systems, and it has been postulated that organismal health and lifespan are enhanced when the oscillation of light stimulus coincides with endogenous circadian periods (Wyse, Coogan et al. 2010). In humans, shift work is associated with negative physical and mental health effects (Knutsson 2003, James, Honn et al. 2017, Moreno, Marqueze et al. 2019), including cancer as well as metabolic, cognitive, and neurodegenerative disorders(Scheer, Hilton et al. 2009, Evans and Davidson 2013). Second, certain types of light have been shown to be beneficial in some contexts. Near infrared light was reported to modestly increase lifespan in *Drosophila* (Begum, Calaza et al. 2015), and it has been used to treat Alzheimer's and Parkinson's disease (Ying, Liang et al. 2008, Peoples, Spana et al. 2012, Purushothuman, Nandasena et al. 2013). Enhanced stress resistance was achieved by neuron-specific overexpression of the major *Drosophila* photoreceptor cryptochrome gene, *cry,* which is involved in resetting circadian rhythms upon sensing light(Solovev, Dobrovolskaya et al. 2019). Third, in the mouse, light perception through photosensitive retinal ganglion cells modulated body temperature and sleep independent of the molecular circadian machinery (Rupp, Ren et al. 2019).

We sought to test whether natural amounts of visible light influence aging in *Drosophila* and, if so, whether such effects involve sensory perception. We discovered that lifespan was robustly extended in both male and female flies when they were aged in constant darkness relative to siblings aged in standard conditions where lights oscillated in a 12hr:12hr on:off pattern. Slowed aging was not due to behavioral differences in the dark, such as self-dietary restriction, changes in locomotion, or reduced reproduction. Flies that lacked light-sensitive neurons and molecules failed to exhibit lifespan extension in the dark, and activation of these same neurons in the absence of environmental light reduced lifespan, suggesting that light modulates lifespan, at least

in part, by visual perception. Lifespan extension in constant darkness was independent of the pace or amplitude of molecular circadian rhythms and also independent of perceived time, as measured by the number of subjective days and nights the flies experienced during their lifetime. These studies suggest that, much like food, light may influence aging through direct physical effects on cells as well as through indirect effects through sensory systems designed to adjust behavior and physiology in the expectation of temporal changes in the environment. Elucidating molecular and neuronal mechanisms underlying sensory-dependent effects of light on aging is an attractive avenue for future research.

Results

Constant darkness increases *Drosophila* lifespan independent of key aging-related behaviors

We first asked whether the complete loss of a light stimulus modulates lifespan in *Drosophila*. We therefore compared the lifespans of flies aged under constant darkness with those aged under conventional conditions comprised of repeated 12 hr:12hr light:dark cycles. Preliminary experiments revealed that incubator-to-incubator variability in temperature and humidity, even among units from the same manufacturer programmed to the same conditions, were sufficient to induce significant changes in lifespan. To avoid having such differences confound effects that might be caused by different light regimes, we constructed light compartmentalization structures in a single incubator within which light was maintained and between which temperature was measurably indistinguishable (e.g., over a 60 day period the mean temperature in the dark compartment averaged 25.34°C [SD = 0.35°C] while the temperature in the 12 hr:12hr light dark cycle compartment was 25.26 °C [SD = 0.27°C]). When we aged flies under constant darkness and a standard 12 hr:12hr light dark cycle,

as has since been reported (De Magalhaes Filho, Henriquez et al. 2018, Nash, Chow et al. 2019), we found that flies of both sexes were significantly longer-lived under constant darkness (Fig 2.1a, b). Mean and maximum lifespan was increased up to 19% and 14%, respectively. This effect was consistent across experimental replicates and genetic strains, suggesting that it is robust and not a genotype-specific phenomenon (Fig 2.1c, d).

We next investigated behavioral changes that might indirectly slow aging in constant darkness. First, we measured food consumption to test the hypothesis that flies were behaviorally limiting their nutrient intake in the dark, thereby executing self-dietary restriction (Yu, Masoro et al. 1985, Chapman and Partridge 1996). We observed that total food intake, as measured over a 24-hour period by a modified version of the ConEx feeding assay (Shell, Schmitt et al. 2018), was not significantly different between female flies previously maintained for 14 days in constant darkness vs. siblings maintained in a standard 12hr:12hr light-dark cycle (Fig 2.2a). Interestingly, males subjected to constant darkness consumed modestly but significantly more food than their siblings that were exposed to light (Fig 2.2b). Second, we measured total activity, which does not directly affect lifespan but may impact caloric balance and long-term health (Sujkowski, Bazzell et al. 2015). Flies were first maintained for 14 days in either constant darkness or light:dark conditions, after which time they were transferred to activity tubes, placed in Trikinetics *Drosophila* Activity Monitors, and measured for 5 days in their experimental light conditions. We found that flies aged in constant darkness maintained similar overall levels of activity as did flies aged in a 12hr:12hr light:dark environment (Fig 2.2c). Third, we examined fecundity as a measure of potential reproductive costs of extended lifespan (Travers, Garcia-Gonzalez et al. 2015). Flies aged in constant darkness for 14 days showed no differences in fecundity over a subsequent 7-day period compared to their sibling control flies aged in light:dark

conditions (Fig 2.2d). Fourth, we asked whether constant darkness affected the decline in circadian rhythms that normally occurs when flies are aged in standard light:dark conditions (Luo, Chen et al. 2012). We observed that flies aged in constant darkness for 21 days exhibited measures of rhythm strength and circadian periodicity that were statistically indistinguishable from their siblings maintained in light:dark conditions (Fig 2.2e, f). We concluded that the extended lifespan observed in flies maintained in constant darkness is not due to diet-restriction, changes in locomotion, reduced reproduction, or improved circadian function.

Slowed aging in constant darkness is modulated, at least in part, through sensory perception of light.

We next asked whether the perception of light is necessary and/or sufficient to modulate fly lifespan. To determine necessity, we took advantage of visually blind flies that lack eyes and photoreceptor cells. These flies express the proapopotic gene *hid* under the control of the *GLASS Multimer Reporter* (GMR) promoter element, which expresses in the photoreceptor cells and downstream neurons (Bergmann, Agapite et al. 1998). Flies carrying two copies of the *GMR-hid* transgene are completely eyeless (Hsu, Adams et al. 2002, Laverty, Li et al. 2011). We found that constant darkness did not increase the lifespans of male or female *GMR-hid* flies compared to siblings aged in standard 12hr:12hr light:dark conditions (Fig 2.3a, b). To test sufficiency, we sought to mimic light perception while avoiding potential damaging effects of physical light. We therefore decided to manipulate the activity of light-perceiving neurons and to measure effects on lifespan in the absence of external light. We again targeted *GMR*-expressing neurons, as well as neurons that specifically express the blue light photoreceptor, *Rh1,* because of the documented effects of this wavelength on lifespan (De Magalhaes Filho, Henriquez et al. 2018, Nash, Chow et al. 2019). Spatiotemporal activation was accomplished by employing the *GAL-4/UAS* system to express the temperature

sensitive cation *TrpA1* selectively in *GMR* and *Rh1* neurons, respectively. The *Drosophila* TRPA1 channel promotes neuron depolarization only at elevated temperatures (>25°C) and allows for temporal control over cell activation (Hamada, Rosenzweig et al. 2008). All experimental flies were aged in constant darkness, and to mimic conventional 12hr:12hr light:dark conditions, we cycled temperature from 18°C to 29°C on a 12hr:12hr period to activate targeted neurons. Oscillatory activation of all visual neurons with the *GMR* driver and *UAS-TrpA1* proved to have sexual dimorphic effects; male flies were unaffected (Fig 2.3c) but female flies exhibited a shortened lifespan (Fig 2.3d). More restricted activation of *Rh1*-expressing neurons reduced lifespan in both males and females (Fig 2.3e, f). These results suggest that light modulates lifespan, at least in part, by visual light perception.

It has been reported that exposure to high amounts of visual light, specifically in the blue range, can directly induce cellular damage and reduce lifespan in the nematode, *Caenorhabditis elegans* (De Magalhaes Filho, Henriquez et al. 2018), and in *Drosophila* (Nash, Chow et al. 2019). To evaluate whether broad-scale light-induced damage is involved in the lifespan differences that we observed in our standard rearing conditions, we studied the effects of variable exposure time, intensity, light:dark transitions, and wavelength. First, we reasoned that if light energy itself was directly damaging, perhaps by inducing senescence or cell death in visual neurons, then exposure time would be negatively correlated with lifespan. We therefore compared the lifespans of flies aged under constant light to those aged in 12hr:12hr light:dark conditions and in constant darkness. While constant darkness reliably extended male lifespan, we found that flies aged in constant light were not shorter-lived than those aged in 12:12 conditions (Fig 2.4a). The same result was observed with female flies (Sup 2.1a). Moreover, we found no significant difference in lifespan between male flies exposed to a 12hr:12hr light:dark schedule with dim light (300 lux) and those similarly

exposed to 5x brighter light (1050 lux; Fig 2.4b), although in females the dim light treatment had a reduced effect on lifespan compared to bright light (Sup 2.1b).

It is possible that lifespan is subject to threshold modulation in which a small amount of light triggers a maximum effect on lifespan. Or it may be that transitions from light to dark (and *vise versa*) are important. To test these ideas, we aged flies in conditions where darkness was interrupted by two one-hour light pulses each day from 8am-9am and 7pm-8pm. We chose this design so that the light pulses would coincide with the first and last hours of light under our standard 12hr:12hr light:dark cycle and so that the number of transitions that the flies experienced would be doubled. Male flies aged in these conditions lived significantly longer than flies exposed to 12hr:12hr cycles but shorter than flies aged in constant darkness (Fig 2.4c). Female flies did not show the same trend (Sup 2.1c). These data suggest that the number of light:dark transitions is not causal for changes in lifespan and that a straightforward damage model is unlikely to account for the observations that we report. This leaves open the possibility that light shortens lifespan as a result of perception and the threshold at which light induces damage is above the levels used in these experiments.

Cell autonomous, light-induced damage has been shown to be wavelength dependent, and we asked whether different wavelengths of high intensity light were capable of modulating lifespan in our conditions and whether such effects were dependent on perception. Flies exposed to high intensity monochromatic blue (470 nm), green (527 nm), and red (640 nm) were all significantly shorter lived than constant darkness (DD) reared flies. Shorter wavelengths had larger effect (Fig 2.4d, Sup 2.1d), which is consistent with published studies that have demonstrated that blue light is detrimental to lifespan (De Magalhaes Filho, Henriquez et al. 2018, Nash, Chow et al. 2019). Notably, however, eyeless *GMR-hid* flies exhibited a similar response to intense blue

light as did control animals suggesting that this treatment influences lifespan independent of visual perception, likely through mechanisms that are distinct from the effects caused by normal levels of visible light (Sup 2.2a-d).

The effects of light perception are independent of molecular circadian clock and of daily time perception.

Given that sensory perception of light is responsible, at least in part, for extended lifespan in constant darkness, we next asked whether this effect was modulated by mechanisms involved in specifying endogenous circadian rhythms, which are entrained by light patterns. The genes *period (Per)* and *timeless (Tim)* are essential components of the repressive limb of the molecular clock, and their loss leads to molecular arrhythmicity. Although these mutants are capable of masking, which is showing behavioral rhythms that correspond with the light cycles without light anticipatory behavior, and will exhibit similar activity patterns as wild-type flies, they will not entrain to the light cycle and are unable to predict the onset of light. We observed that male flies homozygous for a complete loss of function *Per* allele (Per^{01}) exhibited a significant increase in lifespan under constant darkness, as did male animals carrying a deletion in *Tim* (Tim^{01}) (Fig 2.5a, 5b). To more thoroughly explore whether clock function mediates lifespan extension in the dark, we tested the positive limb by loss of the gene *cycle* (Cyc^{01}), which also results in behaviorally arrhythmic flies. Cyc^{01} did not abolish increased lifespan in constant darkness in males, establishing that the positive limb of the clock was also dispensable for this effect (Fig 2.5c). Finally, we tested whether the circadian-light sensor, *Cryptochrome (Cry),* was required for lifespan extension in constant darkness. Male flies homozygous for the null mutation, Cry^{b}, displayed a significant lifespan extension of similar magnitude to control flies (Fig 2.5d). Female flies exhibited comparable trends to males (Sup 2.3a-d). These results indicate

that lifespan extension in constant darkness is independent of molecular circadian rhythms.

The effects of sensory perception on lifespan are often more pronounced when information provided by sensory systems is uncoupled with the experiences that they were designed to predict (Harvanek, Lyu et al. 2017). For example, flies that smell food during periods of food scarcity or that detect the opposite sex in the absence of mating opportunities are significantly short-lived (Libert, Zwiener et al. 2007, Gendron, Kuo et al. 2014). In the context of light and time perception, this situation might be represented by a discordance between the predicted pattern of light cycling provided by the molecular clock and the realization of actual environmental light patterns. Indeed, it is currently thought that such asynchrony reduces lifespan, reproduction, and metabolic health (Pittendrigh and Minis 1972, Libert, Bonkowski et al. 2012, Boomgarden, Sagewalker et al. 2019, Horn, Mitesser et al. 2019).

We therefore investigated how different forms of uncoupling between light schedules and the circadian clock impact *Drosophila* lifespan. We began by exploring the effects of repeated exposure to a shifting light cycle (Fig 2.6a). We chose a light schedule that mimicked human shift workers who travel 4 days a week or who work nights several days a week and then experience a different schedule on weekends. A similar schedule had been shown to be detrimental to mouse lifespan (Davidson, Sellix et al. 2006). This was executed by exposing flies to a standard 12hr:12hr light-dark schedule, with lights on from 9am-9pm, on Monday-Thursday, imposing a 6-hour phase delay on Friday (i.e., with lights on from 3pm-3am), and restoring the normal cycle by applying a 6hr phase advance on Sunday. This shifting light paradigm had no meaningful effect on fly lifespan, with *yw* flies exhibiting a mean reduction in lifespan of 3.6% and w^{1118} exhibiting a mean reduction in lifespan of 3.7% (Fig. 6c, d).

We next tested different patterns of light oscillation, including oscillation rates that were equivalent to the flies free running period as well as those that exhibited difference degrees of discordance. To do this we expressed mutant variants of the *doubletime* kinase, which is responsible for the phosphorylation of PER and thus the amount of time it takes for the molecular clock to cycle (Price, Blau et al. 1998, Nash, Chow et al. 2019). In this way, we created flies with endogenous periods of 18, 24, and 27 hours, which we refer to as short-day, normal-day, or long-day flies, respectively. Short-day flies expressed *UAS-doubletime-short* (*UAS-DBT^{S}*) in clock neurons (using *Clk856-GAL4*), which are the master circadian neurons whose output serves to synchronize all body clocks. Long-day flies expressed *UAS-doubletime-long* (*UAS-DBT^{L}*) in those same cells (Gummadova, Coutts et al. 2009), and flies carrying *Clk856-GAL4* but no UAS element served as control. Flies of each free-running period were exposed to each of three different environmental light:dark conditions of 9hr:9hr, 12hr:12hr, and 13.5hr:13.5hr hours in a factorial design (Fig. 7a). This design was chosen to allow direct, within-strain comparisons among treatments in which one environmental light cycle was in line with its endogenous free running period (termed the control environmental condition for that genotype), and two environmental light cycles that were distinct from it. Our design also allowed us to determine whether the magnitude of the difference between environmental and endogenous periods correlate with lifespan effects (Fig. 7a).

Before executing the lifespan experiments, we sought to establish that our genetic manipulations were effective in maintaining distinct free-running periods throughout the lifespan and that our disparate environmental light cycles were effective in masking them. We found that short-day flies exhibited a mean period length when young of 17.8 hours (SD= 0.34) and that long-day flies showed a mean period length of 26.8 hours (SD

= 0.61). As expected, normal-day *Clk856-GAL4* animals, which did not express either altered version of DBT, exhibited a mean period of 23.9 hr (SD= 0.19) (Fig 2.7b). We also found that each genotype effectively entrained to each of the different light cycles and had an activity period that was within 0.1 hr of the diurnal cycle (Fig 2.7b), even the most disparate. Short-day flies, for example, exhibited 27-hour behavioral rhythms (mean = 26.93 hr, SD = 0.03) when exposed to the 13.5:13.5 hr light:dark regime, and long-day flies expressed an 18 hour behavioral rhythm rhythms (mean = 17.98 hr, SD = 0.02) when exposed to the 9:9 hr regime. When transferred to constant darkness after aging for three weeks in each light environment, flies reverted to their expected genotype-specific free-running periods (Fig 2.7b), establishing that endogenous rhythms were retained until older ages independent of light condition and that extended durations at different periods did not differentially affect rhythmicity.

We then measured lifespan of each of the three genotypes in each of the three light conditions. We observed that short-day flies exhibited statistically indistinguishable lifespans in conditions of 9hr:9hr, 12hr:12hr, and 13.5hr:13.5hr light:dark regimes (Fig 2.7d). Similar results were obtained for both long- and normal-day flies: neither genotype exhibited differences in lifespans when aged across the three light regimes (Fig 2.7e, f). In other words, neither the magnitude nor direction of misalignment between oscillations of environmental light and of the endogenous clock affected lifespan in our experiments.

To examine the hypothesis that perception of time, *per se*, modulates lifespan, we aged the short-, normal-, and long-day flies in constant darkness, which, for a given amount of chronological time, would result in each genotype experiencing a different number of subjective days (Fig 2.8a). We reasoned that short-day flies with their 18hr period might therefore perceive a more rapid passage of time than would normal-day or long-day

flies with their 24hr and 27hr periods, respectively, and that a comparison among them would not be confounded by entrainment. We observed that short-, normal-, and long-day flies exhibited similar lifespans in constant darkness (Fig. 8b).

When taken together, our results from manipulations that were designed to mimic shift work, to study the effects of concordance between endogenous and environmental rhythms, and to examine the effects of perceived time all indicate that the extension of lifespan observed in constant darkness is independent of the molecular clock, that the relationship between circadian timekeeping and external light has little effect on patterns of fly aging, and that the length of life is independent of the number of subjective days.

Discussion

We discovered that flies aged under constant darkness lived longer than those aged under typical laboratory conditions (i.e., a 12hr:12hr light:dark cycle). This effect was independent of behavioral changes often associated with lifespan including: feeding, activity, and fecundity. Interestingly, blind flies did not show a darkness-mediated lifespan extension, and activation of photoreceptor neurons was sufficient to shorten lifespan and phenocopy the effects of light when flies were kept in constant darkness. These results indicate that there is a perceptual component to the ability of light to modulate aging. The light effect was independent of circadian rhythms. Further, when we uncoupled the molecular circadian clock from the environmental light:dark cycles, we found *Drosophila* to be resilient to circadian perturbation. Neither shifting the light cycle nor changing the period of the molecular clock had a meaningful effect on lifespan determination.

We present several lines of evidence indicating that light-induced effects on lifespan are not caused by cell autonomous damage alone. First, the effects of light on lifespan did not scale with exposure time or intensity: doubling light exposure time had no further effect on lifespan and increasing light levels five-fold, from 300lux to 1500lux, had only modest effect. It should be noted that these light levels are below those of standard *Drosophila* incubators, which are usually measured around 2000lux, so any potential damaging effects would be less than one might expect in a typical laboratory setting. Second, we considered the possibility that lifespan may be modulated by light in a threshold-type manner, where small amounts of light trigger an effect on lifespan. Just two hours of light exposure, given as 1hr pulses at the beginning and end of day, was sufficient to shorten lifespan in males, but not females. So, either the threshold is lower than two hours of exposure, or more likely, lifespan is reduced by damaging effects of light that emerge at greater light levels than those used in our experiments, as seen in recently published work (De Magalhaes Filho, Henriquez et al. 2018, Nash, Chow et al. 2019). We also considered the possibility be that abrupt light:dark transitions are damaging, perhaps through repeated startle responses. This does not appear to be the case because flies exposed to twice-daily light pulses, which are startled twice as often, do not show a reduced lifespan when compared to those under a standard light cycle. Third, the effects of our light regime required visual perception. When light perception was muted through genetic ablation of the eyes and photoreceptors, the effect of light on lifespan was lost. However, at high intensities of blue light, ablation of the eyes and photoreceptors was not sufficient to rescue lifespan. Based on these data, together with published studies, we conclude that natural light modulates lifespan in *Drosophila* through sensory systems designed to detect it and that at excessively high intensities, particularly of shorter wave lengths, physical damage is pervasive and effects on lifespan are largely independent of sensory perception.

We hypothesized light is similar to food, which has both a direct effect (through cell autonomous nutrient signaling pathways) and an indirect effect (through sensory perception) on lifespan(Gendron, Chakraborty et al. 2020). Much like food/nutrients, where perception of high calorie food acts through odorant receptor activation and results in shorter-lived animals, we predicted that activation of photoreceptor neurons would reduce lifespan(Libert, Zwiener et al. 2007), which is what we observed. Furthermore, activation of only blue photoreceptors was sufficient to shorten lifespan. Costs of light exposure in our experiments therefore result from the sensing or interpretation of the light cues themselves. These results taken together suggest a biological cost of light perception that is independent of the circadian system and light-induced damage. Further dissection of the molecular and neural mechanisms of light perception may reveal specific photoreceptors and optical processing centers that are required for modulation of lifespan.

Some of our results are inconsistent with previous studies that showed significant effects on lifespan when flies were aged in conditions where external light cycles were discordant with endogenous rhythms (Pittendrigh and Minis 1972, Boomgarden, Sagewalker et al. 2019). The circadian resonance hypothesis, which states animals' health and lifespan will be impacted if endogenous period is not synchronized with the environmental period, has been influential, although effects on aging *per se* have not often been examined (Nash, Chow et al. 2019) (Spoelstra, Wikelski et al. 2016). Contrary to the predictions of this hypothesis, we found creating discordance between circadian inputs and the internal clock through shifting the flies' light environment or modulating endogenous period had no meaningful effect on lifespan. These discrepancies may be due to one or more of the following factors. First, to our knowledge, previous studies were performed in multiple incubators with different light environments (Pittendrigh and Minis 1972, Boomgarden, Sagewalker et al. 2019). We found that environmental

variables known to influence lifespan, such as temperature and humidity, were highly variable across different incubators, even when they were programmed to hold identical conditions. Our studies were done within the same incubator, which allowed for precise control over such factors. Second, it is possible that the circadian resonance hypothesis is more relevant in conditions where there are concurrent stressors – such as mating and predation. Under these conditions, animals must anticipate feeding times of predators and times when mates are most receptive in order to maximize fitness. Similarly, our experiments measured only lifespan not inclusive fitness, which might be examined by allowing flies of different free running periods to compete. Indeed, it was recently demonstrated in *Drosophila* that measures of fertility and offspring survival were maximized in competitive conditions when the free running period was matched with environmental day length (Horn, Mitesser et al. 2019). Lastly, the circadian resonance hypothesis may be incorrect, and lifespan and health are unaffected by discrepancies between endogenous and environmental periods.

To our knowledge, this is the first study in which *Drosophila* lifespan has been measured while manipulating both circadian period and environmental day length in concordance. Our experimental design provided us with the ability to investigate whether the number of subjective days, and thus a form of perceived time, affects the rate of *Drosophila* aging. We found no relationship between lifespan and subjective time passed, suggesting that circadian time perception does not influence the rate of aging.

Overall, our results suggest that, like other sensory systems, light perception through visual photoreceptors deserves attention as an intervention that may modulate healthy aging. The possibility that light, or the perception of light, could be used to improve healthy aging has begun being explored. Near infrared light may improve cell proliferation in culture, and ATP synthesis (AlGhamdi, Kumar et al. 2012, Tsai, Yin et

al. 2015), and far red therapy may ameliorate symptoms of Parkinson's and Alzheimer's as well as improve pain management and flexibility in rheumatoid arthritis (Johnstone, Moro et al. 2015), (Schiffer, Johnston et al. 2009), (Oosterveld, Rasker et al. 2009). It is unknown whether the effects of light on lifespan result from damage to sensory neurons, which may lead to systemic effects that reduce lifespan, or from adaptive responses to light perception *per se*, which may recruit signaling pathways that directly modulate aging. Future research on the relationship between light exposure and aging will benefit from focusing on how light impacts the health of visual neurons and how information about the light environment is transduced from the eyes to impact health and aging.

Methods

Fly husbandry

All *D. melanogaster* used in this paper were reared using the same method. Experimental flies were age-synchronized using a 3 step procedure. First, mated females and males were placed on a grape juice agarose plate supplemented with live yeast paste for 18-22 hours. The eggs laid during this period were collected briefly in PBS, distributed in 32ul aliquots into culture bottles containing a modified Caltech Medium (CT food) (E.B. 1960), and reared at 25°C with a standard 12hr:12hr LD cycle. Second, flies that eclosed within a 24hr window were collected into bottles and maintained on a 10% sucrose/yeast food (SY10) for 2-3 days at 25°C with a 12hr:12hr LD cycle, unless otherwise noted. After the 2-3 day mating period, flies were sorted under light CO_2 anesthesia into single-sex groups of 20 or 25, unless noted otherwise, and placed into vials containing SY10 media, which was changed every 2-3 days for the duration of their lifespans.

Fly strains

Canton-S, *yw*, and w^{1118} fly strains were obtained from Bloomington *Drosophila* Stock Center. We thank the Todd Lab at the University of Michigan for providing us with *GMR:Hid, GMR-Gal4*, and *Rh1-Gal4* fly stocks. The Giebultowicz lab at Oregon State University generously shared Per^{01}, Tim^{01}, Cyc^{01}, and Cry^{B} mutants with us. The Shafer lab was kind to give us *Clk856-Gal4, UAS-*DBT^{L}, and *UAS-*DBT^{S} lines.

Media recipes

The modified CT food recipe is as follows: 1 L water, 10 g agar, 6 g cornmeal, 30 g sucrose, 55 g dextrose, 45 g yeast, 15 mL tegosept solution (20% tegosept in 90% ethanol), 3 mL propionic acid. Sugar-yeast 10% (SY10) food recipe is: 1 L water, 20 g agar, 100 g sucrose, 100 g yeast, 15 mL tegosept solution (20% tegosept in 90% ethanol), 3 mL propionic acid, and 4 mL antibiotic supplement (1% tetracycline and 2.5% kanamycin in water).

Environmental control

Carefully controlled environmental parameters were key for the success of these experiments. As such, all experiments involving comparisons across environmental light cycles were carried out within the same Percival incubator. Individual light cycles were maintained within 3, light-tight, cabinet drawers installed in the incubator. The lights used were DIODER LED strip lights (item model #: 202.194.18), and they were controlled by a digital timer. To compensate for heat produced by the lights, the dark cabinet drawer also contained the same lights on the same schedule, but they were contained within a light-tight aluminum box. Individual cabinet drawer temperature, humidity, and barometric pressure were determined to be statistically indistinguishable in all 3 boxes. Unless noted, temperature was maintained at 25°C +/- 0.5°C.

Lifespan assays

Adult male and female flies (aged 2-3 days post-eclosion) were collected from controlled larval cultures and mating conditions (see above), sorted, and transferred into standard vials (20 or 25 flies per vial) containing SY10 media. The number of flies used per treatment group at the start of the lifespan is given in the figure legends. Actual number used in the analysis is noted in the figure legends. Cohort censuses were taken every 2-3 days, at which time flies were transferred to fresh SY10 media. Experiments were coordinated using DLife computer software(Linford, Bilgir et al. 2013). For experiments in which flies were aged under dark conditions, transfers occurred under indirect, dim red light (5 lux).

Temperature-dependent neuronal manipulations

Temperature was used to activate *GMR* or *RH1* expressing neurons in adult flies. Parental crosses for activation strains were *GMR-Gal4 x UAS-TrpA1* and *Rh1-Gal4 x UAS-TrpA1*, while the control crosses were *GMR-Gal4 x* w^{1118} and *Rh1-Gal4 x* w^{1118}. For all crosses, eggs were collected and raised in 18°C 12hr:12hr LD. This temperature was maintained until the beginning of the lifespan experiment to ensure there would be no neuronal activation during development. At the beginning of the lifespan experiments flies were transferred to a Percival incubator in which they were maintained in constant darkness under temperature oscillations of 12hr:12hr, 18°C:29°C. *TRPA1* activation was designed to mimic day time light perception, and therefore flies were transferred to new media during the 29°C period.

Activity measurements

Adult male flies (aged 2-3 days post-eclosion) were collected from controlled larval cultures and mating conditions (see above), sorted, and transferred into standard vials (20 flies per vial) containing SY10 media. They were subsequently maintained in either

12hr:12hr light-dark (control) conditions or in constant darkness for 14 days. Flies were then sorted individually into 5 mm × 65 mm polycarbonate tubes (Trikinetics part # PPT5x65), with the same sugar-yeast media placed at one end of the tube. Activity tubes were then loaded into *Drosophila* Activity Monitors (Trikinetics part # DAM2), and monitors were then transferred back into their respective light condition. Recording began 24 hours after the flies were loaded into the activity tubes to allow for acclimation to experimental housing conditions. Data was collected using TriKinetics DAMfilescan, and they were subsequently summed into 30 minute bins. Flies that died during the recording period were excluded from the analysis.

Circadian period, and rhythm calculation

Rhythm and period experiments were conducted using activity measurements as an output of circadian rhythms. Briefly, adult male flies (aged 2-3 days post-eclosion) were collected from controlled larval cultures and mating conditions (see above), sorted, and transferred into standard vials (20 flies per vial) containing 10% SY10 media. Flies were aged for 21 days under 12hr:12hr light-dark (control) conditions or in constant darkness and then were sorted into 5 mm × 65 mm polycarbonate tubes (Trikinetics part # PPT5x65) with SY10 food at one end of the testing tube before being assigned to the *Drosophila* Activity Monitors (Trikinetics part # DAM2). All monitors were placed in the same incubator where they received two days of 12hr:12hr LD then 7 days of DD. TriKinetics DAMfilescan was used for data processing. Actimetrics ClockLab Analysis 3 was used for data analysis. A chi-square periodogram analysis was used to determine period, and fast Fourier transformation analysis was used to calculate rhythmicity values. Flies that died during the recording period were excluded from the analysis.

Fecundity assay

Adult male and female flies (aged 2-3 days post-eclosion) were collected from controlled larval cultures and mating conditions (see above), sorted, and transferred into standard vials (5 flies of each sex/vial) containing SY10 media. They were subsequently maintained in either 12hr:12hr light-dark (control) conditions or in constant darkness for 14 days, during which time they were transferred to new SY10 media every 2-3 days. After this 14-day acclimation period, fecundity was measured by maintaining each group of flies in their corresponding light/dark conditions and transferring flies to new media daily, after which the number of eggs laid in each vial was recorded.

Feeding assay

To determine the effects of visible light on feeding we performed a modified version of the ConEx blue feeding assay where only excreted blue is measured (Shell, Schmitt et al. 2018). Adult male and female flies (aged 2-3 days post-eclosion) were collected from controlled larval cultures and mating conditions (see above), sorted, and transferred into standard vials (15 flies of each sex/vial) containing SY10 media. Flies were aged 21 days in their respective light environments. Afterward, flies were transferred to new empty vials, which were topped with plastic caps containing SY10 media with 1% FD&C Blue #1. Flies were allowed to feed on dyed food for 24 hours, after which they were frozen for subsequent analysis (see also, ref (Shell, Schmitt et al. 2018)). Briefly, flies were removed from each vial and the food cap was discarded. Milli-Q water (3 ml) was added to each vial, after which vials were covered (using parafilm) and vortexed. 200 uL of solution was transferred into a flat bottom 96 well plate, and absorbance at 630nm was determined for each well using a BioTek Senergy 2 microplate reader and Gen5 software. Absorbance values were converted to micrograms of food consumed per fly by interpolating a standard curve.

Statistics

Group and pairwise comparisons on survivorship data were performed using the DLife computer software, and the statistical software R (Linford, Bilgir et al. 2013). P-values for survivorship data were obtained using log-rank tests. Pairwise comparisons for feeding, fecundity, circadian period, and rhythmicity, and activity data were evaluated using a two-sided independent-samples t-test. Group comparisons of circadian period data were evaluated with a one-way ANOVA. All non-lifespan statistical calculations were run in the statistical software OriginPro. For all box plots, box represents Standard Error of the Mean (SEM, centered on the mean), whiskers represent 10%/90%, and the horizontal line represents the median.

Acknowledgements

We would like to thank the members of the Pletcher Lab who provided input on the experimental design and analysis. In particular, we thank BY Chung for his critical advice. This research was supported by US National Institutes of Health (R01 AG051649, R01 AG030593, R01 AG063371 S.D.P.), the Glenn Medical Foundation (to S.D.P.), and the University of Michigan Career Training in the Biology of Aging Training Grant (T32-AG000114, to J.C.J.).

Author Contributions

J.C.J. and S.D.P. conceived the project and designed the experiments; J.C.J. and H.M.R. performed the experiments J.C.J. and S.D.P. wrote the paper.

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Figures

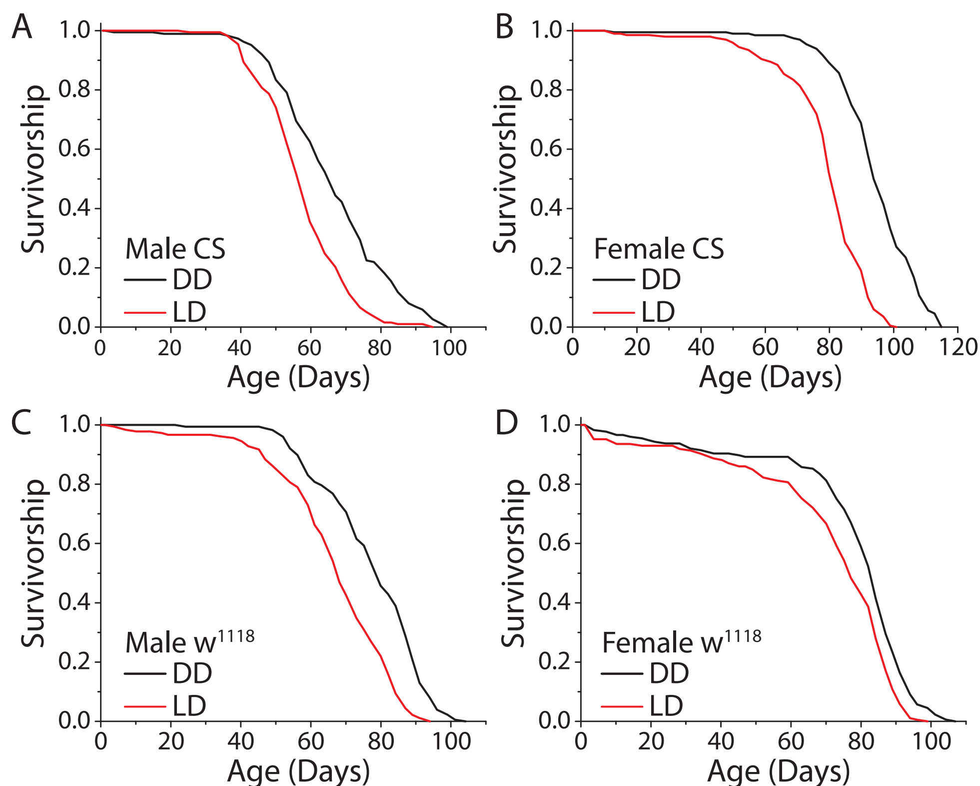


Figure 2.1. Constant darkness increases fly lifespan
(A) Removing flies from a standard 12hr:12hr light cycle (LD) and housing in constant darkness (DD) increased fly lifespan in WT Canton-S (CS) male flies (LD n = 197, DD n =188; $P < 0.0001$). (B) This affect was robust and replicated in female flies (LD n = 200, DD n = 196; $P < 0.0001$). (C, D) Light causes significant lifespan shortening in a second laboratory strain w^{1118} strain. Both male (C) (LD n = 181, DD n = 171; $P < 0.0001$) and female (D) (LD n = 186, DD n = 176; $P = 0.00017$) flies showed lifespan extension when aged under DD as compared to LD conditions.

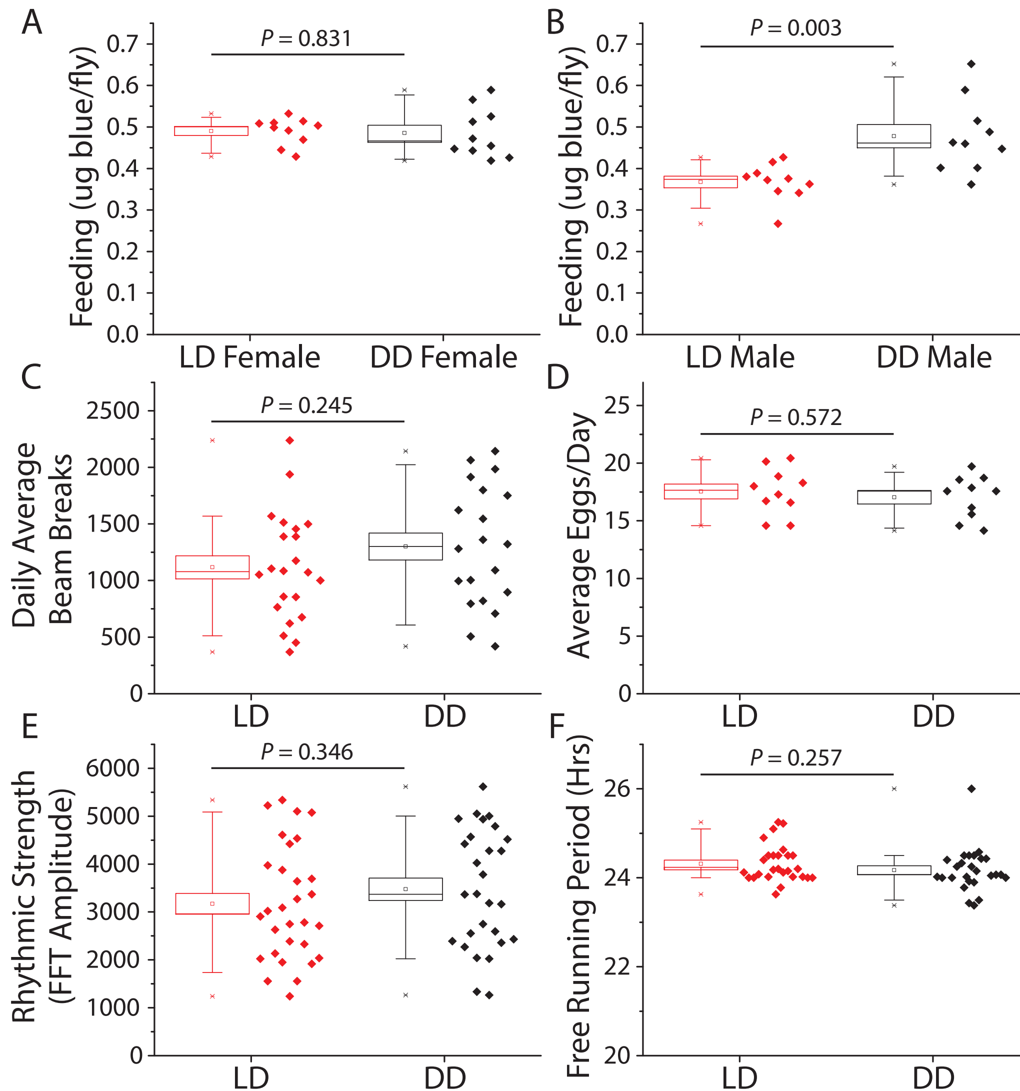


Figure 2.2. Constant darkness has no effect on feeding, locomotion, fecundity, or circadian function.

Several aging-related behaviors were measured after 14 days (activity and fecundity) or 21 days (feeding and circadian measures) under LD or DD conditions. (A) Female dye labeled food consumption over 24 hours was not significantly different in dark reared flies as measured by dye excretion after (n = 10 per treatment group of 15 flies each, P = 0.83). (B) Males reared and kept under dark conditions ate, as measured by dye excretion, significantly more than those under LD conditions (n = 10 per treatment group of 15 flies each, P = 0.003). (C) There was no significant difference in average daily activity as measured by beam breaks in the Trikinetics DAM system over 5 days (LD n = 22, DD n = 20; P = 0.245). (D) Number of eggs laid across 7 days was not significantly altered by a 14 day LD cycle when compared to flies reared in DD (n = 10

per treatment group of 5 females each; $P = 0.572$). (E, F) Circadian health was measured by exposing both male LD and DD pretreated flies to a two-day 12h:12hr LD schedule then placing both under free running (DD) conditions to assess rhythmic strength and free running period. Neither rhythmic strength as measured by FFT amplitude (E) (LD n = 30, DD n = 28; $P = 0.346$) or free running period (F) as measured by chi-square periodogram (LD n = 27, DD n = 26; $P = 0.257$) showed an effect of prior light environment.

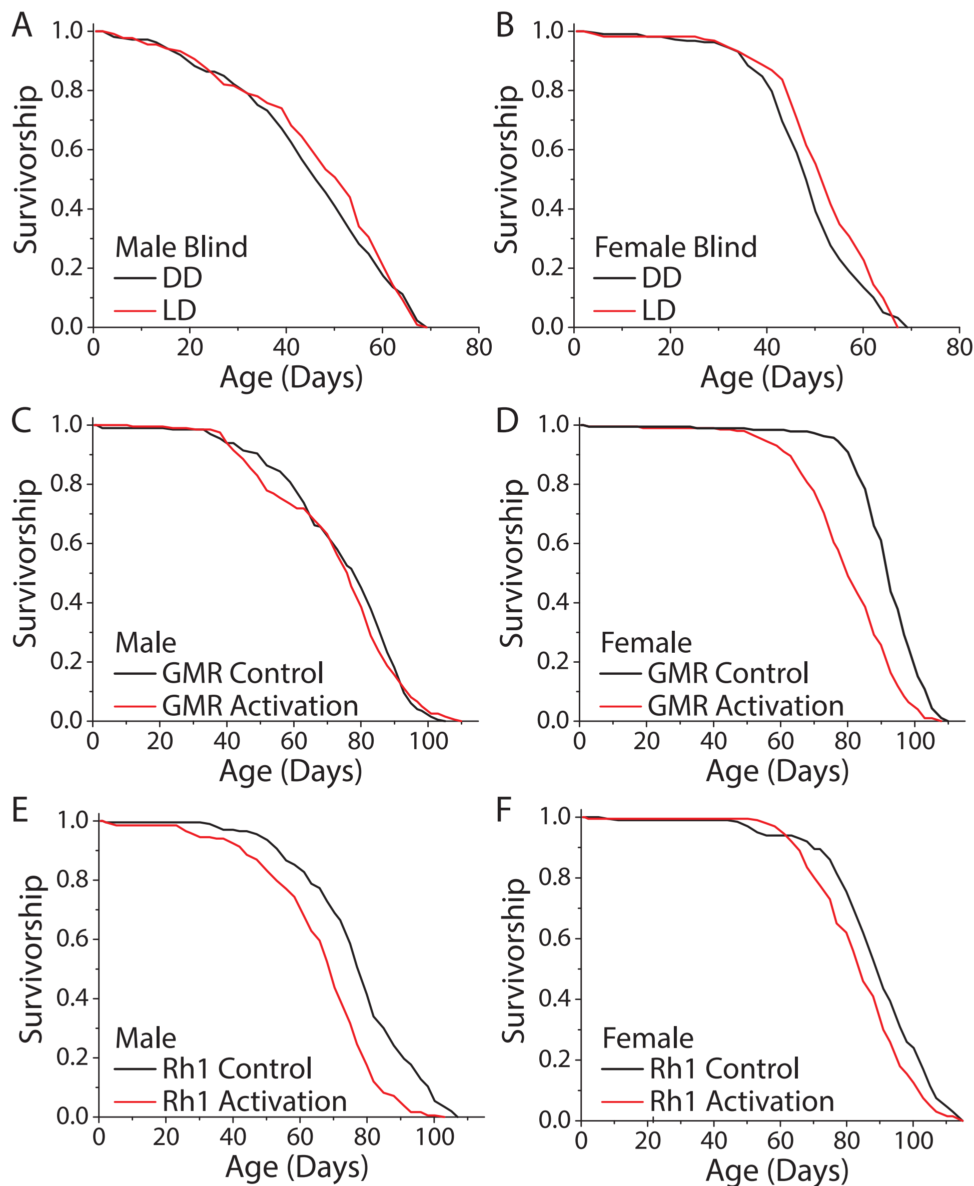


Figure 2.3. Activation of visual neurons is necessary and sufficient to mediate the dark lifespan extension.
(A, B) Flies carrying two copies of the *GMR:hid* transgene, which lack light perception, failed to exhibit an extended lifespan in constant darkness (A) males (LD n = 213, DD n = 223; $P = 0.288$) and (B) females (LD n = 222, DD n = 217; $P = 0.006$). The next 4 panels were conducted in an environment meant to mimic light perception in a standard 24-hour day. Flies carrying a copy of a temperature sensitive cation channel (*UAS-TrpA1*) were used to obtain neuronal activation when at 29°C, and the Gal4 lines were used as background controls. Flies were aged in constant darkness with temperature oscillating 12hr:12hr, 18°C:29°C. (C, D) When aged in constant darkness, activation of *GMR*-

expressing neurons had no effect in male flies (C) (*GMR-Gal4 x* w^{1118} n = 200, *GMR-Gal4 x UAS-TrpA1* n = 198; $P = 0.388$) but was sufficient to shorten lifespan in females (D) (*GMR-Gal4 x* w^{1118} n = 189, *GMR-Gal4 x UAS-TrpA1* n = 202; $P < 0.0001$). (E, F) Similarly, spatiotemporal activation of blue light photoreceptor *Rh1* neurons was sufficient to cause a significantly shorter lifespan. This was observed in both male (E) (*Rh1-Gal4 x* w^{1118} n = 202, *Rh1-Gal4 x UAS-TrpA1* n = 203; $P < 0.0001$), and female flies (*Rh1-Gal4 x* w^{1118} n = 200, *Rh1-Gal4 x UAS-TrpA1* n = 200; $P = 0.0002$).

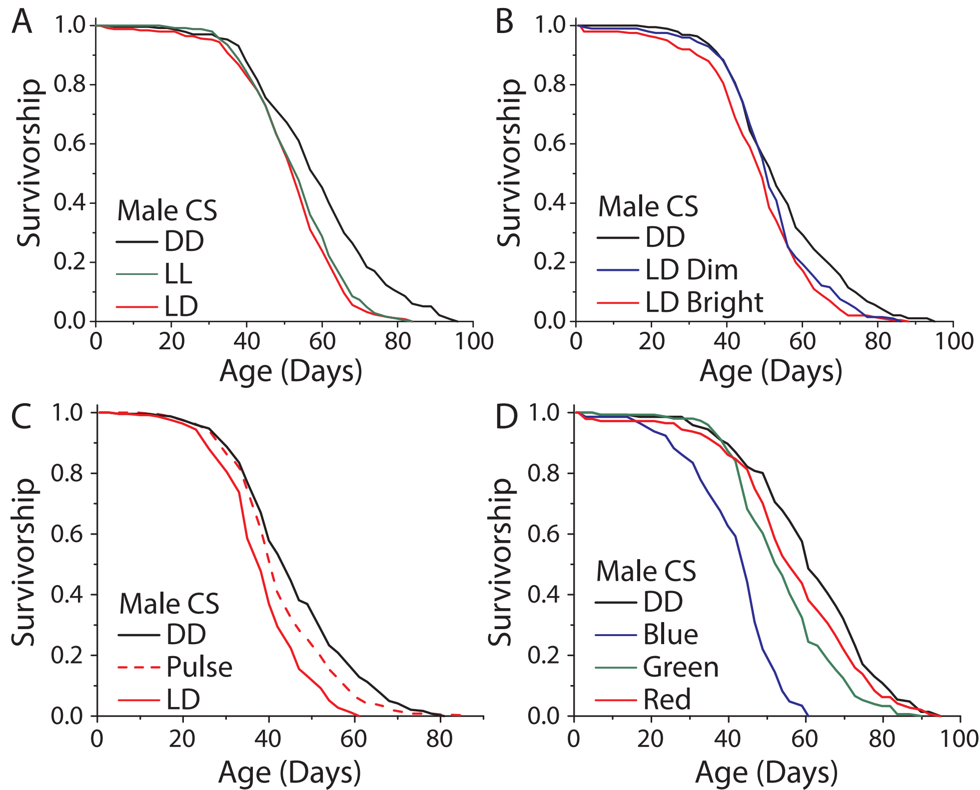


Figure 2.4. Light induced damage alone does not account for the dark lifespan extension.

(A) Male flies exposed to either 12 hours of light daily or constant light (LL) were significantly shorter lived than those aged under DD (LD n = 251, LL n = 247, DD n = 234; *P* < 0.0001). However, there was no significant difference between flies aged under 12 and 24 hours of light (*P* = 0.0304). (B) Similarly, there was a significant lifespan shortening effect when flies were aged under 300 and 1050 lux and compared to DD aged flies (300 lux n = 198, 1050 lux n = 200, DD n = 192; *P* = 0.0003). When making pairwise comparisons to DD there was a significant effect of both 1050 lux (*P* < .0001) and a significant effect of 300 lux (*P* = 0.018). (C) When exposed to either LD or two, one-hour light pulses a day there was a significant light effect (LD n = 251, light pulse n = 240, DD n = 249; *P* < 0.0001). LD exposed flies were significantly shorter-lived than light pulse exposed flies (p < 0.0001) and light pulse exposed flies were significantly shorter lived than DD (*P* < 0.0051). (D) When flies were aged under monochromatic light, there was a significant effect of wavelength on lifespan (blue n = 145, green n = 151, red n = 145, DD n = 146; *P* < 0.0001). Blue, green, and red light-exposed flies were each significantly shorter-lived than those kept in constant darkness (blue *P* < 0.0001, green *P* < 0.0001, and red *P* = 0.048).

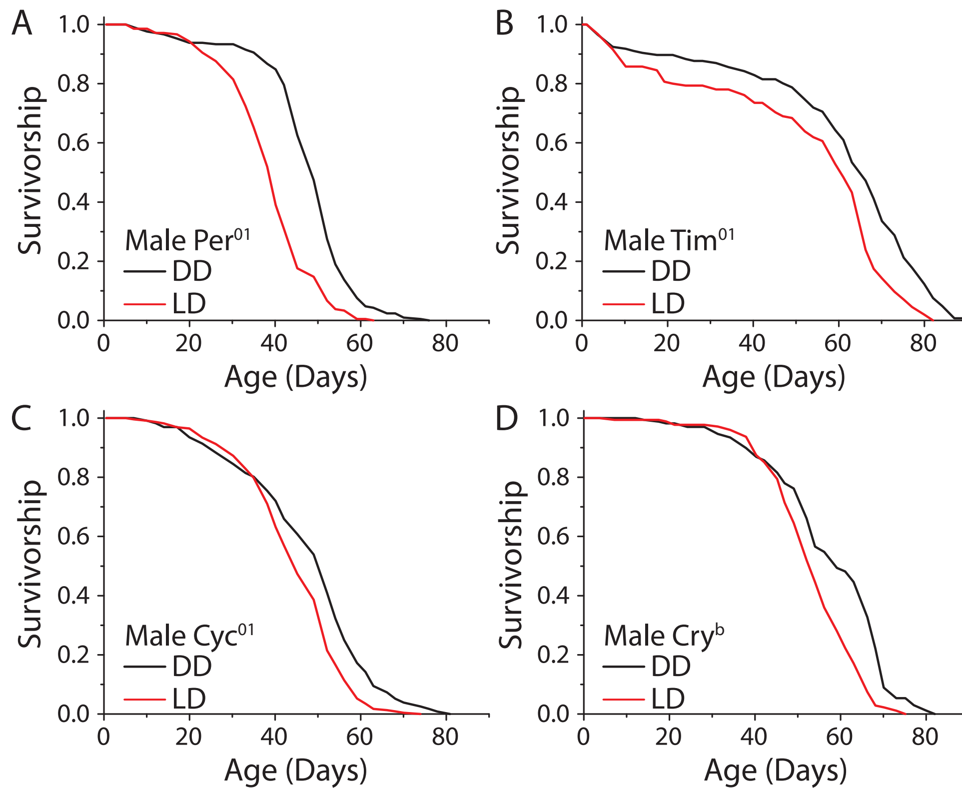


Figure 2.5. The molecular circadian clock is dispensable for extended lifespan in constant darkness.

Loss of function mutations in the molecular circadian clock were assessed for their effect on the dark lifespan extension. (A) *Per^{01}* flies showed a significant lifespan effect when aged under DD conditions (LD n = 220, DD n = 211; $P < 0.0001$). (B) *Tim^{01}* mutants were also significantly longer-lived under DD conditions (LD n = 155, DD n = 146; P = 0.00026). (C) *Cyc^{01}* flies showed a significant lifespan extension when aged under DD conditions as compared to LD (LD n = 228, DD n = 232; $P < 0.0001$). (D) *CryB* flies also showed a lifespan extension when aged in DD conditions (LD n = 175, DD n = 168; $P <$ 0.0001).

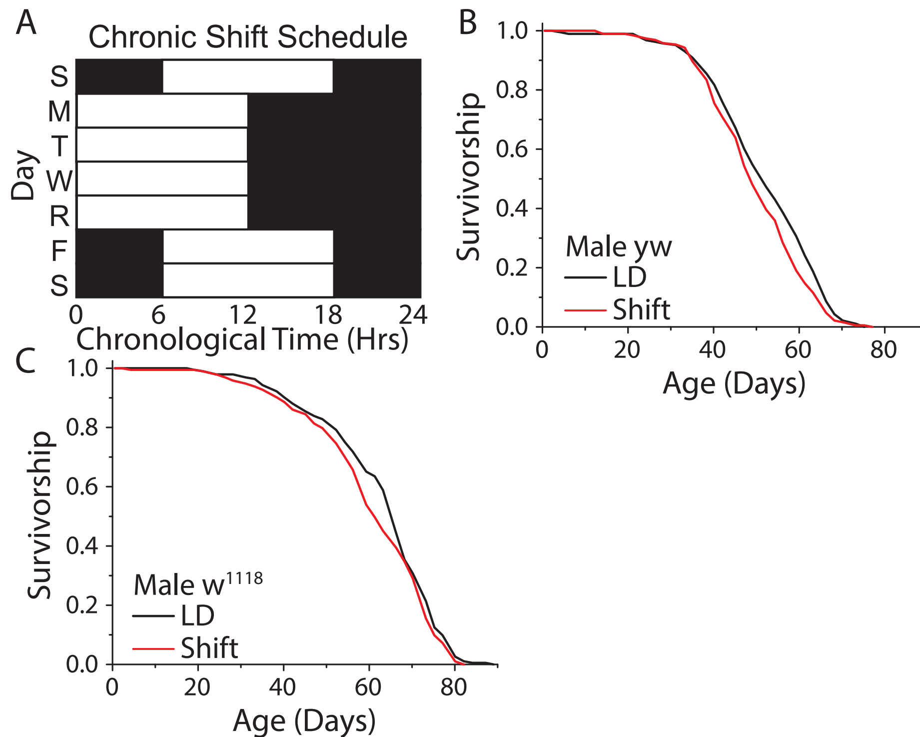


Figure 2.6. A weekly 6hr phase advance and delay had no influence on *Drosophila* lifespan.

(A) Experimental design used to subject flies to a light cycle similar in nature to frequent jet lag, or a shift worker who works 4 days a week. Flies were subjected to a six-hour phase advance, then four days later a six-hour phase delay, with individual days always having 12hr:12hr light:dark schedule. (B, C) The shifting light schedule had little to no effect on male WT lifespan, in both *Yellow White* (B) (12:12 LD n = 188, shift-schedule n = 193; $P = 0.036$) and w^{1118} (C) (12:12 LD n = 195, shift-schedule n = 194; $P = 0.099$).

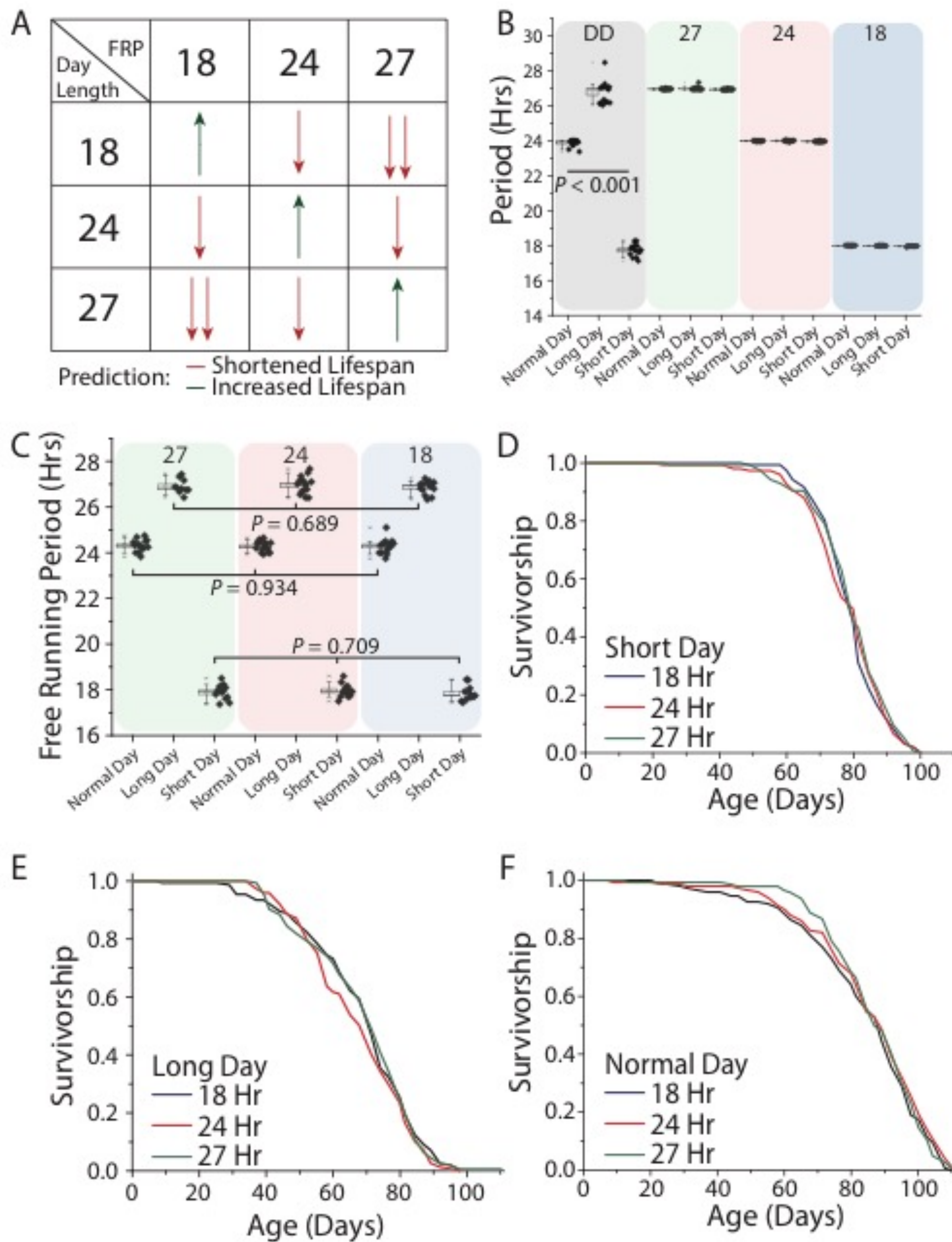


Figure 2.7. Uncoupling between light schedules and the circadian clock does not affect *Drosophila* lifespan.

(A) Experimental design and lifespan predictions when *Drosophila* with free running periods (FRPs) of 18, 24, and 27 hours were exposed to corresponding light cycles. We predicted as day length further deviates from FRP, that lifespan will be negatively impacted (red arrows), and having a FRP that corresponds with the day length be beneficial to lifespan (green arrows). (B) Free running period of 7 day old flies of the

genotypes used in the lifespan experiments (grey quadrant), and activity period when exposed to the three light cycles used (green, red and blue quadrants). *Clk856-Gal4 x UAS-DBT^S* (short day) exhibited a mean period length of 17.8 hours (SD= 0.34) and *Clk856-Gal4xUAS-DBT^L* (long day) showed a mean period length of 26.8 hours (SD = 0.61). Normal-day *CLK856-GAL4 x w^{1118}* (normal day) had a period length of 23.9 hours (SD= 0.19). When exposed to environmental light all flies had an activity rhythm corresponding with the photoperiod. Light cycle day length during recording period is denoted at the top of each colored box. (C) After three weeks under light cycles all genotypes were placed in free running conditions and all genotypes reverted to their endogenous free running period. When comparing within a genotype there were no effects of rearing photoperiod, *Clk856-Gal4 x w^{1118}* (27 hr n = 15, 24 hr n = 16, 18 hr n = 15; *P* 0.934), *Clk856-Gal4 x UAS-DBT^L* (27 hr n = 10, 24 hr n = 15, 18 hr n = 14; *P* 0.689), and *Clk856-Gal4 x UAS-DBT^S* (27 hr n = 12, 24 hr n = 13, 18 hr n = 12; *P* 0.709). Previous light cycle day length is denoted at the top of each colored box. (D, E, F) Lifespan of *Clk856-Gal4 x UAS-DBT^S*, *Clk856-Gal4 x UAS-DBT^L*, and *Clk856-Gal4 x w^{1118}* was not influenced by environmental light cycle, with all genotypes showing no significant effect of light cycle on lifespan (D) short (18 hr n = 157, 24 hr n = 151, 27hr n = 157; *P* = 0.615), (E) long (18 hr n = 153, 24 hr n = 149, 27hr n = 155; *P* = 0.407), and (F) Gal4 control (18 hr n = 148, 24 hr n = 150, 27hr n = 143; *P* = 0.554).

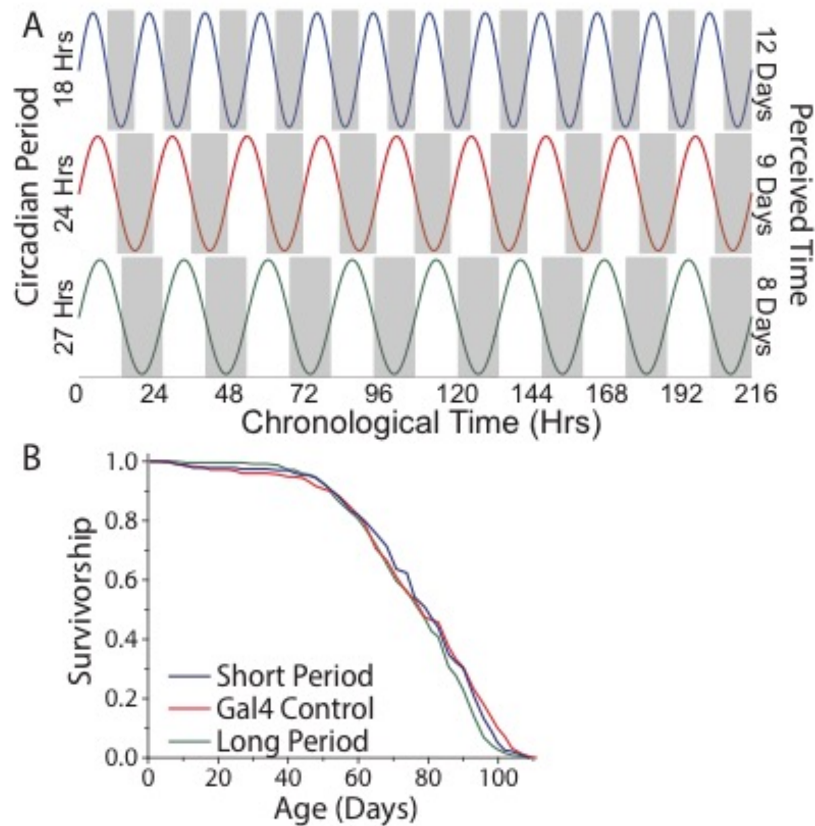
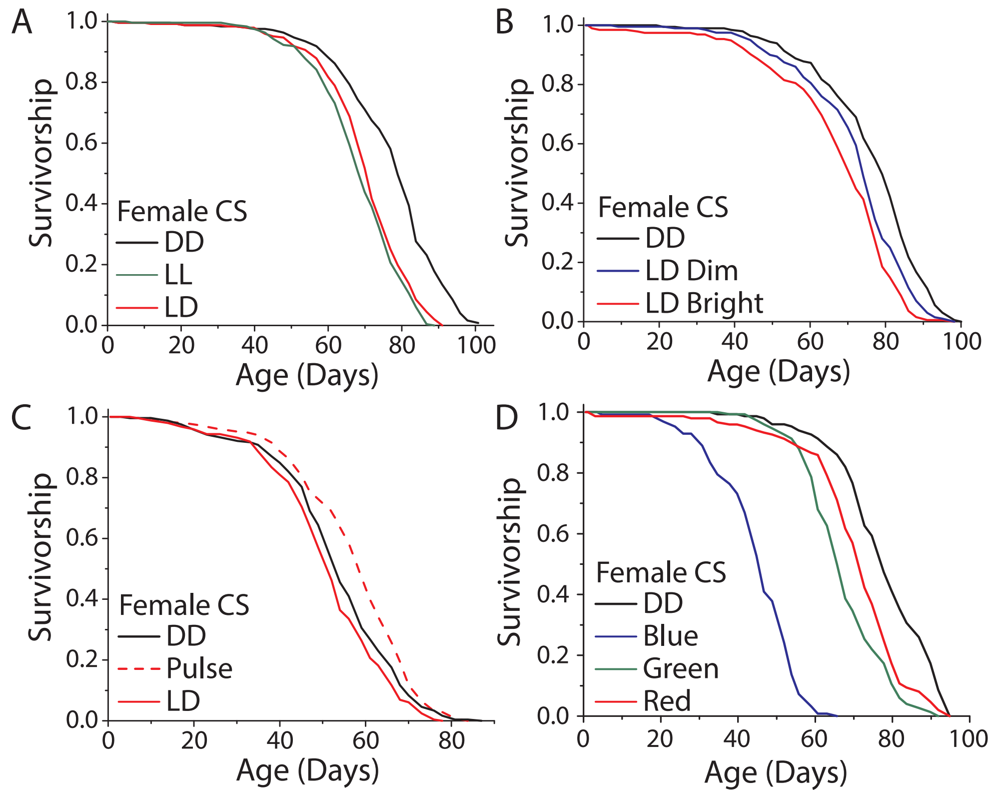
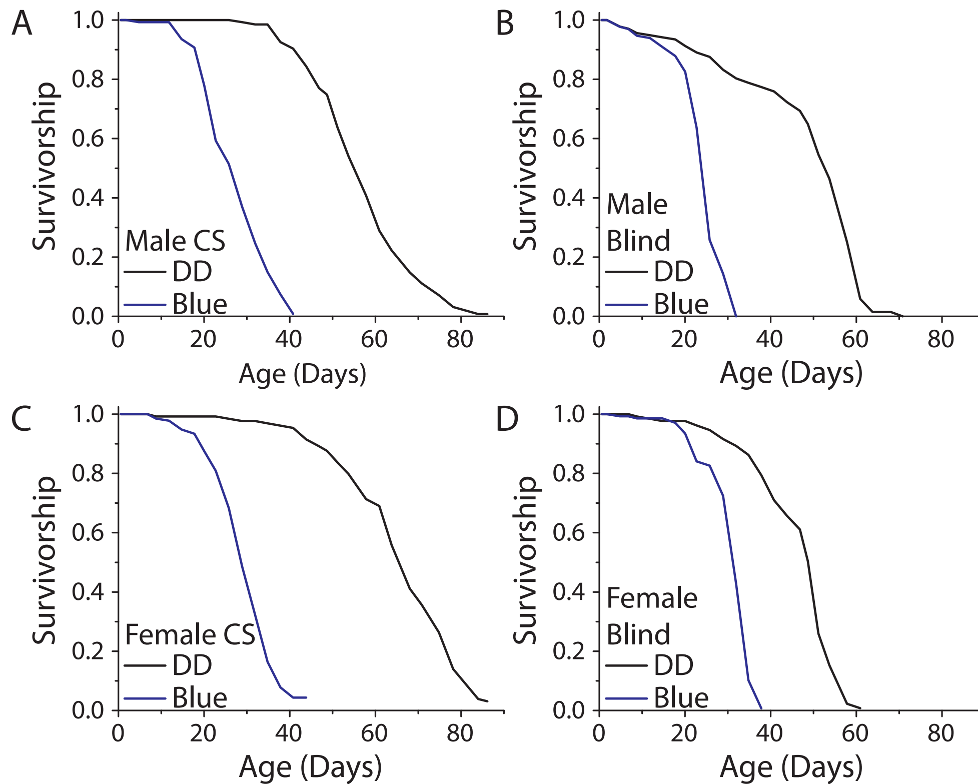


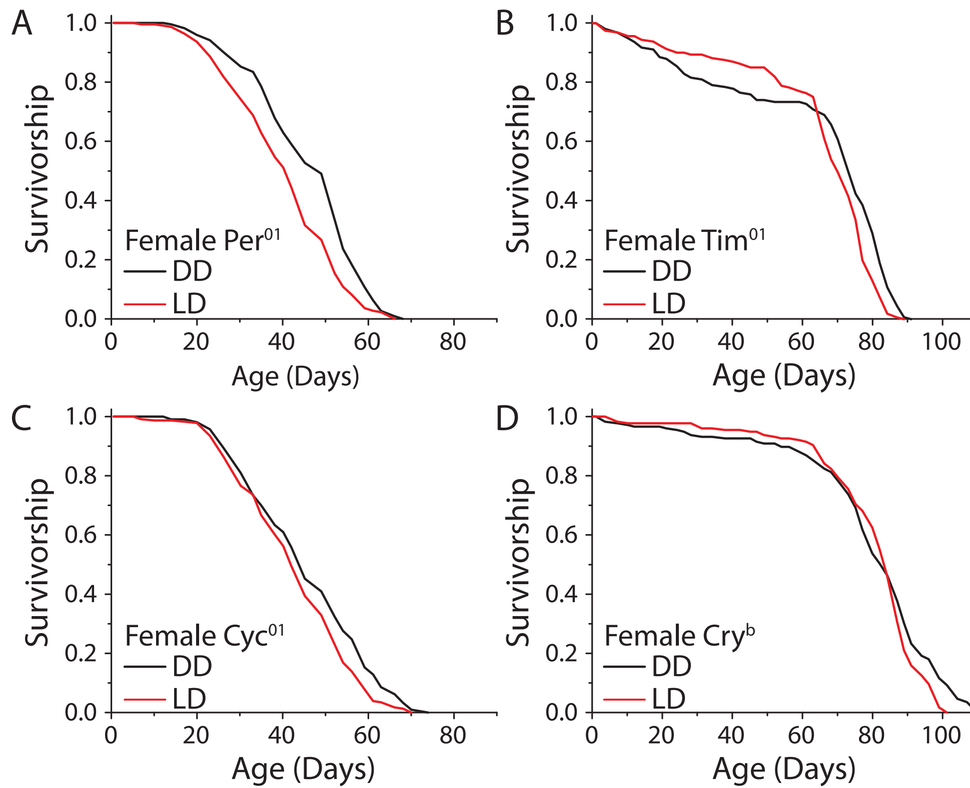
Figure 2.8. Lifespan is independent of the number of subjective days lived.
(A) Relationship between chronological time and perceived days for short-, normal-, and long-day flies. (B) Free running period had minimal effect on lifespan. Animals are aged under free running conditions and a comparison across genotypes was made (short period n = 237, long period n = 252, gal4 control n = 245; $P = 0.022$).



Supplemental Figure 2.1. Light-induced damage alone does not account for the dark lifespan extension.
(A) Female flies exposed to either 12 hours of light daily or constant light (LL) were significantly shorter lived than those aged under DD (LD n = 247, LL n = 246, DD n = 246; *P* < 0.0001). However, in females there was a significant difference between flies aged under 12 and 24 hours of light (*P* = 0.0467). (B) Similarly, there was a significant lifespan effect when flies aged under 300 and 1050 lux or DD (300 lux n = 200, 1050 lux n = 195, DD n = 197; *P* < 0.0001). When making pairwise comparisons there was a greater effect of 1050 lux (*P* < 0.0001), and still a significant effect of 300 lux (*P* < 0.0001). (C) When exposed to either LD or two, one-hour light pulses a day there was a significant light effect (LD n = 247, light pulse n = 248, DD n = 239; *P* < 0.0001), however in females the light pulse exposed flies were longer lived (*P* = 0.003). LD exposed flies were still significantly shorter lived than DD exposed flies (*P* < 0.0253). (D) When flies were aged under monochromatic light there was a significant effect of wavelength (blue n = 152, green n = 150, red n = 149, DD n = 150; *P* < 0.0001).



Supplemental Figure 2.2. Blue light shortens lifespan in sighted and blind *Drosophila*.
Flies were exposed to bright monochromatic blue light on a 12hr:12hr LD schedule or kept under dark conditions. (A, B) Male Canton-S and the blind *GMR:hid* flies lifespan was significantly shortened when aged under monochromatic blue light (CS Blue LD n = 140, CS DD n =135; $P < 0.0001$); (*GMR:hid* Blue LD n = 135, *GMR:hid* DD n =138; $P < 0.0001$). (C, D) Female Canton-S and *GMR:hid* flies also showed significant lifespan shortening when aged under monochromatic blue light (CS Blue LD n = 136, CS DD n =130; $P < 0.0001$); (*GMR:hid* Blue LD n = 138, *GMR:hid* DD n =130; $P < 0.0001$). Plots that end above 0% survival include animals that escaped and were censored prior to the end of the experiment.



Supplemental Figure 2.3. In females, components of the molecular clock may be required for the dark lifespan extension.

Mutations in the molecular circadian clock were assessed for their effect on the dark lifespan extension. (A) *Per^01^* flies showed a significant lifespan effect when aged under DD conditions (LD n = 221, DD n = 224; $P < 0.0001$). (B) *Tim^01^* mutants are not significantly longer lived under DD conditions (LD n = 161, DD n = 158; $P = 0.103$). (C) *Cyc^01^* flies showed small, but still significant lifespan extension when aged under DD conditions as compared to LD (LD n = 231, DD n = 210; $P = 0.0047$). (D) *CryB* flies also showed a similar small but significant lifespan extension when aged in DD conditions (LD n = 176, DD n = 176; $P = 0.03$

Chapter 3

Social Perception Mediates Behavior and Starvation Survival

Introduction

Sensory perception has numerous impacts on organismal life history, from influencing energy homeostasis, tissue physiology, and even dictating lifespan, perception is integral in how organisms interact with each other and the environment. Apfeld and Kenyon first showed ciliated sensory neuron ablation was capable of extending lifespan in 1999 (Apfeld and Kenyon 1999). Since then, the effects of sensory manipulation on physiology and lifespan have been well characterized across sensory modalities and found to include both biotic factors such as diet/nutrients, pain, the opposite sex, and dead conspecifics, and abiotic factors such as hygrosensation and temperature (Lee and Kenyon 2009, Gendron, Kuo et al. 2014, Riera, Huising et al. 2014, Waterson, Chung et al. 2014, Ro, Pak et al. 2016, Chakraborty, Gendron et al. 2019). One biotic factor that has not received much attention is perception of conspecifics, or social perception.

Social perception is a non-canonical and not often thought of perceptual modality which has demonstrated impact on many aspects of life. Most all animals display some semblance of social behavior, even if just by virtue of needing to increase biological success by finding mates. Benefits of sociality can also include reducing metabolic rate, decreasing stress responses, and lowering disease risks (Behringer, Butler et al. 2006, Apfelbeck and Raess 2008, Nadler, Killen et al. 2016). Though *Drosophila* are classified as a solitary species, they show many social behaviors, from cooperative feeding behaviors as larvae and adults (Dombroski, 2017)(Tinette, Zhang et al. 2004), to aggressive

territorial displays (Ueda, 2002), and sophisticated courtship and mating displays (Pavlou, 2013). Further, social isolation leads to shortened lifespan and increased aggression in *Drosophila (Ruan and Wu 2008, Zhou, Rao et al. 2008)*. Courtship is perhaps the most studied of all social behaviors, and the costs associated with reproduction have been well characterized and described across numerous taxa (Reznick 1992). Organisms from simple nematodes, (Van Voorhies 1992) brine shrimp (Browne 1982), and fruit flies, (Smith 1958) to mammals like rodents (Koivula, Koskela et al. 2003), primates (Hoffman, Ruiz-Lambides et al. 2008, Blomquist 2009) and even humans (Westendorp and Kirkwood 1998, Dallerac, Labeur et al. 2000, Min, Lee et al. 2012) all show costs of reproduction. While much is known about reproduction tradeoffs less is known about how social perception, particularly density, may influence lifespan, physiology and behavior.

Both intra- and inter-species competition can be significant drivers in determining life history strategies as resource scarcity doesn't allow for adequate distribution of resources to all areas organisms could utilize (Shaw, Brain et al. 2018). Thus, organisms are likely to utilize limited resources differently based on social situation and population density, and there exist real benefits of social perception. The most often cited example of such resource allocation is the tradeoff between survival and reproduction (Flatt 2011). Aside from reproductive tradeoffs we know *Drosophila* are attuned to other social environmental factors, such as success of similar *Drosophila* species in the environment. Our lab has shown flies are capable of visually recognizing dead flies of the same species, and this is met with shortened starvation survival, reduced fat stores, and decreased overall lifespan (Chakraborty, Gendron et al. 2019). Further, flies exposed to dead flies use olfaction to communicate the potential hazards to naïve flies which then show an avoidance behavior, likely due to the potentially noxious environment that caused the flies to die. Flies also choose to tailor their

environment to optimize health. Isolated flies with tumors live shorter than those kept in groups, and the same cancerous flies are more attracted to tumorless flies than other cancerous flies (Dawson, Bailly et al. 2018). These examples illustrate how social perception provides information about environment quality and lead to changes in life history strategy.

Since social perception involves integrating many sensory modalities, it is likely many sensory inputs allow a fly to determine the density of the environment and allow the species to choose the optimal condition. *Drosophila* make an excellent organism to study social perception since they have both a relatively simple nervous system, and well-characterized genetics, yet exhibit complex behaviors. *Drosophila* use all of the traditional five senses: vision, scent, taste, hearing and mechanosensation, in characterizing the social environment. Vision is used to reinforce cooperative group feeding behaviors (Dombrovski, Kim et al. 2019). Scent is used in group colonization and selection of an optimal environment (Lof, de Gee et al. 2009). Hearing is used in detecting courtship songs and driving mating decisions (Albert and Gopfert 2015). Lastly mechanosensation is used in larval conspecific discrimination (Otto, Risse et al. 2016). Further refining what sensory modalities are key for changing physiology in response to social situation will aid in elucidating pathways by which health may be modulated when organisms have to be isolated or group housed.

In this section, we begin by showing how a well investigate sensory modality, pheromone perception, can be used to uncover neural circuits that influence lifespan in a context-dependent manner. Specifically, we show how corazonin, the invertebrate equivalent of gonadotropin releasing hormone (GNRH), is required for mating recue of pheromone perceptions' effects on lifespan. We then document how density and social perception leads to behavioral and physiological consequences. We do this by exploring

both social isolation and increased densities in an attempt to dissect the perceptual modalities that mediate social perception. We show that *Drosophila* are sensitive to social environment, and change physiology in response to isolation. We also show the threshold at which *Drosophila* begin to recognize they are not isolated. Social crowding and crowd perception had little impact on longevity, and smell but not vision was causal in social perception phenotypes.

Results

Female pheromone exposure shortens lifespan in male flies and mating rescue of this phenotype is dependent on the neuropeptide corazonin

In order to establish how social perception may lead to mechanistic insights on how perception influences longevity, we started by investigating social perception in a well-established model, that of pheromone perception and the costs of reproduction. Costs of reproduction were once thought to be due to a life history choice: invest resources in procreating or in maximizing lifespan. However, recent work shows costs of reproduction are not solely due to physiological or energetic tradeoffs that come from limited resource allocation to somatic tissues or reproduction (Wit, Sarup et al. 2013). Recently our lab published that in male flies the costs of reproduction are partly due to the males' perception of female pheromones (Gendron, Kuo et al. 2014) but the mechanism is not yet well understood. In females however, costs of reproduction are due to the sex peptide transferred along with seminal fluids during copulation (Wigby and Chapman 2005). No similar molecular or neural mechanism is known in male flies, but we do know the costs of pheromone exposure can be ameliorated by mating (Harvanek, Lyu et al. 2017).

We sought to elucidate the mechanism by which mating rescues the negative effect of female pheromone exposure on male lifespan. We hypothesized pheromone exposure leads to an expectation, that mating will occur, and that failing to meet this expectation results in shortened lifespan. In order to test this hypothesis, we measure the lifespan of experimental male flies exposed to pheromone donor flies of both sexes (Fig 3.1a). The donor flies expressed either their natural pheromone profiles or those of the opposite sex, meaning we have male flies expressing a female pheromone profile, and female flies expressing a male pheromone profile in addition to flies expressing the pheromone profile indicated by their sex. This allows us to control for both ability to mate, as the experimental males won't be able to make with male flies expressing female pheromone profiles, and pheromone perception. To measure effects of mating without changes in pheromone perception, we compared experimental males housed with either male or female donor flies that expressed the same pheromone profiles. Then to measure lifespan effects of sexual perception independent of mating, we compared experimental males housed with male donor flies that expressed pheromone profiles characteristic of the same or the opposite sex.

Since we hypothesized the sensory environment sets up an unmet expectation we believed we could target the neurons necessary for the release of seminal fluid to changes flies' perception of whether or not mating had occurred. To do this we either activated or inhibited the experimental flies corazonin neurons, as corazonin is responsible for seminal fluid expulsion (Tayler, Pacheco et al. 2012). Inhibition of *crz*-expressing neurons in adult experimental males (using a combination of a temperature-sensitive *Gal80* and the potassium rectifying channel *Kir2.1* to inhibit neurons in adult male flies only) (Hardie, Raghu et al. 2001, McGuire, Le et al. 2003) prevented the ability of mating to rescue the deleterious effects of pheromone perception on lifespan (Fig. 3.1b). Activation of *crz*-expressing neurons (achieved by exposing adult *crz-Gal4;UAS-*

TRPA1 males to 29°C) (Hamada, Rosenzweig et al. 2008), on the other hand, potentiated the benefits of mating to the extent that pheromone costs were completely reversed by mating and exposure to female flies had no effect on male lifespan (Fig. 3.1c). Notably, activation of *crz*-expressing neurons was not sufficient to increase lifespan in same-sex cohorts suggesting that this peptide only mediates the effect of mating on lifespan (Fig. 3.1c).

Density perception has minimal effects on lifespan and social environment is capable of modulating behavior and physiology

We asked whether flies lifespan would be altered through changing perceived density, with the hypothesis that a greater perceived density would indicate more competition and lead to a shortened lifespan. There has been much theoretical speculation on how population density affects longevity, with the main theories speculating that increased density might shorten lifespan through resource competition, or do the opposite and cause increased lifespan in a dietary restriction like manner (Graves and Mueller 1993). While it has been shown that there is an optimal density threshold that needs to be met optimal for larval development and lifespan, little is known about how adult density is capable of modulation longevity (Luckinbill and Clare 1986, Sorensen and Loeschcke 2001). We therefore sought to determine if lifespan would be altered with a higher perceived social density. In order to alter perceived density, we glued mirrors to the top of the vials. The mirrors create a visual sensory environment as if there is double the density of flies. In order to maximize any potential effects, we used vials with flies at a higher density (45 flies per vial) than standard (20 or 25 flies per vial). The mirrored vials did cause a small but significant lifespan effect in both sexes, indicating there may be a density perception dependent lifespan phenotype (Fig 3.2 a, b). However, the effects were small (4.7% median lifespan extension in females and 10.7% in males) and would not be large enough to hold up to investigation using genetic techniques.

Further, the flies were shorter lived than those reared at a lower density. It is also possible the lifespan effect was not due to the visual change resulting from the mirrors. To determine if vision was causal we repeated the experiment, and included a control that was reared in constant darkness. We also reduced the number of flies per vial to a more typical density of 25 flies. In order to increase sample size only females were used. We again saw a small, yet significant mirrored vial effect in LD, and under dark conditions the trend was actually reversed (Figure 3.2 c). These data indicate vision likely plays a minor role in density perception but a more thorough investigation is necessary.

We next intuited density and social perception may not have had a large effect at such high numbers as the signal could effectively have been maxed out under the standard vial conditions of 25 flies per vial. We set out to determine the number of flies required to show an effect of being group housed. In many species locomotor behavior is altered when reducing the number of animals housed together. In vertebrates including fish (Gomez-Laplaza and Morgan 1991), mice (Essman 1968), rats (Korn and Moyer 1968) and even primates (Spencer-Booth and Hinde 1966) show that there is often a reduction in locomotion upon social isolation. In insects, less in known about how social isolation changes locomotor behavior. We sought to determine if locomotor behavior could be used as a method to determine if flies were perceiving different densities. To determine at what population density *Drosophila* behave as if they are group housed, and explore how isolation affects behavior we isolated flies late in the pupal stage, then upon eclosion kept them isolated or group housed them in groups of 2, 5 or 10. We then aged the flies for 10 days before exposing them to an open field arena to measure behavior. We observed a significant effect of housing density, and flies that were isolated or kept in groups of only two had a significantly greater distance traveled and more time spent moving when compared to those housed in groups of 5 (Fig 3.3a, b). Though these

experiments were preliminary, they did indicate locomotor behavior is influenced in a density dependent manner. Our data do not correspond with vertebrate literature where isolation reduces locomotion, but there is evidence suggesting this may be in line with other work performed on isolated insects (Liu, Nath et al. 2018).

In an attempt to determine if these behaviors are characteristic of a depressive or anxiety like state induced by social isolation we also looked at center crosses in an open field and a forced swim test. Both measures have been used to assay anxiety and depressive behaviors in murine mammals (Kitada, Miyauchi et al. 1981, Choleris, Thomas et al. 2001). Although there was a trend toward increased center crosses in isolated animals, which would signify increased risk-taking behavior, there was no statistical significance (Fig 3.3c). We would benefit by repeating these experiments with greater sample sizes. In addition, isolation had no significant effect on time spent struggling during the forced swim test, or on time spent immobile after a forced swim test (Fig 3.3d). Taken together the behavioral changes in the form of increased mobility and trends toward decreased risk-taking behavior indicate social density has impacts on behavioral state.

***Drosophila* recognize conspecifics through scent and independently of vision**

To determine which sensory modality is used to perceive conspecifics, experimental flies were isolated as pupae and aged 7-8 days. The flies were then transferred to clear, polycarbonate capillary tubes with an air impermeable cap at one end and cotton plug at the other. The tubes were attached to empty vials or vials containing a group of 15 age- and sex-matched conspecifics (exposure flies). The orientation of the capillary tube in the vial allowed us to control for sensory environment (Fig 3.4a). Flies spent 2 days on SY10 food in the exposure condition before being used in behavioral or survival assays.

To determine if social isolation had an effect on physiology we used starvation resistance as a short term and reasonably high throughput assay. Starvation resistance is a suitable assay due to its short experimental turnaround time and links to fat stores, metabolism, and longevity (David, Cohet et al. 1975, Rose, Vu et al. 1992). In order to test the effects of social perception in the context of starvation flies placed on 2% agarose and given the group sensory cues of vision, or vision and olfaction together, control flies were isolated and able to smell the food in the empty vials. Isolated flies of both sexes showed increased starvation sensitivity when compared to flies that were isolated but could perceive both visual and olfactory information from the fly cohort (Fig 3.4b, c). However, there was a sexually dimorphic effect in vision. In male flies, visually perceiving conspecifics resulted in flies being more starvation sensitive than isolation alone (Fig 3.4b). In females, vision caused no additional detriments to starvation resistance when compared with isolation alone (Fig 3.4c). These experiments had the confound that sight and scent were never completely separated, and that the smell of the CT food may influence starvation resistance.

To assay the effects of food odorants and humidity given off by the food we exposed flies to vials that were empty, contained CT food or 2% agar. There was no effect of agar compared to the empty vial so it is unlikely agar smell or humidity effects starvation resistance, however CT food scent caused a lifespan extension (Sup 3.1a). To isolate what component of the CT food was causal flies were exposed to plain 2% agar and compared starvation resistance to those exposed to individual components of CT food in agar. Neither 10% sucrose or 10% yeast had a significant effect over plain agar (Sup 3.1b). Despite that, the antifungal compounds tegosept and propionic acid in agar were capable of producing a starvation survival increase (Sup 3.1b). We posited that as tegosept and propionic acid are not volatile it is likely the ethanol used as a solvent for

tegosept causing the effect. As such, we measured starvation resistance with an agar solution containing the same concentration of ethanol as CT food. Ethanol was sufficient to extend starvation resistance (Sup 3.1c).

With the knowledge CT food components can alter starvation resistance, and seeking to test the sufficiency of smell in group increased starvation survival, we repeated the group exposure starvation; this time including an isolation control for food smell, and a group treatment to test smell alone. Again, flies that had the scent and visual stimuli from a group were longer lived than isolated flies (Fig 3.4d). Smell was both necessary and sufficient to extend survival and the group scent group phenocopied the sight and smell treatment group (Fig 3.4d). Isolated flies exposed to CT food were longer lived than those exposed to room air, confirming our caps are indeed impermeable. Lastly, vision had no effect on starvation resistance as flies exposed to visuals from a group had no change in starvation to isolated flies.

To ease the investigation of future high throughput physiologic screens we developed a simple paradigm to autonomously measure starvation survival and activity. By using Trikinetics DAM monitors in conjugation with a custom R analysis package we are able to continuously measure survival based on activity counts. This assay can be placed in a closed environment or used with an olfactory deliver system to control sensory environment (Sup 3.2a). When monitors containing *Canton-S* flies were delivered odorants from a Tupperware box of containing 400 conspecifics lifespan was extended (Sup 3.2b). However, when the same odorants were filtered with a desiccant, sodasorb to remove CO_2, and cotton filter the lifespan extension effect was mitigated (Sup 3.2b), again implicating olfaction as the sensory que causal in social perceptions' effects on physiology.

Discussion

We discovered the rescue of pheromone perception lifespan shortening by mating was due in part to release of the peptide corazonin. This shows it is beneficial to investigate how social and density perception may influence lifespan and physiology. While we did not see large effects of density perception on lifespan, we were able to determine the population threshold at which isolated flies begin behaving as if they are grouped. Isolated flies displayed some behavioral markers of depressive-like symptoms, but not others, and these symptoms could be rescued by giving the olfactory sensory environment from a group of flies. This work builds on a budding field demonstrating how *Drosophila* may be used to explore how primitive emotions affect behavior and health (Gibson, Gonzalez et al. 2015, Gu, Wang et al. 2019). Past studies have shown unpredictable stressors can induce depressive-like states in flies that manifest in reduced exploration and increased immobility in the forced swim test (Araujo, Poetini et al. 2018). Further, we showed there is a physiological impact of isolation in that starvation survival is reduced. This reduction in starvation resistance can be rescued through olfactory but not visual perception of conspecifics. In addition, we devised a simple and high-throughput assay by which survival can be measured autonomously through activity recordings.

The first major discovery, showing consequences social perception via pheromone exposure can be utilized to identify discrete sets of neurons which can be targeted to provide a longevity benefit and ameliorate the pheromone exposure phenotype. The neurons we identified were those that release corazonin, the peptide required for *Drosophila* ejaculatory response (Tayler, Pacheco et al. 2012). Corazonin, the insect homologue of the mammalian gonadotropin-releasing hormone, is known to be a

mediator of stress, and ablation of corazonin producing neurons has been shown to confer resistance to metabolic, oxidative, and osmotic stress (Zhao, Bretz et al. 2010). These actions may likely be due to corazonin neurons interfacing with *Drosophila* Insulin-Like Peptide (DILP) neurons which are known to regulate survival (Kapan, Lushchak et al. 2012). However, in the context of pheromone exposure silencing corazonin did not allow for mating to be beneficial, and thus shortened lifespan. In the previous studies corazonin silencing or ablation increased survival under stress contexts (Zhao, Bretz et al. 2010). Differential responses may indicate a context-dependent role of corazonin and that mild stressors may, under certain contexts, increase lifespan. Or, perhaps sensory mismatches are detrimental. Pheromone exposure sets the organism up to mate, then when no mating is perceived a sensory mismatch is created. Though corazonin may mediate the effect of other stressors, in this circumstance the two stressors, pheromone exposure and mating, are aligned and thus make biological sense. Therefore, they are no longer detrimental as the sensory mismatch is resolved.

Several lines of evidence support the hypothesis that mismatches between sensory systems cause physiological or lifespan consequences. For example, in our data we show isolation shortens starvation survival, and male flies that can visually perceive a group but not smell them are shorter still. This may be a similar situation where visual information indicates a group but olfaction indicates isolation. Biological benefits to having sensory information aligned may also observed in nutrient and protein sensing. Flies kept on a choice diet, where they are responsible for consuming the carbohydrate and protein macronutrients separately, are shorter lived (Ro, Pak et al. 2016). Part of this may be due to the flies spending more time on one food while eating more of the other. This would involve increased gustation of one food but increased nutrient consumption from the other and thus creates a sensory imbalance. While the neural mechanisms

underpinning such an effect are unknown, it is acknowledged proper excitatory-inhibitory neural balance is required to maximize lifespan, and modulators of neural excitability have potent effects on longevity (Zullo, Drake et al. 2019).

Another key takeaway from this work is that there is a threshold that must be met for flies to perceive conspecifics. Much like there is a working range for drugs, so too do sensory manipulations have an optimal working range. In the case of social perception, it seems isolation phenotypes are rescued when there are at least 5 conspecifics. Other work done in our lab supports that flies are sensitive to perceiving conspecifics in similarly low numbers. In order for flies to elicit an avoidance response to dead conspecifics at least 5 dead flies were required (Chakraborty, Gendron et al. 2019). Yet during the density perception lifespan experiments flies showed little effect of high density perception. There may be several reasons these experiments did not show a lifespan phenotype. First, using mirrors to increase density relies on flies recognizing others through the mirror and responding as if they were conspecifics. Second, though mirrors double the number of flies perceivable by vision the space is also doubled, leaving the flies per volume identical to an un-mirrored vial. Lastly, the density lifespan experiments were performed while we were operating under the assumption vision was causal in social perception, and we went on to show olfactory cues carry signs of conspecifics. Our initial prediction vision would be the modality used to interpret surrounding conspecifics was not without reason; both mammals and insects use vision to recognize kin (Parr 2011, Sheehan and Tibbetts 2011). Only after performing the density lifespan experiments did we discover olfaction, but not vision, was required in determining the starvation resistance change in response to conspecifics, and it is likely a similar mechanism exists for higher densities. This may be an evolutionary conserved phenomenon, as *C. elegans* larval density dependent lifespan is mediated by secreted

factors (Ludewig, Gimond et al. 2017). In future experiments, it would be beneficial to alter the perceived olfactory environment while testing density perception.

Social environment, and thus social perception may be an indicator of the overall ability of an environment to support a species. It follows then that sensing conspecifics thriving could lead to an increased ability to endure starvation, as it serves to indicate favorable environmental conditions are near. *Drosophila* use olfaction to both aggregate, and colonize new areas based on food and fly smells (Lof, de Gee et al. 2009, Lebreton, Becher et al. 2012). It would be interesting to measure whether a metabolic rate change occurs when flies under nutrient-depleted conditions are given odorants of thriving conspecifics. This would show social environment is capable of dictating metabolic state. It would also be worthwhile to measure exploratory behavior in starving animals when given olfactory signals indicating food or thriving flies are nearby. It seems olfactory signals from other animals serve as a stronger or more reliable indicator of environmental quality than olfactory food smells alone. When *Drosophila* were unable to smell environmental yeast odors they were longer lived than flies able to smell the yeast odors (Libert, Zwiener et al. 2007). This is may be an adaptive benefit as conspecific odors indicate an environment can support life, when food odors alone may be misleading. Thus, future research on social perception may benefit by focusing on what metabolic and neurological states are altered in these conditions, and the downstream responses in peripheral tissues.

Methods

Fly husbandry

All *D. melanogaster* used in this paper were reared using the same method. Experimental flies were age-synchronized using a 3-step procedure. First, mated females and males were placed on a grape juice agarose plate supplemented with live yeast paste for 18-22 hours. The eggs laid during this period were collected briefly in PBS, distributed in 32ul aliquots into culture bottles containing a modified Caltech Medium (CT food) (E.B. 1960), and reared at 25°C with a standard 12hr:12hr LD cycle. Second, flies that eclosed within a 24hr window were collected into bottles and maintained on a 10% sucrose/yeast food (SY10) for 2-3 days at 25°C with a 12hr:12hr LD cycle, unless otherwise noted. After the 2-3 day mating period, flies were sorted under light CO_2 anesthesia into single-sex groups of 20 or 25, unless noted otherwise, and placed into vials containing SY10 media, which was changed every 2-3 days for the duration of their lifespans.

Fly strains

The control laboratory stocks *yw*, *w^{1118}*, and *Canton-S* were obtained from the Bloomington Drosophila Stock Center. The *OK72-Gal4* line, *UAS-Kir2.1* line, and *UAS-Gal80^{ts}* line were also obtained from the Bloomington Stock Center. *UAS-TRA* and *UAS-TRA^{DSRNA}* were provided by B.J. Dickson. The *Crz-Gal4* line was provided by E. Johnson. The *UAS-TrpA1* line was provided by P. Garrity.

Media recipes

The modified CT food recipe is as follows: 1 L water, 10 g agar, 6 g cornmeal, 30 g sucrose, 55 g dextrose, 45 g yeast, 15 mL tegosept solution (20% tegosept in 90% ethanol), 3 mL propionic acid. Sugar-yeast 10% (SY10) food recipe is: 1 L water, 20 g agar, 100 g sucrose, 100 g yeast, 15 mL tegosept solution (20% tegosept in 90% ethanol), 3 mL propionic acid, and 4 mL antibiotic supplement (1% tetracycline and 2.5% kanamycin in water). Sugar 10% (SY10) food recipe is: 1 L water, 20 g agar, 100 g

sucrose. Yeast 10% (Y10) food recipe is: 1 L water, 20 g agar, 100 g yeast. Agar with tegosept and propionic acid recipe is: 1 L water, 20 g agar, 15 mL tegosept solution (20% tegosept in 90% ethanol), 3 mL propionic acid. Agar with ethanol recipe is: 1 L water, 20 g agar, 15 mL ethanol solution (90% ethanol). The 2% agarose solution recipe is as follows: 1 L water, 20 g bacto agar.

Pheromone donor fly production

Male donor flies were produced by crossing either w^{1118};*UAS-TRA;*+ virgin females or *yw;UAS-TRA;*+ virgin females to either w^{1118};*OK72-Gal4;*+ males (to create ♂$^{(♀)}$ donor flies) or to w^{1118};+;+ genetic controls (to create ♂$^{(♂)}$donor flies). Female donor flies were produced similarly, except w^{1118};+;*UAS-TRA*^{DSRNA} virgin females were crossed to w^{1118};*OK72-Gal4;*+ males to generate ♀$^{(♂)}$ donor flies and to w^{1118};+;+ genetic controls to create ♀$^{(♀)}$ donor flies.

Pheromone donor fly exposure

Experimental flies were exposed to donor animals in a ratio of 5 experimental flies to 25 donor flies, unless otherwise stated (see also (Gendron, Kuo et al. 2014)). For lifespan experiments, experimental flies were exposed to donor animals beginning on day 2 following eclosion (after sexes had been separated), and exposure continued over the lifetime of the flies.

Social isolation and exposure fly production

Experimental flies for social isolation experiments were collected as pupae and placed individually into SY10 vials. Once emerged flies were either kept isolated or grouped depending on the experiment. Flies were maintained in SY10 vials for 9-10 days before being used for behavioral and starvation experiments. Exposure flies were age matched

with experimental flies and sorted within 4 hours of emergence to ensure flies had not mated. Exposure fly sex was matched with donor fly sex.

Lifespan assays

Adult male and female flies (aged 2-3 days post-eclosion) were collected from controlled larval cultures and mating conditions (see above), sorted, and transferred into standard vials (20 or 45 flies per vial) containing SY10 media. The number of flies used per treatment group at the start of the lifespan is given in the figure legends. Actual number used in the analysis is noted in the figure legends. Cohort censuses were taken every 2-3 days, at which time flies were transferred to fresh SY10 media. Experiments were coordinated using DLife computer software(Linford, Bilgir et al. 2013). For experiments in which flies were aged under dark conditions, transfers occurred under indirect, dim red light (5 lux).

Temperature-dependent neuronal manipulations

Temperature was used to activate or inhibit *Crz* expressing neurons in adult flies. Parental crosses for activation strains were *Crz-Gal4 x UAS-TrpA1*, while the control crosses were *Crz-Gal4 x* w^{1118}. Parental crosses for inhibition strains were *Crz-Gal4 x UAS-Kir2.1;tub5-Gal80*^{ts}, while the control crosses were *Crz-Gal4 x* w^{1118}. For all crosses, eggs were collected and raised in 18°C 12hr:12hr LD. This temperature was maintained until the beginning of the lifespan experiment to ensure there would be no neuronal activation during development. At the beginning of the lifespan experiments flies were transferred to an incubator kept at 29°C, the restrictive temperature at which neuronal activation or inhibition occurs, for the rest of their lives.

Isolation social exposure setup

Exposure to the sensory environment of a group of flies was carried out by housing experimental animals within 5 mm × 65 mm polycarbonate tubes (Trikinetics part # PPT5x65) filled with SY10 food or 2% agar and connected to a vial using an acrylic adaptor (Fig 3.4a). Sensory environment was manipulated by changing the orientation of the polycarbonate tube, which was impermeable to air on one end but capped with permeable cotton on the other (Fig. 3.4a). Experimental flies were exposed to donor animals in a ratio of 1 experimental fly to 15 donors; the total number of experimental animals used in each experiment is recorded in the figure legends. Donor flies were the same sex as the experimental animal. For behavioral analyses conducted in another arena (forced swim and video assays) flies were aspirated from the polycarbonate tube into the new arena without the use of anesthesia.

Conventional starvation resistance experiments

Experimental flies were isolated and kept in either 5 mm × 65 mm polycarbonate tubes or standard vials filled with 2% agar during the starvation period as noted in the figure legends. Experimental flies were exposed to donor animals in a ratio of 1 experimental fly to 15 donors; the total number of experimental animals used in each experiment is recorded in the figure legends. Donor flies were the same sex as the experimental animal. The number of dead flies was recorded every 3-6 hours until no experimental flies remained alive.

High throughput starvation resistance assay

High throughput starvation resistance was measured using a TriKinetics *Drosophila* Activity Monitors (DAM). In brief, isolated experimental flies were placed into 5 mm × 65 mm polycarbonate tubes containing 2% agar and placed into DAM monitors (Trikinetics part # DAM2). Two monitors of 32 flies each were placed in 3 large vented Tupperware containers supplied with air from an odor delivery system. The treatment

group were as follows: social isolation (room air delivery), conspecific exposure (air from Tupperware containing two bottles of *Canton-S* flies (~400 mixed sex flies)), and filtered conspecific exposure (the air from the tupperware containing *Canton-S* flies was filtered through a cotton filter, drierite, and sodasorb). Activity counts (beam breaks) were recorded every 30 seconds, and time of death was interpolated based on the most recent census time in which the activity count was greater than zero. To allow for bumps or jostling of the monitor up to two counts of non-zero activity within a six-hour window would be ignored, more than 2 counts and a new time of death would be calculated based on the most recent beam break. This algorithm allowed an accurate determination of death time without frequent census checks. This assay was validated with visual observation. A free copy of the analysis software is made available at: https://github.com/PletcherLab/DAMSurvival.

Video analysis

Nine to ten day old experimental flies were aspirated and placed into circular video arenas containing 2% agar. The arenas were placed into a 25°C incubator with backlighting and video cameras. Flies were recorded at 1 frame per second for 4 hours. Videos were analyzed for total time spent moving, total distance moved in pixels, and center crosses using the DTrack software. Data was log transformed.

Forced swim test

After being separated as pupae female *Canton-S* flies were housed individually or grouped with 5 others for 9-10 days. Forced swim test was performed in a 6 well plate. Flies were aspirated into a costar 6-well flat-bottom plate containing 6 mL water. Time to cessation of movement was recorded. Upon ceasing movement, the flies were removed with a paint-brush and set on a Kimtech wipe and time to regain movement was recorded.

Statistics

Group and pairwise comparisons on survivorship data (both starvation and aging survival) were performed using the DLife computer software, and the statistical software R (Linford, Bilgir et al. 2013). P-values for survivorship data were obtained using log-rank tests. Pairwise comparisons for behavioral assays were evaluated using a two-sided independent-samples t-test. Group comparisons for behavioral data were evaluated with a one-way ANOVA and post-hoc Tukey's test for individual comparisons. All non-lifespan statistical calculations were run in the statistical software OriginPro 2016. For all box plots, box represents Standard Error of the Mean (SEM, centered on the mean), whiskers represent 10%/90%, and the horizontal line represents the median.

Acknowledgements

We would like to thank the members of the Pletcher Lab who provided input on the experimental design and analysis. In particular, we thank Z.M. Harvanek and B.Y. Chung for their critical advice. Z.M. Harvanek contributed conceptual motivation and provided figure 3.1 (Harvanek, Lyu et al. 2017). T.S. Chakraborty and Y. Luo assisted with starvation experiments, and H.M. Richardson assisted with lifespan assays. This research was supported by US National Institutes of Health (R01 AG051649, R01 AG030593, R01 AG063371 S.D.P.), the Glenn Medical Foundation (to S.D.P.), and the University of Michigan Career Training in the Biology of Aging Training Grant (T32-AG000114, to J.C.J.).

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Figures

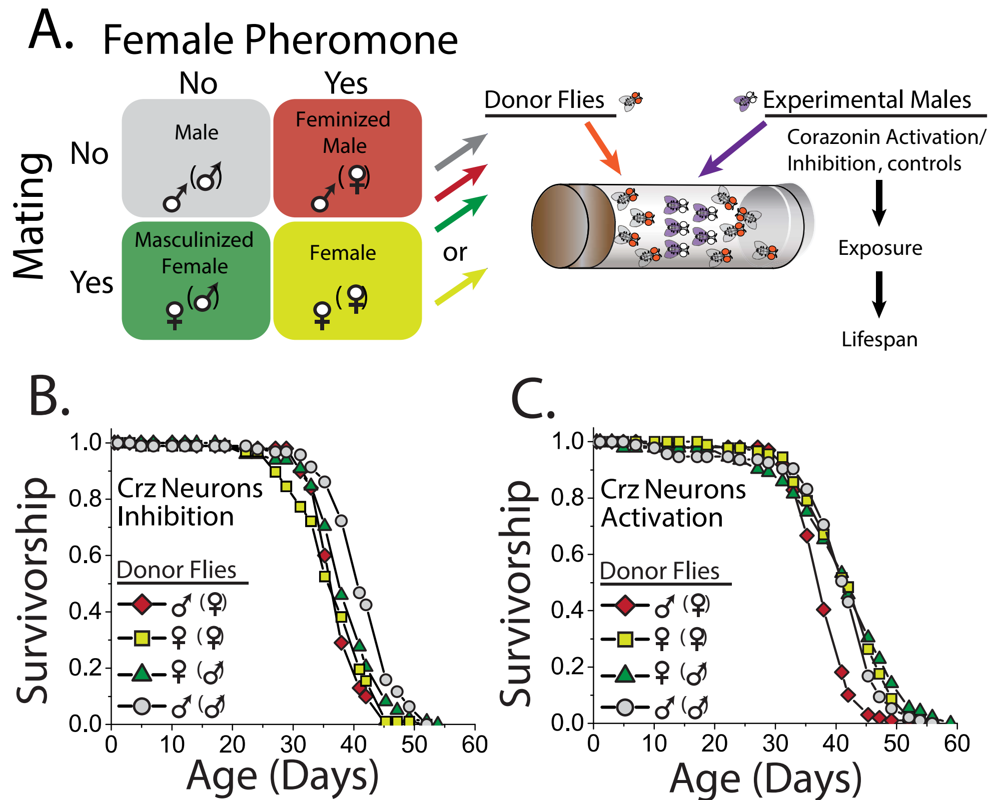

Figure 3.1: Costs of reproduction are mediated by corazonin peptidergic neurons[2]**.** (A) Experimental design used to distinguish effects of pheromone perception from effects of mating. (B) Spatiotemporal inhibition of *crz*-expressing neurons in adult male flies (*UAS-Kir2.1;tub5-Gal80^{ts}* x *crz-Gal4*) eliminated the beneficial effects of mating in the presence of female pheromones (n = 100 per treatment group, *P* = 0.48, comparing feminized male and control female groups). (C) Spatiotemporal activation of *crz*-expressing neurons (*UAS-TrpA1 x crz-Gal4*) potentiated the beneficial effects of mating such that the reduction of lifespan caused by pheromone exposure was completely reversed by allowing mating (n = 100 per treatment group, *P* = 0.31 comparing exposure to control females to control males).

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[2] Zachary Harvanek (ZMH) created this figure as part of the manuscript (Harvanek, Lyu et al. 2017). Jacob Johnson (JCJ) assisted with experimental setup and data collection.

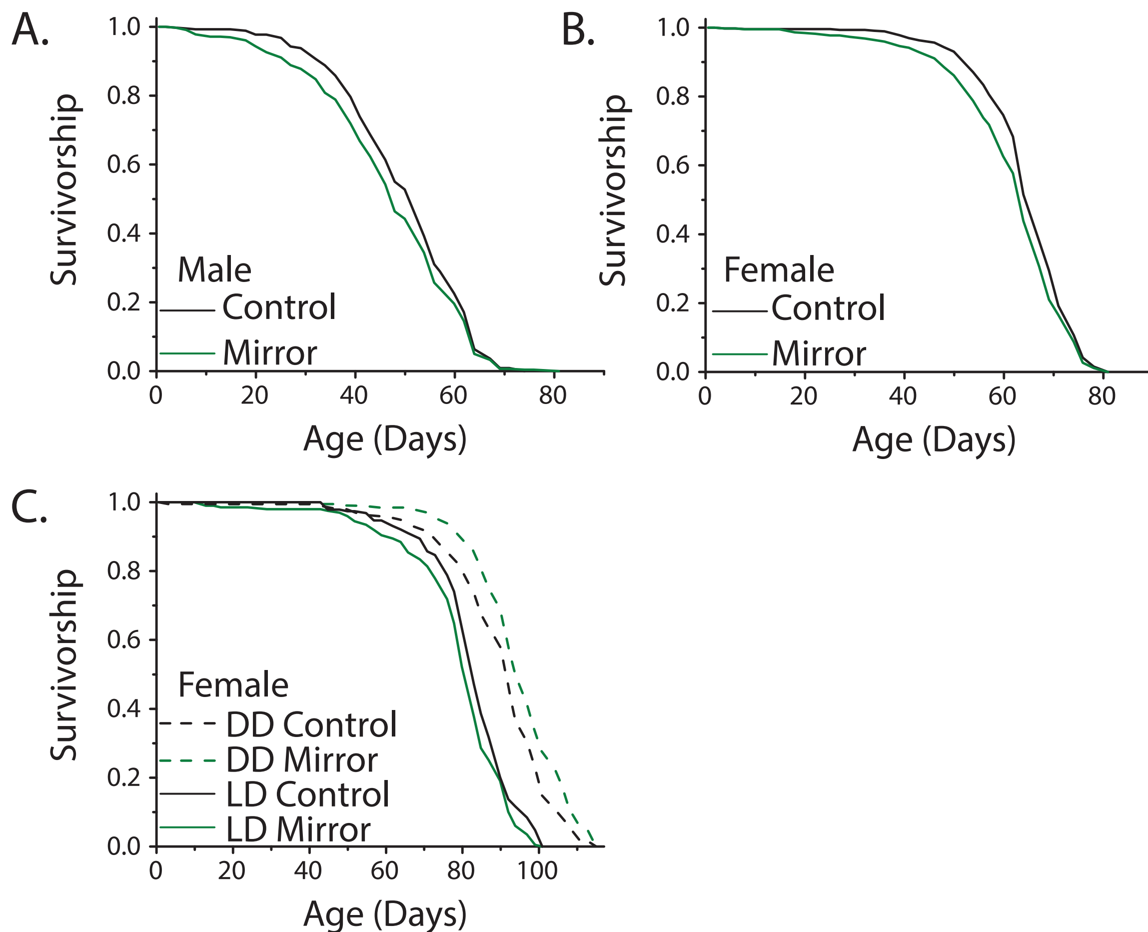


Figure 3.2: Density perception has a small, yet significant effect on lifespan determination.
In order to test effects of density perception on longevity *Canton-S* flies were aged in vials with a mirrored cotton topper and compared to those topped with a white acrylic disc. (A) Mirrored vial toppers shorten male fly lifespan when compared to an acrylic disc control, both with 45 flies per vial (Control n = 438, Mirror n = 460; $P = 0.042$). (B) Female flies also show a shortened lifespan with a mirrored cotton topper and 45 flies per vial (Control n = 459, Mirror n = 448; $P = 0.0043$). (C) Under light:dark (LD), but not dark (DD) conditions mirrors resulted in a shortened lifespan for flies kept at 25 flies per vial (LD Control n = 189, LD Mirror n = 200, $P = 0.013$; DD Control n = 197, DD Mirror n = 196, $P = 0.0007$).

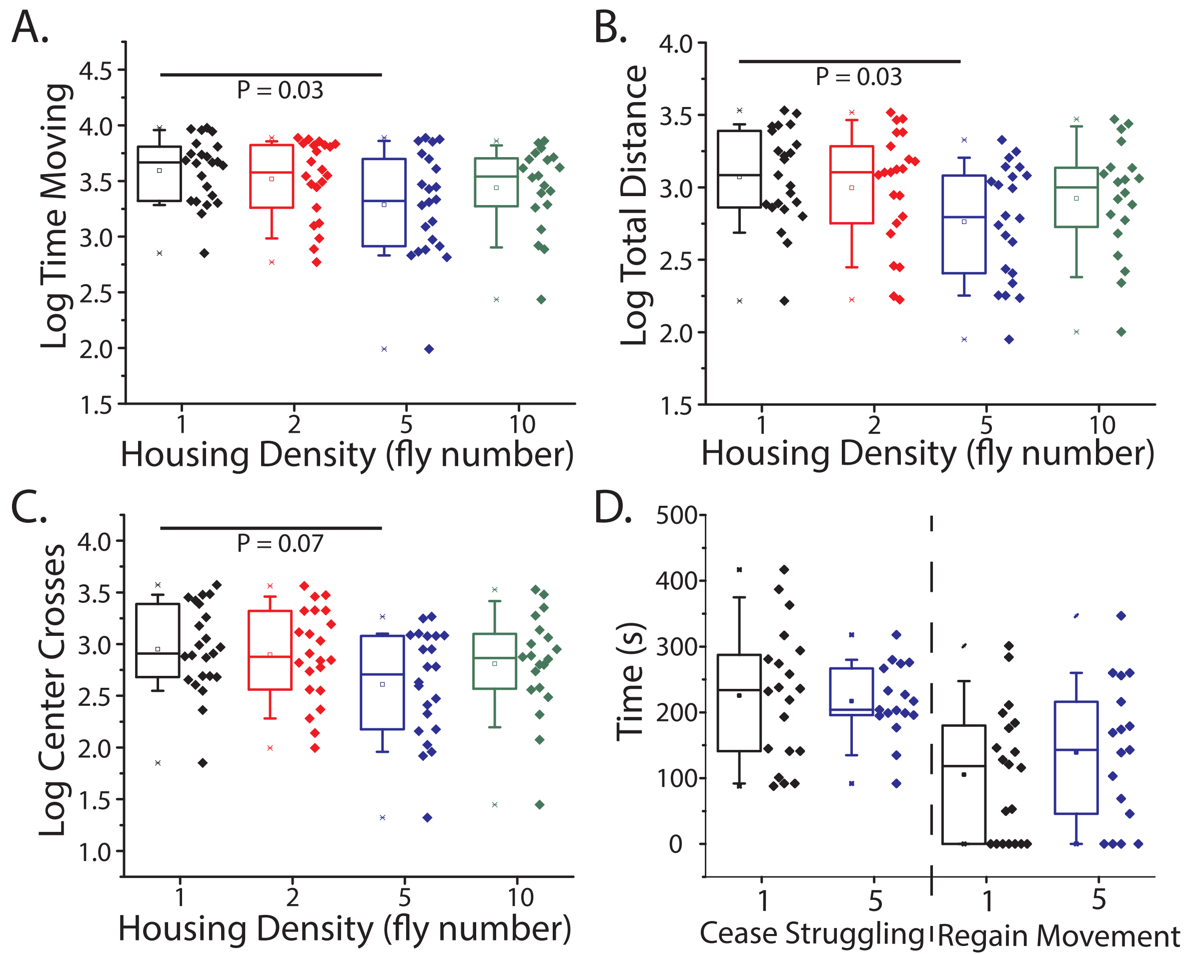


Figure 3.3: Housing density causes locomotor changes, with low housing densities resulting in hyperlocomotion, but no changes in centrophobism or forced swim behaviors.

To determine whether housing density effects locomotor behaviors we housed female *Canton-S* flies collected as pupae individually or in groups of 2, 5, or 10 for 9-10 days before performing video analysis in a circular arena. (A) Housing density had significant effect on time spent moving (P = 0.048) with only groups of 5 being significantly lower than individually housed flies (P = 0.03). (B) Housing density also had a significant effect on total distance traveled (P = 0.046) with only groups of 5 being significantly lower than individually housed flies (P = 0.03) (C) There was no housing density effect on centrophobism (P = 0.086). For panels A-C: 1 n = 23, 2 n = 22, 5 n = 22, 10 n = 20) (D) Housing density had no significant on time to cease struggling (n = 20 per treatment group, P = 0.76) during a forced swim test, or time to regain movement (n = 20 per treatment group, P = 0.33) after a forced swim test.

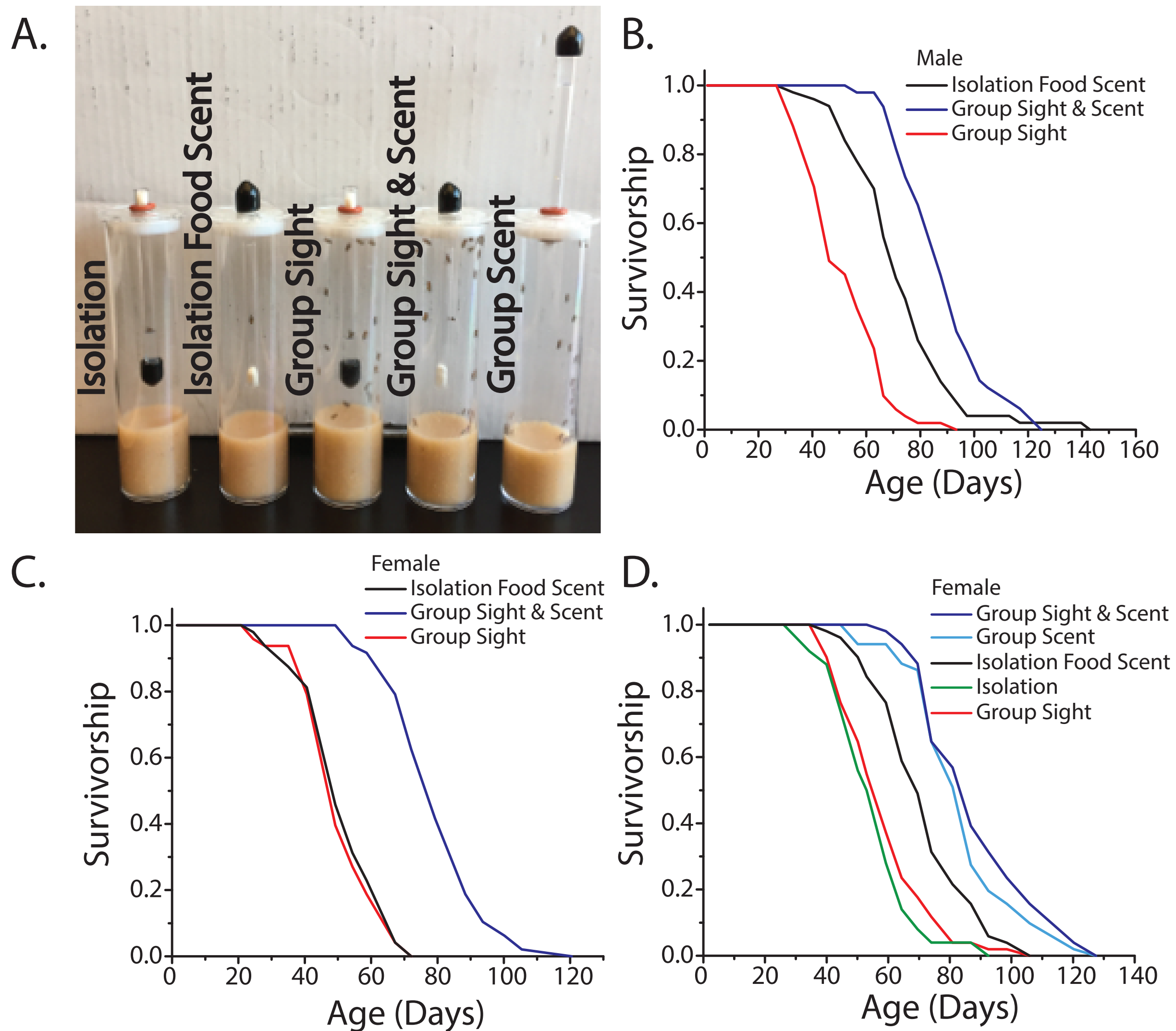


Figure 3.4: Starvation resistance can be altered through perception of conspecifics. (A) Image showing sensory exposure setup. Black cap is gas impermeable and orientation determines olfactory environment within the capillary tube: when the cap is oriented inside the vial flies in the capillary tube are considered isolated from the vial scents. One experimental isolated fly was placed in the capillary tube and 15 conspecifics were used for the group environment. (B) Male *Canton-S* flies showed a significant starvation resistance extension when given the sights and scents of a group of conspecifics and compared to those that are isolated (n = 50 for all treatments, $P < 0.001$). Interestingly, vision of a group was sufficient to shorten starvation resistance when compared to isolated flies ($P < 0.001$) (C) Female *Canton-S* flies also showed a significant starvation resistance extension when given the sights and scents of a group of conspecifics and compared to those that are isolated (n = 50 for all treatments, $P < 0.001$). However, vision of a group was had no effect on starvation resistance when

compared to isolated flies (P = 0.273*)* (D) Starvation survival of female *Canton-S* flies. A group olfactory environment alone significantly extended starvation resistance when compared to an isolated fly exposed to CT food (n = 51 flies per group, $P < 0.001$). There was no difference between group scent and group scent with sight (n = 51 flies per group, $P = 0.273$). Isolated flies were longer lived when given food smell (Isolation n = 50, Isolation + food scent n = 51, $P < 0.001$). Sight of a group had no impact on starvation survival when compared to isolated fly (group sight n = 51, isolation n = 50, $P = 0.215$).

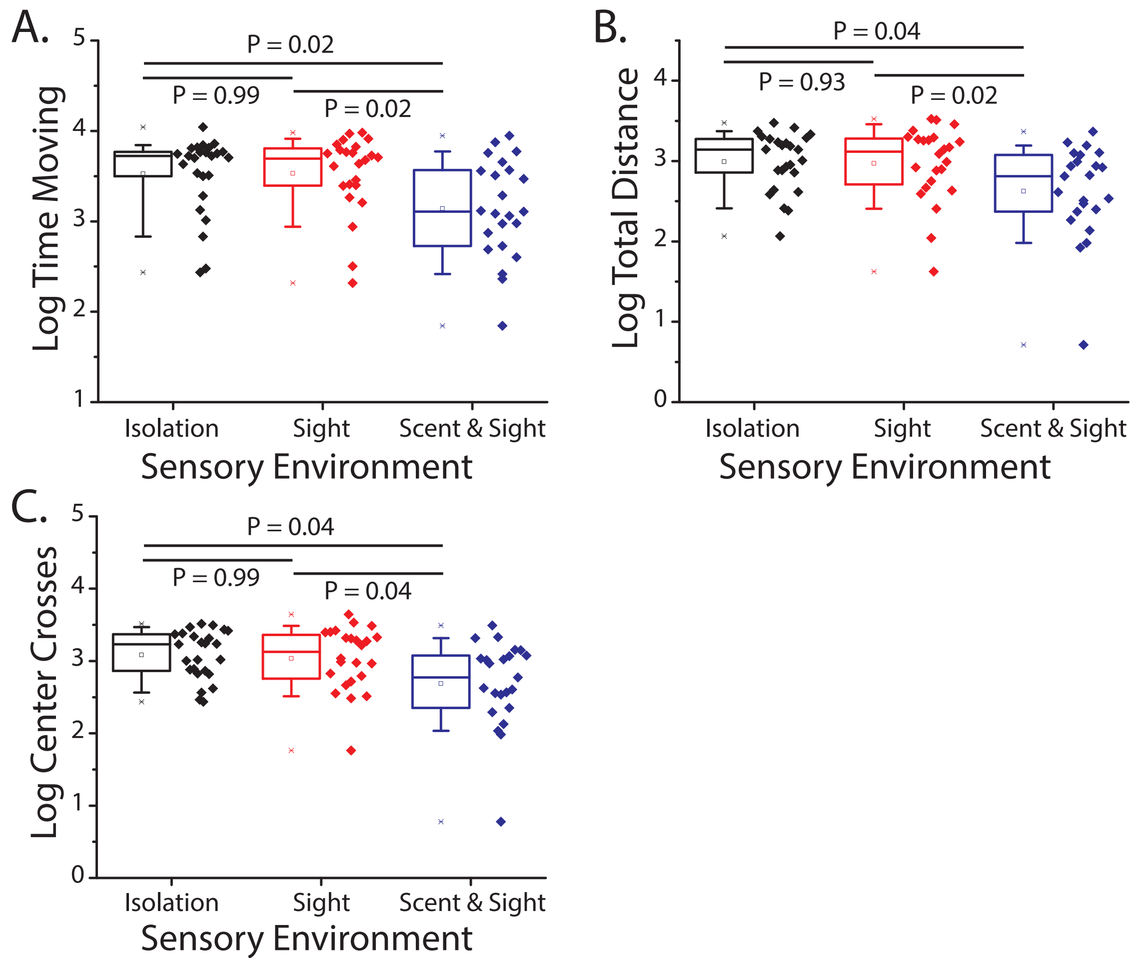
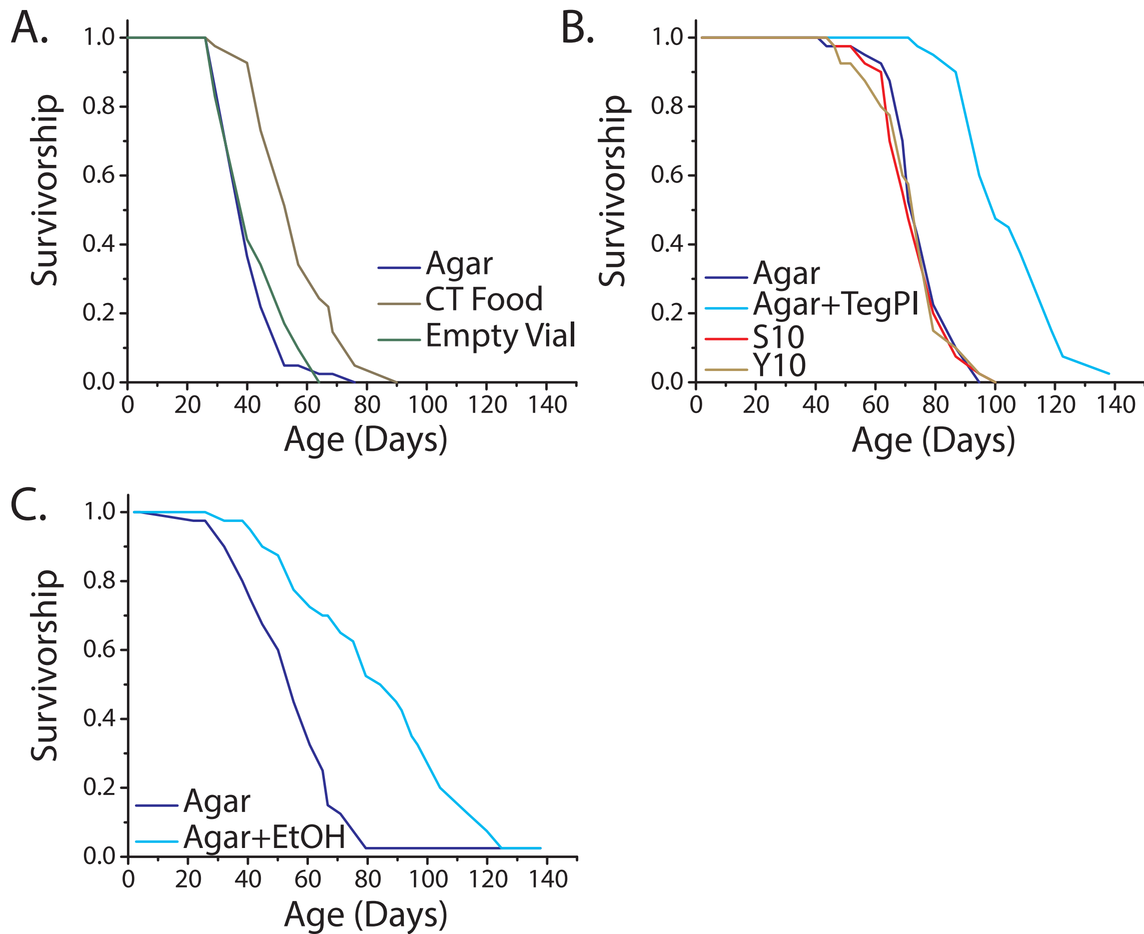


Figure 3.5: Social perception impacts behavior through olfaction.
In order to determine if the same sensory cues causal in starvation resistance changes were also causal in the group behavior we subjected isolated female *Canton-S* flies to the sight or sight and scent of 15 conspecifics then measured behavior in a circular video analysis arena. For all plots, pairwise comparisons listed on plot (Isolation n = 25, Sight n = 24, Smell & Sight n = 23). (A, B) There was a significant effect of sensory environment on time spent moving ($P = 0.009$) and total distance traveled ($P = 0.015$); sight of conspecifics was not sufficient to decrease time spent moving or distance traveled, scent and sight of conspecifics did cause a significant reduction in time spent moving and distance traveled. (C) Social exposure had a significant effect on center crosses ($P = 0.024$), again with group sight and scent environment causing a significant reduction.

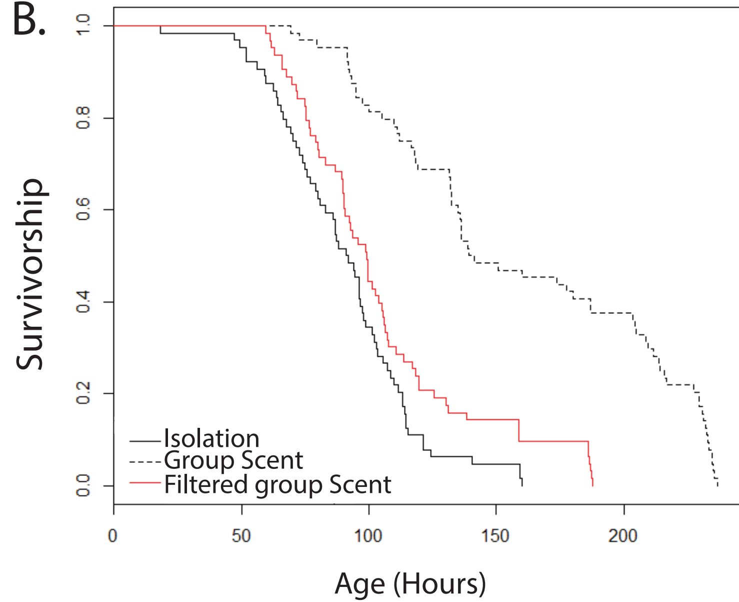


Supplemental Figure 3.1: Humidity and macronutrient odorants have no impact on starvation resistance; ethanol extends starvation survival.

In order to determine if either humidity or odorants from the food could be causal in the group starvation resistance phenotype we subjected isolated female *Canton-S* flies to vials containing CT food components using the same social exposure setup with permeable cotton on the inside of the vial. (A) The scent and humidity contributed by 2% agar had no survival benefit over an empty vial (Agar n = 41, Empty Vial n = 41, P = 0.41), however starvation resistance was improved by the presence of CT food when comparing to agar (Agar n = 41, CT Food n = 41, P = 0.0001), (B) Exposure to food macronutrient scents did not have an effect on starvation survival, however the food components tegosept in ethanol and propionic acid caused extended survival (all treatments n = 40; P < 0.0001 for the pairwise comparison between Agar and Agar+TegPI) (C) Ethanol containing agarose significantly extended starvation survival (Agar n = 40, Agar+EtOH n = 40; P < 0.0001).

A.

B.



Supplemental Figure 3.2: Starvation resistance assays can be made high-throughput with DAM monitors.

(A) Example experimental setup with DAM monitors placed inside Tupperware containers (odor delivery system not pictured). (B) Example output from R package showing survival extrapolated based on continuous activity measurements. *Canton-S* flies supplied gas that has passed through a group of flies (400 mixed gender flies) are longer lived than both flies kept in isolation, and flies supplied gas that has been filtered after passing through the group (n = 64 for all treatment groups, $P < 0.0001$ for both comparisons).

Chapter 4

Conclusions and Future Directions

Sensory systems are fundamental to optimize survival, and are employed by all organisms from the simplest prokaryotes to complex multicellular organisms with intricate nervous systems. In day-to-day life, sensory systems help organisms adapt and quickly respond to threats or opportunities; over time these signals are integrated to make complex decisions to optimize biological success, be it through deciding to change location for the season, choosing to mate, or changing metabolic state to maximize longevity. Despite the immeasurable benefits of sensory perception, there are also costs associated with environmental perception, particularly when environmental signals portray unfavorable conditions, even if those conditions are never realized. The first demonstration of this phenomenon in *Drosophila* was when our lab demonstrated yeast odorants, which signify a high-calorie high-protein food, were capable of shortening lifespan, even in flies kept on a lower-calorie diet (Libert, Zwiener et al. 2007). It was also shown that eliminating the sensory receptors required to perceive yeast odorants resulted in increased lifespan when on the high-calorie and high-protein diet. This was groundbreaking for two reasons. First, it showed there is a sensory component to the dietary restriction longevity response. Second, and perhaps more importantly, it demonstrated that longevity can be manipulated with targeted interventions to a small group of neurons without requiring dietary change. The ability to modulate lifespan through targeting the senses opened up a whole field of inquiry exploring how

perception, rather than reality itself is key in shaping life history strategies. In the work presented here I investigated both recognized and non-canonical forms of environmental perception, light and social perception respectively, in the context of health and longevity.

Light Perception Influences Longevity Independent of Circadian Rhythms

When starting my dissertation work, my main goals were to understand how longevity could be influenced through circadian sensory inputs, and investigate how endogenous rhythms interact with sensory signals to affect an animals' health and lifespan. Initially I wanted to tackle this goal in two ways: through shifting light cycles, and shifting food availability with time restricted feeding. Links between circadian rhythms, health and lifespan have been hypothesized since Jürgen Aschoff, the father of chronobiology, published numerous papers on the potential survival value of diurnal rhythms in 1963 and 1964 which are well reviewed (Emerson, Bradshaw et al. 2008). As light is the strongest entrainment factor, it was the logical place to start exploring how rhythms could be manipulated. Surprisingly, I was not able to show substantial lifespan effects when I perturbed rhythms in several ways. First, constantly shifting a 12-hour light cycle in a jet-lag like manner resulted in no meaningful lifespan reduction. Second, I did not observe a change in lifespan when aligning or misaligning day length with circadian period. Last, even under constant light conditions where flies are behaviorally and molecularly arrhythmic, I did not observe a drastic lifespan reduction. This serves to show circadian rhythms may be less beneficial than previously believed, or just less beneficial under laboratory conditions. These experimental results taken together indicate *Drosophila* clocks are either more flexible than once thought, or perhaps not as influential in lifespan determination. I believe it likely the relatively benign

experimental conditions did not put enough temporal selective pressures on flies to show the true benefits of circadian rhythms. If I had put constraints on the flies, be it in the form of reduced temporal food ability, reduced mate choice, or predators with circadian feeding rhythms, the benefit of rhythms may have become more apparent.

The second, and far-fetched, goal of my thesis work was to determine if time perception through the circadian clock could be altered or slowed in such a way as to benefit lifespan. Testing hypotheses like these is important not just to identify novel paths of lifespan extension, but also to provide insight into seasonal disorders such as Seasonal Affective Disorder (SAD) and Seasonal Depression. In order to test such a hypothesis we needed to identify a neuronal population used to track long time scales, then target those neurons while separating the animal from environmental time cues. The obvious answer was that circadian neuron would be used to track time. They integrate light cues to keep an animal in line with the daily rotation of the earth in addition to indicating seasonal changes. Though there are neuronal populations used for tracking times of shorter scales, there are no identified circuits that track time on timescales longer than that of which circadian neurons are capable (Heys and Dombeck 2018). We predicted the circadian neurons would be key in tallying days lived, and if this tally could be slowed we could conceivably slow aging. Despite slowing rhythms in all clock neurons using established molecular modifications, we found no relationship between lifespan and subjective time passed. This leaves open the possibility that there are other neuronal populations tracking time, or that time perception is not relevant in lifespan. The later explanation seems more likely as there would be little adaptive benefit to tracking timescales longer than the time during which an animal is reproductive.

Another interesting research question would be to ask how much of an animals' circadian rhythms are due to behavioral choice. *Drosophila* display daily changes in

temperature preference, preferring locations warmer during the day and cooler at night (Kaneko, Head et al. 2012, Goda and Hamada 2019). When allowed to behaviorally regulate location based on time and temperature, flies have a poikilotherm like body temperature rhythm. Conceivably the costs associated with circadian rhythms could come from the behavioral choice of when to be active, and not the entrainment itself. For example, if a fly were in an environment where there was always a light area, and totally dark area accessible would the fly regulate locational preference to align with the time of day and circadian phase? As *Drosophila* are crepuscular, meaning they are active around dawn and dusk, I believe it likely the flies would choose to spend more time in the light sector around dawn and dusk, and limit their light exposure during the middle of the day and night. I would predict that their behavioral rhythms would become longer in period due to a late day light induced phase delay. Establishing behavioral choice in environmental light would be interesting for several reasons. It would show *Drosophila* regulate their light exposure based on circadian time, and it provides a more natural context in which we could test the impact of rhythms on longevity. It could also provide an environment still largely devoid of the traditional selective pressures circadian rhythms assist with that we could use to further test the circadian resonance hypothesis.

Though I performed a thorough investigation into how asynchronous light treatments affect longevity, one area my research failed to address is how multiple circadian input factors interact with each other to influence health and lifespan. It would be worthwhile to determine whether light : food input can be optimized to maximize lifespan. Early in my tenure in the lab I had planned to entrain flies with a time restricted feeding regime then shift the regime to be asynchronous with the light cycle. However, the technology was not fully developed and we were limited in our abilities to both run experiments long enough to reentrain flies, and in the number of flies available for analysis. A

combinatorial experiment where both food and light are shifted could uncover how multiple inputs are integrated to contribute to both entrainment, periodicity, and synchrony of clocks across tissues. These types of study could also provide mechanistic insights into what inputs can be altered without affecting health, and could perhaps be used to optimize feeding schedules or off day light cycles for shift workers, a population prone to circadian : environmental discordance. But perhaps Drosophila are not the optimal model organism in which to conduct these studies as they seemed largely resilient to circadian perturbation when it comes to altered light cycles.

Having observed no circadian-dependent longevity changes when it came to shifting light cycles, I did show that light itself caused a lifespan shortening effect. Although recently there have been a flurry of publications on the topic, little is known about why visible light is capable of having such drastic lifespan consequences. In invertebrates, it appears the prevailing theory is that visible light, much like higher energy UV light, cause photochemical damage or neurodegeneration and this damage is responsible for the lifespan shortening effects (De Magalhaes Filho, Henriquez et al. 2018, Nash, Chow et al. 2019, Shen, Zhu et al. 2019). My work fits in nicely with this published literature from both worms and flies, in that it supports that visible light is not innocuous, and that shorter wavelengths are more detrimental to lifespan. In addition, my work goes to show that much like scent and other perceptual systems, light perception itself is partially responsible for the longevity response. I have several lines of evidence to support this theory. The first is that when I doubled the amount of time flies were exposed to light there was no concordant doubling of the lifespan shortening effect. Second, increasing light levels from 300 to 1500 lux only modestly decreased lifespan. Third, when flies have ablated visual neurons they are no longer sensitive to light-induced lifespan reductions. Most convincingly, the light-induced lifespan reduction can be phenocopied in a dark environment by activating a subset of retinal neurons

genetically. I would like to follow this work up by determining what neurons downstream of the eye are causal in this lifespan phenotype. This would allow us to determine if this response is due to general neural activation, or specific to visual processing. Further, it would allow us to investigate and identify signaling pathways that are recruited and may directly modulate aging.

Aside from any lifespan shortening effects light may have, darkness itself may promote health and longevity through several mechanisms. First, it may act through reducing the amplitude of circadian rhythms. Living in an environment devoid of entrainment cues for extended periods causes a loss of synchronicity and dampened activity rhythms. Having both dampened molecular and behavioral rhythms is traditionally thought of as being detrimental, but perhaps entrainment stimuli are stressors when they come at unanticipated times. Dampening rhythms reduces the expectation that entrainment events will occur and provides more flexibility. Second, the lack of light perception is still perception of darkness. Perhaps activation of the dark perceptual pathway is itself beneficial to lifespan. Third, darkness removes most visual inputs that may either cause stress, or alter expectations about the environment. In many sensory lifespan effects expectations are made based on sensory information, and when the expectations are not fulfilled there is a lifespan consequence. This is seen in *Drosophila* when pheromones of the opposite sex are perceived but mating is not allowed(Harvanek, Lyu et al. 2017). Living in darkness results in an environment where vision cannot be used to set expectations. It may also alleviate social burdens as aggressive displays and courtship dances are not seen. Lastly, the living in darkness may cause a switch in sensory processing. Humans often close eyes to focus on a sensory system aside from vision. For example, if you want to localize a faint sound or explore an object with touch, eye closure reduces the dominance the visual system has over other senses. There is scientific basis for this switch in sensory processing. Eye

closure, even under dim and dark conditions, improves tactile discrimination abilities (Brodoehl, Klingner et al. 2015). It is possible prolonged living in darkness promotes a sensory balance or enhances senses that are more beneficial for lifespan.

Though certain wavelengths of visible light are deleterious, there may be contexts under which visible light is beneficial. A well-known instance where specific wavelengths of light are beneficial is in the treatment of humans displaying SAD. Both short wavelength blue lights, and long wavelength infrared and near-infrared light have been used to reduce the risk of SAD incidence in humans(Nussbaumer-Streit, Forneris et al. 2019). We have preliminary evidence showing limited doses of red light (3 minutes per hour) are capable of extending longevity beyond that of what complete darkness is capable of doing (Figure 4.1). Though there have not been thorough longevity studies on effects of red or near infrared light, there are well documented cases where long wavelength light is beneficial. Red light therapy has been used to improve both retinal and neural tissue health, and treat disorders such as stroke, Alzheimer's, and other neurological diseases (Fitzgerald, Hodgetts et al. 2013, Johnstone, Moro et al. 2015). Near-infrared light has also been shown to improve cognitive, motor, and visual decline in aged *Drosophila* (Weinrich, Coyne et al. 2017). Mechanistically, these benefits appear to come from the ability of longer wavelength light to improve mitochondrial complex activity. In fact, red light therapy has been used to improve age-related visual decline in humans through improvements in mitochondrial function (Shinhmar, Grewal et al. 2020). Aside from the benefits red light provides mitochondria, it may also act through minimizing or counteracting the effects of blue light on the Rh1 photoreceptor, which require a photon of red light to turn off (Hillman, Hochstein et al. 1983, Montell 2012). This corroborates both our, and others' data showing monochromatic blue light as being inimical to lifespan. However, even in darkness red, but not green, light increases lifespan, showing its beneficial effects are

not only through muting blue photoreceptors (Figure 4.1). Rather than focusing on detriments of short-wavelength light, future research will benefit from exploring how long-wavelength light acts to improve health and longevity.

Social Perception Modulates Physiology and Lifespan

In chapter 3, I showed that social perception via female pheromones shortens lifespan in the absence of mating and that the neuropeptide corazonin mediates the mating rescue. This shows the power social perception can have on lifespan and how it may be used to determine downstream pathways used in modulating lifespan in response to sensory cues. We also found density perception is threshold-dependent and is largely based on olfactory cues. Surprisingly, crowd perception occurs independent of vision. We also showed social isolation increases activity and decreases starvation resistance, both of which can be rescued by introducing an olfactory component from a group of conspecifics.

One interesting finding from the social isolation experiments was that isolation reduced a flies' starvation resistance, and that this reduction could be rescued by providing the olfactory sensory environment of a group of flies. This is an interesting finding as it goes to show the power social signals have in determining physiology. The scent of flies alone was sufficient to cause a metabolic change and increase resiliency under starvation conditions. It makes sense that signals from conspecifics could be more reliable indicators of environmental quality than the sensory signals directly from the environment. For example, when moving to a new environment a fly would have to evaluate the food quality and availability, reproductive opportunities, predator and disease risk, and protection from the elements. Evaluating all these parameters likely

takes more time and resources than determining whether conspecifics in the area are thriving or not. Several environmental factors have already been shown to be communicated to nearby conspecifics. The presence of dead flies or predators causes the exposed flies to broadcast a signal that reduces longevity or fecundity respectively (Kacsoh, Bozler et al. 2015, Chakraborty, Gendron et al. 2019).Therein lies the power of social perception, it serves as an efficient means to assay likelihood of survival.

The rapid nature of starvation survival is useful in conducting high throughput screens, and could be utilized in order to determine what environmental information is conveyed by conspecifics. It would be interesting to investigate whether starvation response is dependent on the nature of the message communicated by the group of flies. It is likely starvation resistance will change based on the nutrient or caloric quality in the fed fly environment, and thus it makes sense that flies would be able to improve starvation resistance knowing high calorie food is near. However, flies would likely choose a different survival strategy knowing a nearby group of flies are also starving, perhaps by increasing activity or exploratory behavior. I would also like to explore whether flies exposed to pathogens, predators, or other noxious factors communicate a signal that promotes increased exploration or movement that is interpreted differently? Once we have determined the range of responses and identified those that promote and dampen survival we may begin screening downstream neurons and pathways with the goal of identifying neuronal circuits that can be targeted to improve health and longevity.

Our work with density was novel, and though we have yet to observe a lifespan phenotype, the starvation and behavioral data hint that with more thorough investigation where we test a wider range of fly densities, we may find a density perception lifespan phenotype. It was only after conducting the density perception

lifespan experiments that we identified olfaction as the sense that was both necessary and sufficient to convey a group effect. It would be worth further investigation into the effects of density on lifespan using an apparatus that allows olfactory signals from a dense group to be sensed by experimental flies in a standard vial. Conjoined tubes separated by a mesh screen could be used similar to the yeast exposure apparatus. This field of study has become more relevant recently with COVID-19 quarantine resulting in many feeling socially isolated. It also seems likely there will be a lasting cultural shift towards more remote work, and many people who previously worked in groups, or at least had frequent in-person meeting will now have those meetings virtually. This creates an entirely different sensory experience, and we have yet to determine the mental and physical health implications. Work such as this in model organisms may uncover mechanisms by which we can limit feeling of isolation even under isolation conditions.

Final Perspectives

Through exploring how light and visual inputs impact longevity, this dissertation furthers the field of sensory neuroscience and aging biology by enhancing our understanding on the manners in which light can impact longevity. My dissertation defines how light acts independently of both the circadian system and cellular damage to impact lifespan through perception. Flies are surprisingly resistant to circadian perturbations, and this calls into question the contexts in which the circadian resonance hypothesis is relevant. The work presented here also shows how inputs from conspecifics can serve as an indicator of environmental quality, even when the conspecifics are in vastly different environments. Further visual inputs do not convey as

much weight about crowd size as olfaction. These data will define new approaches to target sensory systems with the goal of increasing lifespan.

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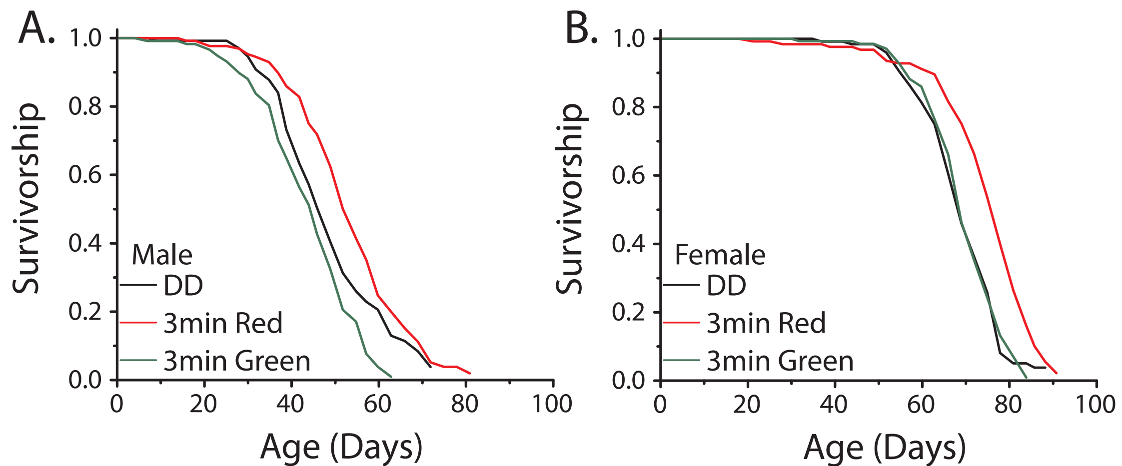

Figure 4.1: Limited red-light exposure improves longevity.
Flies were exposed to brief periods (3 min) of light every hour and otherwise kept in darkness. (A) Male flies exposed to 3 minutes of red light showed significantly increased lifespan over DD housed animals (Red n = 129, DD n = 117, p < 0.0001). Green light had a significant lifespan shortening effect (Green n = 131, DD n = 117, p = 0.0009). (B) This effect was also observed in female flies where brief red light exposure showed significantly increased lifespan over DD housed animals (Red n = 125, DD n = 136, p < 0.0001). There was no significant effect of brief green light treatment in females (Green n = 131, DD n = 136, p = 0.805)