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Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät der Universität Basel.

Auf Antrag von

Prof. Dr. Silvia Arber	**Dr. Joy Alcedo**	**Prof. Dr. Markus Noll**
(Fakultätsverantwortlicher)	**(Referent)**	**(Korreferent)**

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Prof. Dr. Eberhard Parlow

(Dekan)

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1. Summary

The lifespan of an organism is affected by the complex interplay between genetic and environmental factors. In *C. elegans*, this interaction between genes and environment is mediated by the sensory system. Indeed, a subset of gustatory and olfactory neurons has been shown to inhibit worm longevity, while a different subset of gustatory neurons promotes longevity. While olfactory neurons have been found to affect lifespan through signals coming from the worm's reproductive system, the lifespan effects of gustatory neurons are shown to be mediated by the insulin/IGF-1 signaling (IIS) pathway. Recently, the effects of olfaction on lifespan have been reported in *Drosophila,* which suggests that other aspects of the sensory influence on lifespan will also be conserved between these two species.

For my thesis, I show that gustatory inputs not only affect *Drosophila* lifespan but that they also do so in a bidirectional manner. Interestingly, I find that fly gustatory inputs influence lifespan independent of the level of food intake. Indeed, compared to controls, taste-impaired flies have an increase in feeding rates and body weights and show no decrease in lipid storage. Moreover, I show that the gustatory influence on fly lifespan is dependent on food type, *i.e.*, this influence depends partly on yeast in the food source, which suggests that the gustatory system affects longevity in response to a number of food-derived cues.

Consistent with the above hypothesis, I find that the gustatory system affects lifespan partly in parallel to the IIS pathway. I show that taste inputs shorten *Drosophila* lifespan through inhibition of the IIS pathway effector *dFOXO*, while other taste inputs lengthen lifespan via a different pathway that acts in parallel to *dFOXO.* These data suggest that different gustatory cues will modulate the activities of distinct molecular pathways, one of which is IIS, to optimize the animal's survival under different environments.

The findings that the gustatory and olfactory influences on lifespan exist in both *C. elegans* and *Drosophila* raise the intriguing possibility that mammalian lifespan is also subject to the activities of the sensory system. Indeed, in mammals both gustatory and olfactory information are relayed to the hypothalamus, a region in the brain that controls neuroendocrine function and physiology. Thus, the processing of this sensory information by the hypothalamus may consequently affect lifespan.

2. Introduction

Aging is a complex phenomenon characterized by the decline in organismal structure and function over time, which finally results in death. Classical research has largely regarded aging as a linear process. However, it has recently been shown that this process is actually regulated and that this regulation involves several important genetic pathways (Kenyon et al., 1993). Moreover, it has been shown that specific environmental factors, like food composition or sensory cues, could affect aging (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004; Mair et al., 2005; Mobbs et al., 2006; Libert et al., 2007; Skorupa et al., 2008; Maier et al., 2010), which add a level of complexity in studying this process. Yet, despite these findings, a linear view of the process, which originated from classical research within the field, still remains.

It should be understood that aging is a multidimensional, non-linear process, which involves many different individual elements acting at different systemic levels (i.e., specific genes, proteins, signaling molecules acting at the cellular, tissue, organ or organismal level). Indeed, multiple cross-talks and feedback loops are likely to exist among these elements. Furthermore, organismal aging is context-dependent, which means that different environmental conditions affect this process: these environmental factors can influence the interplay between the above-mentioned elements. This in turn can lead to different physiological outputs, such as different lifespans.

One very important, but often neglected, characteristic of the aging process is that it is dynamic. Some researchers often overlook the fact that physiological homeostasis, which presumably could affect aging, is a dynamic rather than a static phenomenon. In support of this view, previous findings indicate that the organism is a complex system of elements that are linked by multiple nonlinear feedback loops: the activities of these elements oscillate

between many dynamic physiological states to promote the optimal response to the environment (Deisboeck and Kresh, 2006).

Some of the signaling pathways within an organismal system are crucial elements that control the behavior of wider genetic networks, and these pathways/elements could be regarded as "control parameters". Control parameters govern the dynamics of macroscopic systems by modulating the status of many factors (i.e., genes or organ functions). One such pathway that could act as a control parameter is the insulin/IGF-1 (IIS) pathway, which affects many organismal functions and acts at several levels—cellular, tissue and systemic (Kenyon, 2010). By altering the activity of this pathway, an animal can shift its physiological state between different dynamic, but stable, states, and thus fine-tune its response to the environment. In addition, the alterations in these activities might have time-dependent readouts, e.g., alterations of key developmental pathways lead to developmental, but not necessarily post-developmental, changes.

Changes in the system context or environment (i.e., food composition) can also affect the value of the control parameter, e.g., food will affect IIS signaling levels, which could shift the optimal physiological state of the animal. Consequently, this shift might lead to alterations in the timing of different life processes (like developmental time or lifespan). Finally, an organism is constantly updated on the status of its environment through its sensory system, which can lead to alterations in the activities of some important genetic pathways (control parameters) that control physiology (Alcedo et al., 2010). Thus, updating the system status through sensory perception of specific environmental cues, with consequent changes in control parameter values, would presumably be beneficial for the animal, since the animal could rapidly and specifically respond to its changing environment.

In summary, there are several important things one should have in mind when thinking conceptually about an animal's physiology, including its aging, since aging is a time

readout of physiology. In this introduction, I will further elaborate on the complex adaptive system view of organismal functions and aging, and then discuss the most important findings in the field regarding the genetic and environmental factors underlying this process, the interaction between these two sets of factors and, finally, the role of the sensory system in mediating these interactions.

2.1. Setting the stage: Why do we age?

Many researchers have tried to answer the question of why we age and eventually die. It was suggested that there are more than 300 different theories of aging (Bengtson et al., 2008). However, most of these are not real theories, but rather mechanistic hypotheses (Bengtson et al., 2008). Although they look quite different superficially, most of them do have one thing in common: they assume implicitly or explicitly that aging is a stochastic process. The sources of this stochasticity are either external factors, like free radicals that cause random damage, or internal factors, like random failure of organs or physiological systems.

As mentioned above, aging is a complex, vis a vis a complicated, process, but the nature of this complexity has been rarely addressed. Complicated processes are those that might be hard to understand because there are many elements that participate in the process. However, in these processes, the elements interact linearly; and with sufficient computational processing power, one can precisely predict the outcome of these interactions. On the other hand, complex processes are those whose outcomes cannot be predicted precisely, not because of the number of participating elements or lack of processing power, but rather because of the nature of the interactions among the elements involved in the process: they interact a in non-linear manner with multiple feedback loops. Thus, aging shares this common feature of complex processes, namely that it is inherently uncertain.

A healthy animal requires the integration of a complex network of genes, proteins and physiological systems that are in constant interplay on multiple levels in space and time. These genetic and physiological systems exist in cells, tissues and organs and enable the constant exchange of different information within the system and between the system and the environment (Deisboeck and Kresh, 2006), making the system a highly dynamic process. This dynamic outlook challenges the concept of homeostasis, which is the activity of a physiological system to maintain the internal static steady state (Guyton and Hall, 2007). However, with the recent technological progress and the increased ability to acquire data continuously from individual experimental subjects, it became obvious that different physiological subsystems are in constant flux, even under homeostasis (Yates, 1993; Deisboeck and Kresh, 2006). These observations gave rise to the concept of homeodynamics, which implies that survival is determined by the dynamic interaction of multiple regulatory systems rather than the constancy within the internal environment (Yates, 1993). Such dynamics would then allow an organism to cope successfully with changes in its internal and external environments. Aging would then be the changes in the dynamics of the system and the underlying regulatory subsystems over time.

Recently, it has been proposed that one of the causes of aging is complexity loss (Lipsitz, 2002). Complexity loss with age is seen in many anatomical systems of an organism. For example, neuronal dendritic arborization displays loss of branches and connectivity over age; bone tissue displays changes in trabecular meshwork, i.e, trabecular loss and disconnections; and in the kidney, there is degeneration and loss of capillary networks (Lipsitz and Goldberger, 1992). The age-dependent loss of complexity is also seen in physiological systems, like in heart rate dynamics, blood pressure dynamics, respiratory dynamics, and posture control (Kaplan et al., 1991; Hausdorff et al., 1997; Peng et al., 2002). These observations lead to a theory that complexity is important for the maintenance of

proper homeodynamic responses to changes in the internal or external environment of an organism. Thus, with aging, it seems that there is loss in the network complexity of signaling pathways and physiological systems, disabling the organism from responding properly to the perturbations within its environment, which can be fatal for survival. Figure 1 below summarizes this hypothesis.

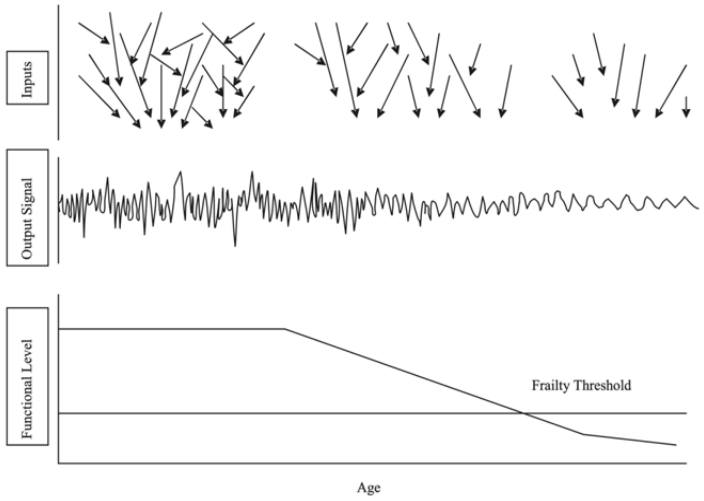

Figure 1. Loss of complexity with aging. In the young healthy organism, there are multiple physiological signals that produce complex and irregular outputs that enable the organism to adapt to the changing environment. These complex and irregular outputs would indicate a high level of functionality within all the systems. However, with age this output becomes simpler and more regular, which leads to a functional decline. Eventually, this decline will reach a point where an organism is unable to respond to stress or other environmental changes and lead to ultimate system failure. This figure is taken from (Lipsitz, 2002).

Accordingly, this view suggests that there are both external and internal factors that drive aging. External factors can be free radicals or other factors that damage the cellular machinery, which is presumably not efficiently repaired and leads to cumulative damage and eventually aging. Since external factors cause damage randomly, this would imply that aging is a stochastic process. However, this is not completely true. Although aging is both stochastic and dynamic in nature, it is still a controllable process, i.e., its rate is controlled by internal factors, such as gene activities. This is supported by the observation that evolutionarily related organisms have significantly different rates of aging (Kenyon, 2010). Indeed, it has been clearly demonstrated across species that aging is a genetically regulated

process and this is perfectly exemplified by mutations that downregulate insulin/IGF-1 signaling (IIS) in *Caenorhabditis elegans*, *Drosophila melanogaster* and mice, which extend the lifespan of these animals (Kenyon, 2010).

The two opposing views, namely that aging is a stochastic process versus it being a controlled process, could be reconciled by adopting a complex system view. This means that aging is essentially driven by stochastic processes, including random failures in the activities and interaction between genetic pathways; yet this process can be controlled by moving the organismal system between different homeodynamic states, and thus confer different levels of resistance to damage. In other words, the process remains dynamic and inherently uncertain (probabilistic). However, by shifting the organism, for example, between different levels of stress resistance, this can change the probability that damage, and consequently system failure, will occur.

In order to understand this fully, one can use the concepts that have already been developed for analyses of critical phenomena in complex adaptive systems (Sornette, 2004). These concepts suggest that one can observe different classes of system behaviors and that the system can be induced to switch between these classes through changes in the values of control parameters (Sornette, 2004). These mathematical equations are used for modeling non-linear systems, whose behavior are regulated by the activities of a small number of parameters (Sornette, 2004). They are particulary important for the mathematical analyses of systems that undergo gradual or sudden behavioral changes as a result of the changing quality of the environment (Sornette, 2004). These mathematical concepts have been successfully translated from the physical sciences into the social sciences, economics, meteorology, seismology, medicine and other fields (Sornette, 2004).

I will try to illustrate how this could work by using a very simple one-dimensional non-linear system. Let us assume that we have a one-dimensional variable x that describes

the system. The system can be controlled through changes in the values of a set of control parameters u (u1, u2...). If the system is nonlinear, the dynamics of this system that is its behaviour in time t, $\frac{dx}{dt}$, is described by the non-linear function f(x,u); thus, $\frac{dx}{dt}$ = f(x,u). The system is in equilibrium when $\frac{dx}{dt}$ = 0 and the values of x, in this particular case, are called attractors or fixed points. These fixed points are determined by the value of the control parameter u; and through these fixed points, which are denoted as x^{st}_n, the system exhibits dynamic stability. This system behavior can be illustrated by Figure 2 below.

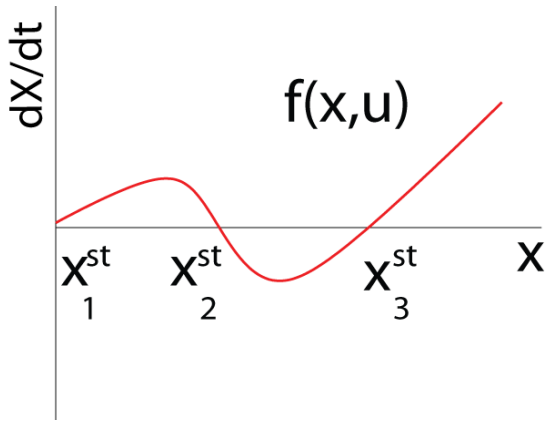

Figure 2. A mathematical description of a non-linear system's dynamics, $\frac{dx}{dt}$, which is determined by the non-linear function f(x,u). The system is stable, when $\frac{dx}{dt}$ = 0. This figure is taken from the lecture 11 of Systems Dynamics and Complexity given by Frank Schweitzer at ETH Zurich, 26.11.2009.

To understand further dynamic complex system behavior in relation to equilibrium states, we could also imagine the movement of a ball along the gradient of a "landscape". Now, imagine that this landscape could be described by some potential function of the system V(x,u), which depends on the vector x and control parameter u. The ball moving along this gradient would always go to the minimal point or the lowest point of this landscape, in the same way as the ball released from the top of the hill would go to its base. This analogy suggests that the stable dynamic equilibrium of the system is achieved in the minima of V(x,u).

Moreover, let us assume that we have a system with two control parameters, and that one of them, u_1, is kept constant, while the other, u_2, fluctuates. This could result in potential "landscapes" that have different minima, which represent different equilibria, x_{stat}. Hence, figure 3 shows the dependence of the minimal (equilibrium) point (x_{stat}) on the control parameter u_2. As we increase the value of u_2, we can see that the equilibrium state does not necessarily change dramatically, cf., u_2 = -400 and x_{stat} ~ 10 with u_2 = -200 and x_{stat} ~ 8 (Figure 3). However, as the value of the critical parameter, u_2, approaches zero, somewhere between -100 and +100, two different equilibria exist. This region of bistability is present where the curves overlap. Finally, further increases in the value of u_2 will drive the system into an equilibrium state (x_{stat} ~ -10) markedly different from the original state (x_{stat} ~ 10).

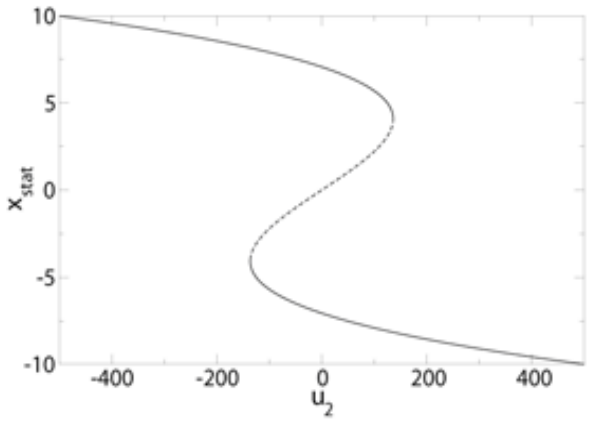

Figure 3. Dependence of value x_{stat} on the value of the control parameter u_2, in the system where u_1 is fixed. The dotted line shows the metastability loop, where the reactive state is unstable. The figure is taken from (Jantsch, 1980).

The question now is how these concepts relate to the aging process. One could translate the fluctuations in animal physiology in time as the behavior of the system. For example, the control parameters u_1 and u_2 could be two independent genetic pathways, like the IIS and TOR pathways, which have been shown to affect stress, metabolism, reproduction, and consequently aging (Guarente and Kenyon, 2000). Let us assume that u_1 is TOR and u_2 is IIS. Vector x could be regarded as the cumulative stress resistance of the individual organism. If TOR signalling levels are more or less constant and IIS levels are

variable, we could reproduce Figure 4 from Figure 3 in the context of an animal's response to its changing environment.

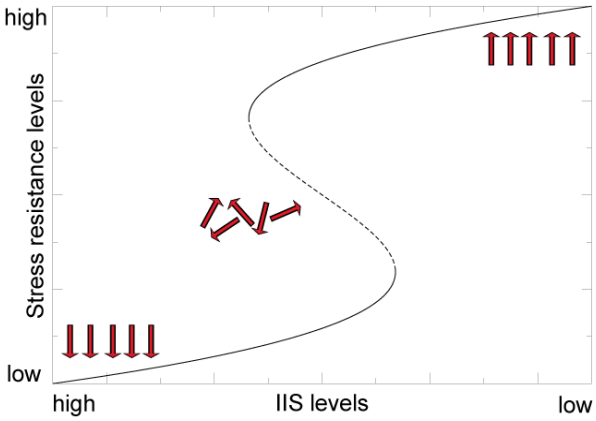

Figure 4. Dependence of x_{stat}, which is presented here as stress resistance levels, on the value of the control parameter IIS levels. When the IIS levels are high, stress resistance in cells, tissues and organs is low (represented with downward red arrows) and cumulative stress resistance level is also low. However, reduction of IIS levels increase stress resistance by synchronising and inducing the activities of genes involved in fighting stress across different cells, tissues and organs.

We could select individual cells as stress resistance vectors that cumulatively produces x levels. So if IIS levels are high, this would reduce stress resistance in both individual cells and the organism as a whole, which means that the value of x_{stat} will also be low. In this organismal system, there is no coupling of the stress vectors. However, alteration in IIS levels alters stress resistance. At intermediate IIS levels, stress resistance is moderate, where two equilibria—moderately low and moderately high stress resistance—can exist and achieved with little physiological fluctuations (Figure 4). This accordingly allows the organism to respond quickly to changes in its environment. Finally, as we decrease IIS values, there is a high coupling of stress "oscillators" at all levels (cells, tissues and organs), which would increase the values of individual stress resistance vectors. The synchronization of all these vectors would consequently lead to very high stress resistance. Nevertheless, it is important to note that the nature of the stress-induced damage that can occur under these conditions remains stochastic and probabilistic, since the physiological processes are still dynamic. However, at a state of high stress resistance, the probability that damage will occur and have permanent consequences is lower.

The occurrence of external and internal damages causes time failures within the organismal system, in which initial "small" events accumulate and reach a critical threshold that eventually lead to a loss of complexity and the ability to respond to external perturbations. However, a lower probability of damage occurrence, under a high stress-resistance state, would lead to a slower decline in function, in contrast to events under the low resistance state. Accordingly, these examples suggest that changes in the control parameters, i.e., genetic pathways, can alter the rate of aging of the animal system. Since the activities of these genetic control parameters change the activity of the system, e.g., its rate of aging, it is important to understand how these parameters are subject to external and internal factors. The interactions between these genetic and environmental factors can be mediated by the sensory system (Alcedo et al., 2010). Thus, I will now discuss how these environmental factors modulate the signals of genetic control parameters and the role that the sensory system plays in this process.

2.2. Genetic pathways that affect longevity

In this section, I will review the most important findings on the effects of two of the most important nutritient-sensing genetic pathways on longevity: the insulin/IGF-1 pathway (IIS) and the TOR (Target of rapamycin) pathway.

2.2.1. Insulin/IGF-1 (IIS) pathway

Many mutations in important and evolutionarily conserved genetic pathways affect lifespan. One of the key pathways that have been implicated in lifespan regulation is the IIS pathway. In *C. elegans*, mutations in the gene *daf-2*, which encodes an ortholog of the human insulin/IGF-1 receptor, double the lifespan of the animal (Kenyon et al., 1993). This lifespan extension is completely dependent on another gene, *daf-16*, which encodes a FOXO family transcription factor (Lin et al., 1997; Ogg et al., 1997). Insulin-like peptides modulate the

activity of DAF-2, which activates an evolutionarily conserved phosphatidylinositol 3-kinase (PI3K) signalling cascade that negatively regulates DAF-16 [Figure 5; reviewed by (Kenyon, 2010)].

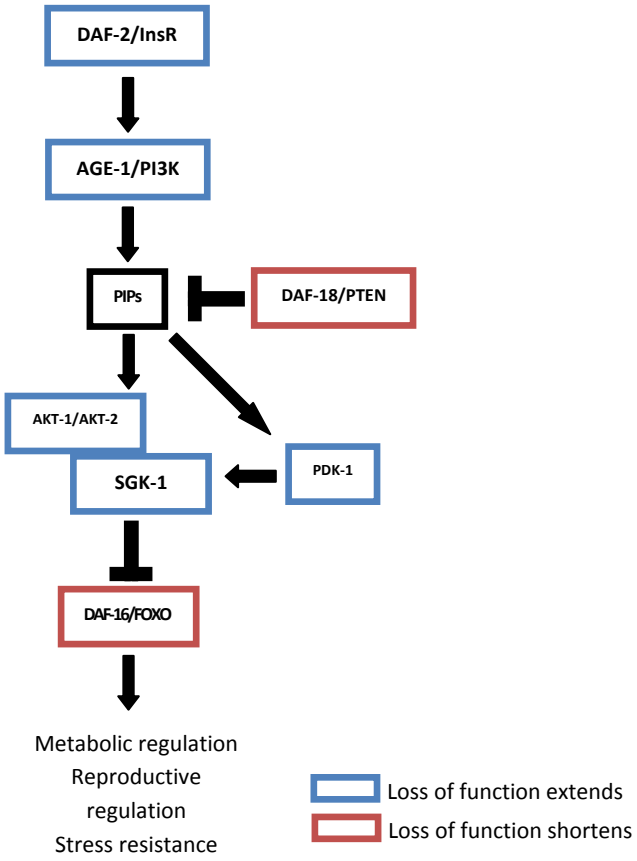

Figure 5. IIS signaling cascade. Studies in *C. elegans* have revealed that mutations in the genes *daf-2*, *age-1*, *akt-1* and *akt-2, sgk-1* and *pdk-1* extend lifespan, while mutations in *daf-16* and *daf-18* shorten lifespan. Adapted from (Gami and Wolkow, 2006).

An activated DAF-2 receptor activates AGE-1, which is a PI3K ortholog in the worm. AGE-1 phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3), which in turn triggers a signaling cascade that consists of AKT-1 and AKT-2, SGK-1 and PDK-1 (Figure 5). Subsequently, this leads to phosphorylation of the transcription factor DAF-16, which inactivates it and restricts its localization to the cytoplasm. On the other hand, DAF-18 inhibits the phosphorylation of PIP2 to PIP3, which downregulates IIS activity and prevents phosphorylation of DAF-16. This in turn promotes

translocation of DAF-16 to the nucleus, where it activates sets of genes involved in metabolic regulation, increased stress resistance and innate immunity. In addition to *daf-2* mutations, those present in *age-1*, *akt-1* and *akt-2*, *sgk-1* and *pdk-1* have been shown to extend lifespan, while mutations in *daf-16* and *daf-18* have been found to shorten lifespan (Klass, 1977; Kenyon et al., 1993; Larsen et al., 1995; Lin et al., 1997; Ogg et al., 1997; Ogg and Ruvkun, 1998; Paradis et al., 1999; Rouault et al., 1999; Hertweck et al., 2004).

The role of the IIS pathway in longevity regulation has been shown to be conserved in many organisms (Figure 6). In *D. melanogaster*, mutations in the insulin-like receptor (InR) (Tatar et al., 2001) and in the insulin receptor substrate *chico* (Clancy et al., 2001), which mediates the interaction between InR and PI3K (Bohni et al., 1999), lead to lifespan extensions. This lifespan extension also appears to be dependent on *dFOXO*, the *Drosophila* orthologue of the mammalian FOXO transcription factor (Jünger et al., 2003), since the overexpression of dFOXO in the adult fat body also extends lifespan (Giannakou et al., 2004; Hwangbo et al., 2004). In addition, ablation of the median neurosecretory cells (mNSCs), which are some of the key cells that produce insulin-like peptides in the fly brain, lengthens fly lifespan (Broughton et al., 2005). Likewise, mutations in the fly's insulin-like peptides produce a long-life phenotype (Grönke et al., 2010).

Unlike flies and worms that have a single insulin/IGF-1 receptor, mice have two separate receptors for insulin and IGF-1. An adipose tissue-specific knockout of the insulin receptor extends mouse lifespan (Blüher et al., 2003). Similarly, mice that are heterozygous for the IGF-1 receptor live longer: ~30 % in females and 16% in males (Holzenberger et al., 2003). Moreover, a whole-body or brain-specific knockout of the insulin receptor substrate *Irs2* increases mouse lifespan up to 18% (Taguchi et al., 2007). Furthermore, in mice, the growth hormone stimulates IGF-1 production and release; and a mutation in the growth

hormone receptor also leads to a longer life (Coschigano et al., 2003). However, the role of FOXO in regulating mouse lifespan remains unclear.

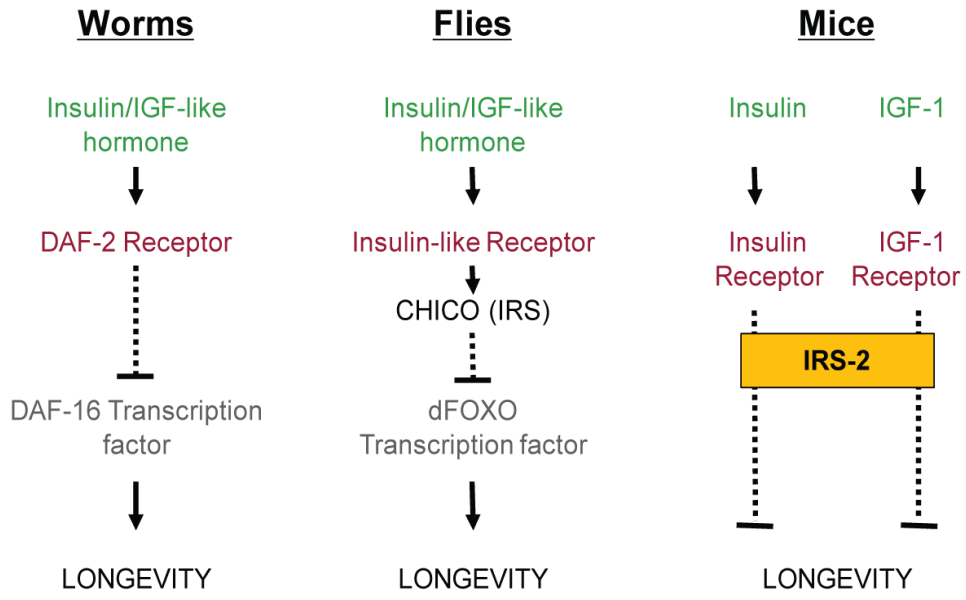

Figure 6. Effects of IIS signalling on longevity are conserved in worms, flies and mice. Adapted from (Kenyon, 2005).

As implicitly suggested above, there is a spatial dynamic in the regulation of lifespan, i.e., it is sufficient to mutate components of IIS in specific tissues to extend lifespan. The adipose tissue seems to be a very important source of signals that mediate longevity. Similar to the downregulation of IIS in mouse adipose tissue, the overexpression of the respective worm and fly FOXO homologs in tissues analogous to mammalian adipocytes is sufficient to extend lifespan (Libina et al., 2003; Giannakou et al., 2004; Hwangbo et al., 2004). Another set of cells that are important for lifespan regulation are the neurons. In worms, downregulation of IIS in neurons increases lifespan (Apfeld and Kenyon, 1998; Wolkow et al., 2000). In flies, as already mentioned above, ablation of mNSCs in the fly brain also prolongs lifespan (Broughton et al., 2005), while in mice loss of *Irs2* specifically in the brain promotes longevity (Taguchi et al., 2007).

It seems that IIS controls both cell-autonomous and systemic signals. Cell-autonomous signals enable single cells or tissues to respond successfully to changes in local

micro-environments, while systemic signals bring together individual cells and tissues to promote a coordinated system-level response to the changing environment. For example, downregulation of insulin signaling by ablation of mNSCs in *Drosophila* leads to translocation of dFOXO to the nucleus of fat body cells (Broughton et al., 2005). Likewise, increase of DAF-16 activity in the *C. elegans* intestine increases DAF-16 activity in other tissues (Libina et al., 2003).

Since many of the downstream genes of DAF-16 and dFOXO are involved in stress response and metabolic regulation (Lee et al., 2003; Murphy et al., 2003), the IIS and DAF-16/FOXO system are hypothesized to function as the central switch of a "longevity module" that regulate the expression of many genes that act together to influence lifespan [Figure 7; (Kenyon, 2005)]. Together with the complex spatial (neurons and adipose tissue; see above) and dynamic temporal [early in adulthood (Dillin et al., 2002; Giannakou et al., 2007)] requirements for IIS in lifespan regulation, this hypothesis would be consistent with a complex adaptive system view of an organism and its lifespan, as previously described above (see Section 1.1). IIS would be a control parameter of the system and IIS activity would determine the state of the whole system.

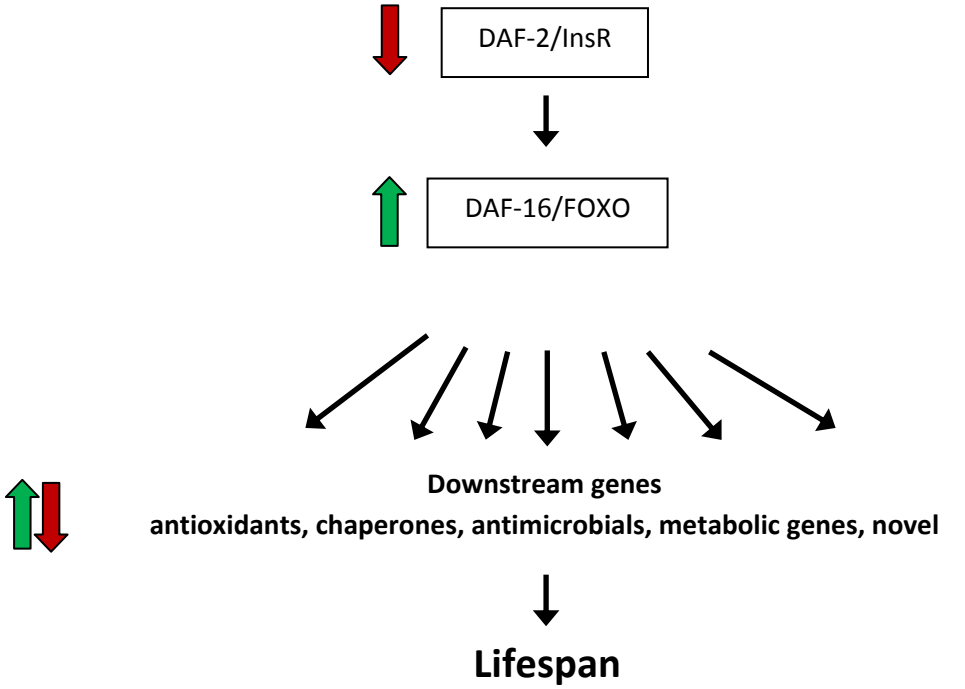

Figure 7. DAF-16/FOXO act as a central switch of a longevity module that regulate expression of many genes that affect longevity. Adapted from (Kenyon, 2005).

2.2.2. TOR pathway

Another important nutrient-sensing genetic pathway that influences lifespan is the TOR pathway (Arsham and Neufeld, 2006). Mutations and pharmacological interventions that decrease the activity of this pathway increase lifespan in yeast, worms, flies and mice (Fabrizio et al., 2001; Vellai et al., 2003; Kapahi et al., 2004; Kaeberlein et al., 2005; Powers et al., 2006; Harrison et al., 2009). Figure 8 shows a model for the regulation of the TOR signaling pathway.

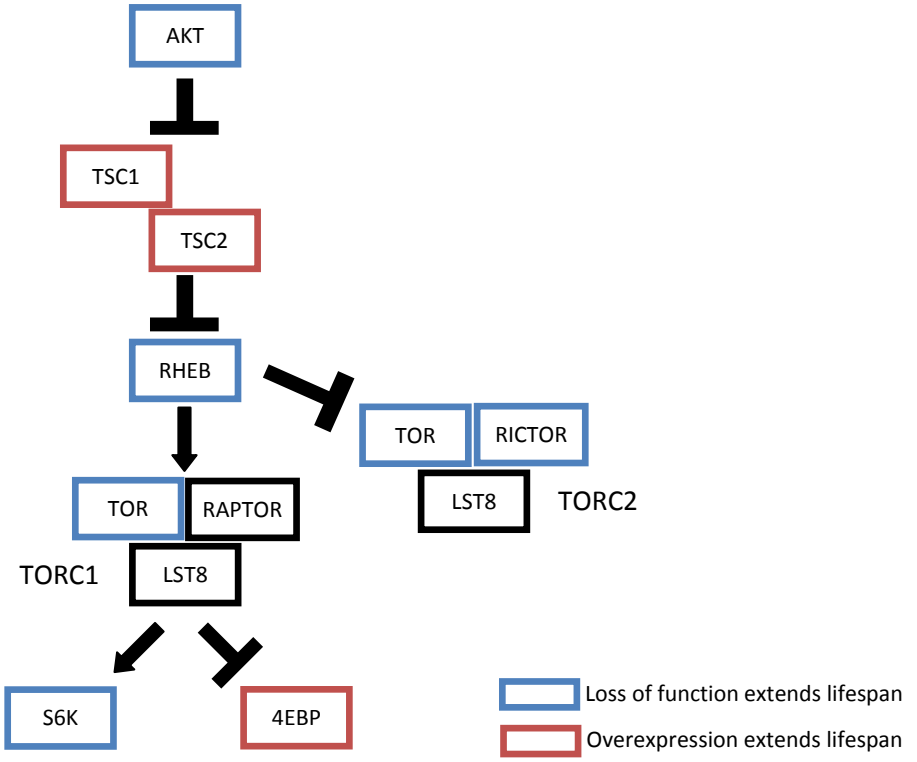

Figure 8. TOR signaling cascade. Insulin and other growth factors inhibit the Tuberous sclerosis complexes 1 and 2 (TSC1 and 2) through an increase in the activities of AKT kinases. TOR Complex 1 (TORC1)-activator RHEB also inhibits TOR Complex 2 (TORC2), although the details of the regulation are not completely clear. TORC1 is also repressed by the energy-sensing AMP-activated protein kinase (AMPK; not shown here). TORC1 consists of three proteins TOR, RAPTOR, LST8, while TORC2 consists of TOR, RICTOR and LST8. Mutations that downregulate or abolish the functions of RHEB, TOR, RICTOR and S6K extend lifespan in various model organisms (blue square), whereas TSC1, TSC2 and 4EBP do so through overexpression. See reviews by (Wullschleger et al., 2006; Stanfel et al., 2009).

In *C. elegans*, reducing the levels of *let-363*, which encodes the TOR otholog, extends lifespan (Vellai et al., 2003). In addition, inhibition of *daf-15*, which encodes the ortholog for the mammalian raptor protein that forms part of the TOR Complex 1 (TORC1), increases lifespan (Jia et al., 2004). Recently, a component of the TOR Complex 2 (TORC2), *rictor*, also appears to be involved in food type-dependent lifespan regulation (Soukas et al., 2009). Mutations in *rictor* leads to alterations in fat metabolism, growth, feeding behavior, reproduction, as well as lifespan on different food sources (Soukas et al., 2009). Moreover, inhibition of S6 kinase activity in worms leads to long life, which is independent of IIS (Hansen et al., 2007). Consistent with the idea that TOR and IIS act in parallel to affect lifespan, some of the developmental phenotypes of *daf-15* mutants also do not require IIS (Jia et al., 2004).

In *Drosophila*, it was shown that overexpression of TSC1, TSC2 or the dominant-negative forms of TOR or S6 kinase extends lifespan (Kapahi et al., 2004). Similarly, overexpression of the activated downstream TOR target, d4EBP, extends lifespan in a yeast level-dependent manner (Zid et al., 2009). Interestingly, *d4EBP* transcription is induced upon starvation in a *dFOXO*-dependent manner (Teleman et al., 2008), whereas the induction of *d4EBP* upon moderate yeast-restriction is independent of *dFOXO* (Zid et al., 2009). Under these yeast-restriction conditions, d4EBP upregulates the translation of mRNAs that have low 5' UTR complexity to increase mitochondrial biogenesis and capacity (Zid et al., 2009). Finally, feeding mice with rapamycin, a chemical inhibitor of TOR, extends lifespan (Harrison et al., 2009).

Together these results show that the TOR pathway has a similar, but not exactly the same, "signature" as IIS. Thus, the alteration of a nutrient-sensitive pathway can be regarded as a control parameter of the system, which changes the status of many effectors by linking them across many cells and tissues through the activity of a single factor (dFOXO or d4EBP). In turn, these events change the homeodynamics of the system, which can consequently affect the lifespan of an animal.

2.3. Nutrients as the environmental signals that affect lifespan

In my description of the environmental effects on lifespan, I will focus specifically on one form of environmental change, dietary restriction (DR), which has shown positive effects on lifespan in many species, ranging from yeast to nonhuman primates [reviewed in (Weindruch and Walford, 1988; Mobbs et al., 2006)]. DR is a procedure that changes the caloric value or composition of the food source, in the absence of malnutrition. In recent years, it has become apparent that the lifespan extension through food manipulation can be achieved in two ways. One is through changes in the food's total caloric value, a

manipulation that I will call in this thesis as food-level restriction; and the second, a food type-dependent form of manipulation, is through changes in food source composition, which would include restriction of specific food components. However, in most of the scientific literature, the term DR does not distinguish between these two forms of manipulations, which can have independent effects on lifespan, as has been recently suggested (Maier et al., 2010).

The first experiment that has demonstrated that DR is a form of environmental perturbation that alter lifespan was performed 70 years ago in rodents (McCay et al., 1935). Subsequently, it has been shown that this phenomenon is conserved in many metazoan species (Guarente and Picard, 2005; Colman et al., 2009). In *C. elegans*, reducing the calories of its bacterial food source through different procedures, like bacterial dilution, increases lifespan (Klass, 1977). For example, the dilution of bacteria by 10-fold extends lifespan by about 60%, which is coupled to a severe decrease in reproductive output (Klass, 1977).

However, these studies have some drawbacks. First, the standard live *E. coli* food source that is fed to worms in the laboratory is slightly toxic, since feeding worms with UV-killed *E. coli* extends lifespan (Gems and Riddle, 2000; Garigan et al., 2002). Second, if the bacterial food source is treated with bacteriostatins, similar effects on lifespan have been observed (Garigan et al., 2002). Third, some cues derived from live bacteria have been found to reduce lifespan (Larsen and Clarke, 2002; Maier et al., 2010). Fourth, a semi-defined axenic non-bacterial medium also promotes worm longevity in liquid culture, although worms grown in this liquid medium, which includes yeast extract, soy peptone enriched with heme from liver extract and sterols, have altered physiology and reproduction compared to worms fed *E. coli* on solid agar plates (Houthoofd et al., 2003). Thus, it is not completely clear whether the previous reported effects of many of the DR regimens on worm lifespan are due to food-level restriction or food-type dependence.

In *Drosophila*, there are two protocols that are extensively used for DR studies. One is performed through a simple dilution of fly food, while another is through reduction of the yeast to sugar ratio (Mair et al., 2005). Both dietary manipulations prolong lifespan in flies at the expense of reproductive output (Mair et al., 2005). Looking at these results, one could simply conclude that DR of specific nutrients is sufficient to extend lifespan (Mair et al., 2005). However, these studies alone do not necessarily suggest such a straightforward conclusion, since these protocols rely on dilution and not on limiting the absolute availability of the food. Thus, it is unclear whether these flies have reduced yeast intake, calories or both. Indeed, several studies have shown that yeast and sugar concentrations affect fly feeding rates (Edgecomb et al., 1994; Min and Tatar, 2006; Skorupa et al., 2008), whereas another study has observed compensatory feeding with food dilution (Carvalho et al., 2005). However, this compensatory effect is disproportional with the degree of the dilution (Carvalho et al., 2005).

A more recent study has reported that the longevity-promoting effect of yeast restriction can be rescued by adding essential amino acids to the fly diet, which also restores the reproductive output of the fly (Grandison et al., 2009). Interestingly, methionine alone is necessary and sufficient to restore reproduction to the level promoted by full feeding without rescuing the long-life phenotype of flies fed a protein-poor diet (Grandison et al., 2009). Nonetheless, two other studies have also found that extreme reduction of methionine extends lifespan in mice and rats (Orentreich et al., 1993; Miller et al., 2005). In addition, another study has shown that, at least in rats, it is possible to extend lifespan by reducing other food components and keeping the protein concentration constant (Masoro et al., 1989). Thus, these latter studies suggest that, in addition to amino acids, there are other food components that affect lifespan.

2.3.1. Genetic pathways that mediate dietary restriction: C. elegans

Regarding the question on how DR extends lifespan, there have been so far many mechanisms that have been proposed, which range from a stochastic process to a regulated one. In recent years, the view has prevailed that DR is mediated by a regulated process, i.e., by one of the key genetic pathways, although controversy remains about the exact molecular nature of this regulation.

In *C. elegans*, genetic epistasis experiments show that the DR effects on lifespan is independent of IIS. For example, it is possible to extend the lifespan of *age-1/pi3k* mutants by DR (Johnson et al., 1990); of *daf-2* mutants by introducing a mutation in *eat-2*, which causes a decrease in food intake (Lakowski and Hekimi, 1998); and of *daf-2* or *daf-16* mutants by growing them in liquid non-bacterial axenic medium (Houthoofd et al., 2003). Moreover, *daf-16* does not suppress the long-life phenotype of an *eat-2* mutant, a worm genetic model of DR (Lakowski and Hekimi, 1998).

Additional experiments suggest that the TOR pathway is a likely candidate for promoting the lifespan extension due to DR, since DR does not extend the lifespan of *C. elegans* TOR mutants (Hansen et al., 2007). Furthermore, both DR and downregulation of TOR activity (i) induce an autophagic phenotype and (ii) increase lifespan in a manner that requires the activities of genes that promote autophagy (Hansen et al., 2008).

2.3.2. Genetic pathways that mediate dietary restriction: D. melanogaster

In *Drosophila*, the pathways that mediate DR are less clear. It seems that mutants in the IIS pathway can still respond to DR but their response is somewhat altered. Flies that carry loss-of-function mutations in *chico* (Clancy et al., 2002) or the insulin-like peptide genes *dilp2*, *dilp3* and *dilp5* (Grönke et al., 2010) or flies that overexpress dFOXO (Giannakou et al., 2008) can live longer after DR treatment, but their responses are shifted

compared to control flies. The typical DR lifespan responses for wild-type and IIS mutants are shown in Figure 9.

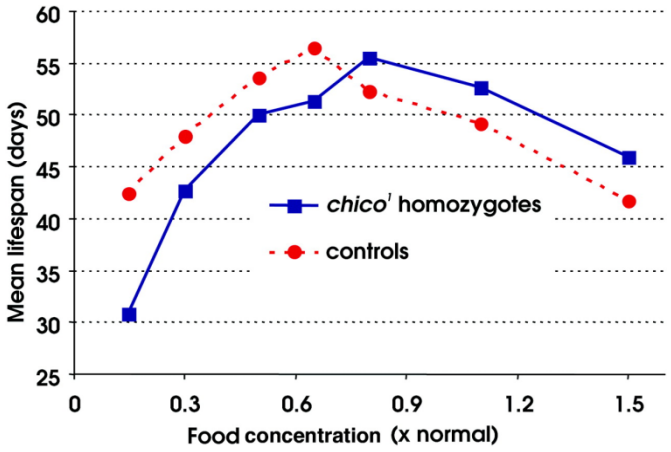

Figure 9. The interaction between IIS and DR. On high food levels, reduced IIS activity increases lifespan. However, on low food levels, flies with reduced IIS live shorter than control flies. The figure is taken from (Clancy et al., 2002).

As can be seen in Figure 9, the mean lifespan curve in response to different food levels is shifted to the right for IIS mutants (Clancy et al., 2002). This means that on high food the IIS pathway reduction-of-function or loss-of-function mutants or dFOXO overexpressors live long, while on low food they live short compared to wild type (Clancy et al., 2002; Giannakou et al., 2008; Grönke et al., 2010). However, *dFOXO* null mutants are able to respond to DR (Giannakou et al., 2008). Thus, these data suggest that the IIS pathway is not required for the DR lifespan response, but that IIS could modulate this response. Yet, recent studies suggest that *dilp5* can mediate the lifespan effects of DR, possibly by acting independent of *dFOXO* (Grönke et al., 2010), which raises an intriguing possibility that the activities of the different *dilps* require different downstream effectors and might explain part of the IIS response to DR. In contrast to IIS, the fly TOR pathway has been found to be required for the lifespan extension due to DR, since (i) the lifespan of *d4EBP* null mutants do not respond to changing levels of yeast concentration and (ii) *d4EBP* levels are induced under these conditions independently of *dFOXO* (Zid et al., 2009).

2.4. *Perception of nutrient levels and/or quality by the sensory system to affect lifespan*

Food levels or the compositions of different food sources are presumably sensed by the animal's sensory system. In the subsequent sections, I will discuss the organization of the nutrient-sensing gustatory and olfactory systems within the fly and the importance of these systems in eliciting the appropriate physiological responses.

2.4.1. *Organization of the fly gustatory system*

The taste organs of the fly are distributed all over the body. Flies have a functional homolog of the vertebrate tongue in their proboscis and taste structures on their legs and wings (Figure 10), as well as within the female vaginal plates (Vosshall and Stocker, 2007).

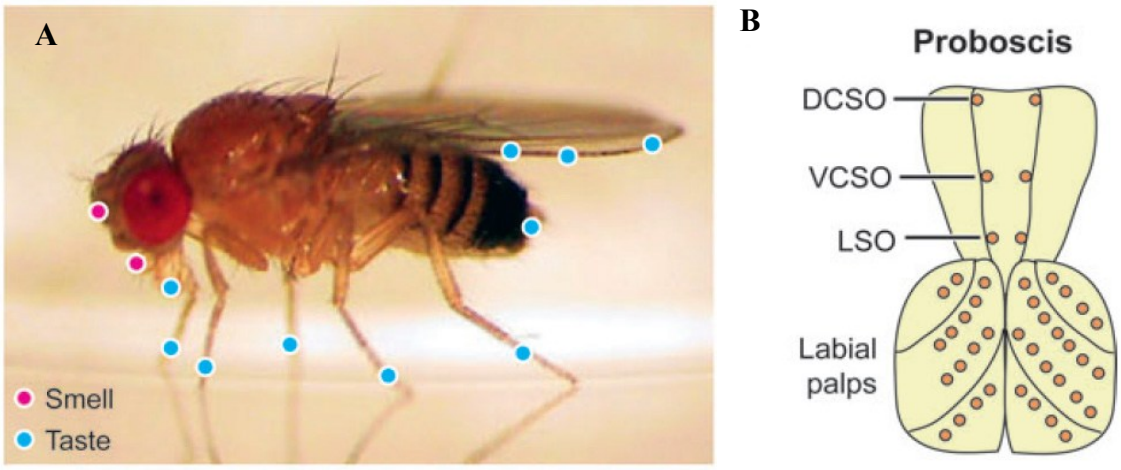

Figure 10. Organization of the fly gustatory system. A) The fly has taste bristles on the proboscises, legs and wings. B) Proboscis view from the ventral side: labial/labellar palps contain taste bristles and taste pegs, while the pharynx contains three bilaterally symmetric organs, DCSO, VSCO and LSO (see text for more details). This figure is taken from (Vosshall and Stocker, 2007)

There are numerous gustatory receptor neurons (GRNs) on the proboscis, the majority of which are located on the labellum (Figure 10). The labellum contains two different taste structures: 31 labellar taste bristles, each of which is innervated by 2 to 4 GRNs; and approximately 30 taste pegs, each of which is innervated by a single GRN (Vosshall and Stocker, 2007). These external sense organs could be classified into three groups, according to size, distribution and number: small (s-type), long (l-type) and intermediate (i-type)

sensilla (Vosshall and Stocker, 2007). Each of the s-type and l-type sensilla has four GRNs: one neuron is activated by sugars (the S cell), another by water (the W cell), a third by low salt (the L1 cell), and a fourth by high salt (the L2 cell) (Vosshall and Stocker, 2007). The bristles that are innervated by two GRNs are missing the W neuronal cell, while the S and L1 neuronal cell activities are located within one cell (Rodrigues and Siddiqi, 1981; Amrein and Thorne, 2005).

The taste bristles and taste pegs on the labella are key taste organs involved in feeding, since they enable the fly to evaluate the quality of the food before its intake. There are also three bilaterally symmetric taste organs within the fly pharynx, the labral sense organ (LSO) and the ventral and dorsal cibarial sense organs [VCSO and DCSO, respectively; (Vosshall and Stocker, 2007)]. These internal organs might act as final checkpoints that allow the fly further assessment of food quality and adjustment of appetite (Vosshall and Stocker, 2007).

Flies also have taste organs on their legs and wings. Males have 50 taste sensilla on the first pair of legs, while females have 37 in comparison (Vosshall and Stocker, 2007). This difference in the number of taste bristles between males and females may reflect the courtship behavior of the males and their perception of female pheromones (Bray and Amrein, 2003; Park et al., 2006). At the same time, both males and females have 30 and 32 taste bristles on their second and third pairs of legs, respectively (Vosshall and Stocker, 2007). Leg taste bristles have a similar structure as the labellar taste bristles and are also innervated by two to four GRNs (Vosshall and Stocker, 2007). In contrast, the fly wing contains 40 taste bristles, each of which is innervated by 4 GRNs (Vosshall and Stocker, 2007). The role of the leg and wing taste bristles in feeding behavior is unclear.

The GRNs from the labella, legs and wings project to different regions of the subesophageal ganglion (SOG), which might suggest that both the quality and positional

information coming from the GRNs are preserved in the SOG (Wang et al., 2004). Different GRNs from the same peripheral tissue can recognize different taste cues: e.g., Gr66a neurons recognize bitter compounds, while Gr5a neurons recognize sweet compounds (Scott et al., 2001; Wang et al., 2004). The projections of Gr5a and Gr66a in the SOG are segregated to form part of a spatial taste map: Gr5a and Gr66a likely connect to different types of SOG interneurons that regulate attractive versus aversive feeding behavior (Wang et al., 2004).

It is worth noting that the molecular and neuronal organization of the gustatory system in flies resembles the mammalian system (Vosshall and Stocker, 2007). First, multiple gustatory receptors (GRs) are expressed in single neurons; second, bitter and taste receptors are expressed in different subpopulations of cells; and third, both flies and mammals have spatial gustatory maps within their brains (Scott et al., 2001; Wang et al., 2004; Vosshall and Stocker, 2007). This suggests that the study of taste and taste-related phenomena in flies should yield valuable lessons for the mammalian systems.

2.4.2. Organization of the fly olfactory system

The olfactory organs of the flies are located in the third antennal segment and in the maxillary palps on the head [Figure 11; reviewed in (Vosshall and Stocker, 2007)]. Both the antennae and maxillary palps are covered with specialized hairs that are also called sensilla and protect the olfactory receptor neurons (ORNs) from external damage [Figure 11; (Vosshall and Stocker, 2007)]. Structurally, the fly neurons share some common features with mammalian neurons (Vosshall and Stocker, 2007). Fly ORNs are bipolar and project a single axon from the basal side and a sensory dendrite from the apical side [Figure 11; (Vosshall and Stocker, 2007)]. Each axon of an ORN projects to an olfactory glomerulus in the antennal lobe [AL; (Vosshall and Stocker, 2007)]. The fly olfactory glomerulus is functionally analogous to the mammalian olfactory bulb (Vosshall and Stocker, 2007).

On the other hand, each sensory dendrite projects into the shaft of a sensillum: each sensillum contains between one and four ORNs that are surrounded by support cells [Figure 11; (de Bruyne et al., 1999; de Bruyne et al., 2001)]. There are different types of sensilla (basiconic, trichoid, and coeloconic) and they differ in size and morphology [Figure 11; (Vosshall and Stocker, 2007)]. Each antenna contains between 1100–1250 ORNs and have sexually dimorphic distribution (Stocker, 2001), although the functional significance of this sexual dimorphism remains unknown.

Unlike the antennal segment, the maxillary palp contains only one class of sensilla, the basiconic sensilla [Figure 11; (Vosshall and Stocker, 2007)]. Each palp contains 60 sensilla, where each sensillum is innervated by two ORNs (de Bruyne et al., 1999; Vosshall and Stocker, 2007). These sensilla can be categorized into six different classes based on their functions (de Bruyne et al., 1999), which reflect the combinations of olfactory receptors expressed in the ORNs of these sensilla (Couto et al., 2005; Goldman et al., 2005). While maxillary palp ORNs project to the AL, it is interesting that their axons also fasciculate with GRNs from the labellar nerve and that their afferents pass through the SOG to reach the AL (Vosshall and Stocker, 2007). The functional significance of this kind of neuronal architecture is currently unknown, but it might suggest an interaction between gustation and olfaction in feeding behavior.

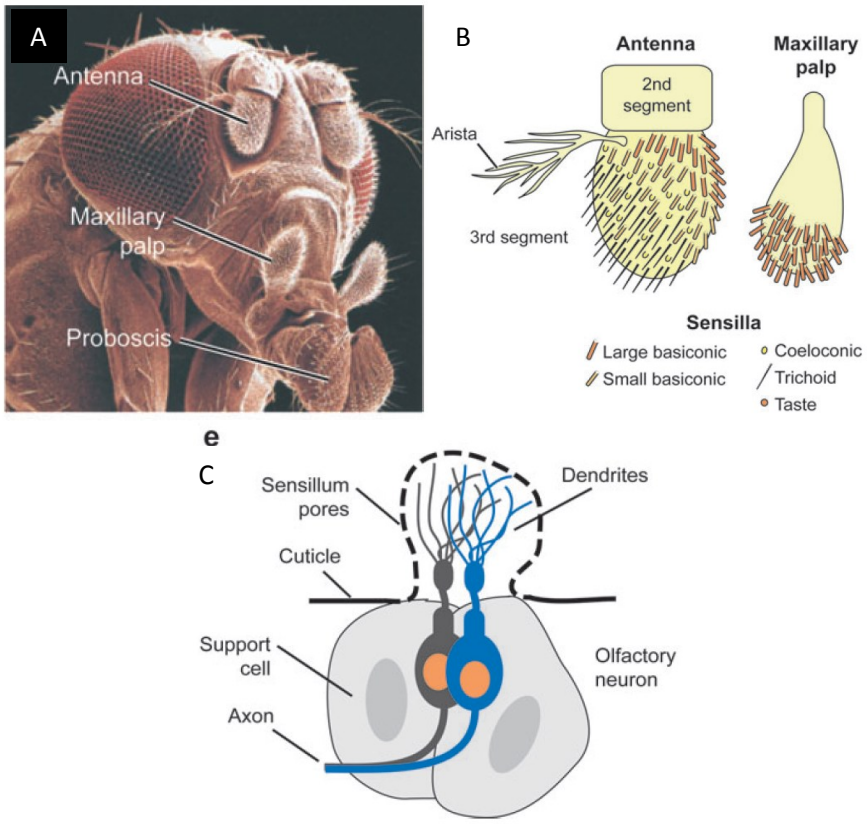

Figure 11. The fly ORNs are located on the fly head. (A) The fly head and its maxillary palps, antennae and proboscis. (B) Schematic representations of the olfactory structures on the 3rd anntenal segment and the maxillary palp. The distributions of of the different sensilla are also depicted. C) Each sensillum is innervated by one to four ORNs that are supported by support cells. This figure is taken from (Vosshall and Stocker, 2007).

2.4.3. The sensory influence on lifespan

What mediates the interaction between environmental and genetic factors to regulate an animal's lifespan? One possibility is that particular food components act as internal signals to activate or inhibit different genetic pathways, which would consequently affect lifespan. Another interesting possibility is that the activities of these pathways are modulated upon sensory perception of specific external environmental signals.

To test this hypothesis, Apfeld and Kenyon (1999) measured the lifespan of *C. elegans* mutant that have disrupted sensory structures. Accordingly, worms that have disrupted sensory cilia or sensory support structures live longer than wild type in a *daf-16*-

dependent manner (Apfeld and Kenyon, 1999). Moreover, this sensory influence on worm lifespan involves a specific subset of sensory neurons under a particular environmental condition (Alcedo and Kenyon, 2004). Laser ablation of specific olfactory neurons extends lifespan, which means that the wild-type function of these neurons is to inhibit longevity (Alcedo and Kenyon, 2004). At the same time, laser ablation of specific gustatory neurons suggests that they have different effects on longevity—inhibitory, promoting or neutral (Alcedo and Kenyon, 2004). Unlike olfactory neurons, the ablation of a pair of gustatory neurons named ASI increases worm lifespan in a completely *daf-16*-dependent manner (Alcedo and Kenyon, 2004). On the other hand, killing another subset of gustatory neurons, ASJ and ASK, suppresses the long-life phenotype due to the ASI ablation, and killing other pairs of gustatory neurons, ASE and ADF, had no effect on lifespan (Alcedo and Kenyon, 2004).

In addition, ablation of ASJ and ASK in long-lived *daf-2* mutants shortens the lifespan of these worms (Alcedo and Kenyon, 2004), which, together with the *daf-16*-dependence of the ASI ablation phenotype, suggests that this gustatory influence on lifespan is mediated by the IIS pathway. Since many sensory neurons express insulin-like peptides (Pierce et al., 2001; Li et al., 2003), this suggests a model where gustatory cues affect lifespan by regulating the release of insulin-like peptides that would modulate the activity of the IIS pathway in worms.

The fact that there is a sensory influence on worm lifespan raises the possibility that such an influence also exists in other animals. Recently, olfactory cues derived from live yeast have been shown to shorten fly lifespan (Libert et al., 2007). As mentioned above, a reduction in yeast concentrations in the fly food lengthens lifespan (Mair et al., 2005). However, exposure of yeast-restricted, long-lived flies to live yeast odors alone is sufficient to decrease their lifespan (Libert et al., 2007). Consistent with the idea that the olfactory

system also influences fly lifespan, loss of olfaction through a mutation in the broadly expressed olfactory co-receptor *Or83b*^{2} (Larsson et al., 2004) prolongs fly lifespan (Libert et al., 2007). However, the genetic pathways that act downstream of olfaction to regulate fly lifespan are unknown to this date. Furthermore, the role of gustatory perception on fly lifespan or the mechanisms involved in such a process remain unanswered and are the aims of this study.

3. Scope of the thesis

Considering the role of gustatory neurons in the lifespan regulation of *C. elegans* and the conserved influence of olfaction on *Drosophila* lifespan (Alcedo and Kenyon, 2004; Libert et al., 2007), I posed the question on whether the taste influence on lifespan is also conserved in flies. Since the gustatory system also affects *C. elegans* lifespan bidirectionally (Alcedo and Kenyon, 2004), I further asked whether there are positive and negative inputs on *Drosophila* lifespan, which are coming from different taste neurons.

Various studies have shown that changes in lifespan can be achieved through alterations in diet, either through a reduction in the number of calories (food-level restriction) or a change in the dietary composition of the food source with little or no effect on caloric value (food-type dependence). The effects of both forms of dietary manipulation on lifespan—food-level restriction and food-type dependence—have been shown to be at least partly mediated by the sensory system in both worms (Bishop and Guarente, 2007; Maier et al., 2010) and flies (Libert et al., 2007). Thus, I also asked whether flies lacking different taste inputs might have altered physiology and lifespan that are due to food-level restriction and/or dependence on food type.

The effects of gustatory neurons on worm lifespan have been shown to be mediated by the IIS pathway (Alcedo and Kenyon, 2004), which next led me to ask whether fly taste inputs would also modulate IIS activity to affect lifespan. Consistent with this idea, fly gustatory neurons have been found to project their axons to the SOG interneurons, which in turn communicate with the insulin-producing mNSCs (Brogiolo et al., 2001; Scott et al., 2001; Ikeya et al., 2002; Rulifson et al., 2002; Melcher and Pankratz, 2005; Agrawal et al., 2009). Accordingly, I determined how different taste inputs affect *dilp* mRNA levels and tested for genetic interactions between taste inputs and the IIS downstream effector *dFOXO*

on regulating fly physiology. I propose that different gustatory cues will promote or inhibit the activities of different ILPs to modulate IIS and dFOXO, which consequently would lead to changes in fly metabolism, stress resistance and lifespan.

4. Results

Positive and Negative Gustatory Inputs Affect *Drosophila* Lifespan Partly in Parallel to *dFOXO* Signaling

Ivan Ostojic[1], Werner Boll[2] and Joy Alcedo[1]*

[1]Friedrich Miescher Institute for Biomedical Research

Maulbeerstrasse 66, CH-4058 Basel, Switzerland; [2]Institute of Molecular Life Sciences,

University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

*Correspondence: joy.alcedo@fmi.ch

Tel. no.: ++41 61 697 5173

Fax. no.: ++41 61 697 3976

4.1. Abstract

In *C. elegans*, a subset of gustatory neurons, as well as olfactory neurons, shortens lifespan, while a different subset of gustatory neurons lengthens lifespan. Recently, the lifespan-shortening effect of olfactory neurons has been reported to be conserved in *Drosophila*. Here we now show that the *Drosophila* gustatory system also affects lifespan in a bidirectional manner. We find that taste inputs shorten lifespan through inhibition of the insulin pathway effector *dFOXO*, whereas other taste inputs lengthen lifespan in parallel to this pathway. In addition, we note that the gustatory influence on lifespan is independent of food intake levels but depends on the type of food sources, which involve yeast-dependent and yeast-independent effects. Together our study suggests that different gustatory cues can modulate the activities of distinct signaling pathways, including different insulin-like peptides, to promote physiological changes that ultimately affect lifespan.

4.2. Introduction

Aging is a universal process that causes deterioration in the biological functions of an organism over the progression of its lifetime. This process is affected by genetic and environmental factors, whose interaction could be mediated by the sensory system, which perceives and transmits environmental information to modulate the signaling activities of downstream target tissues. Accordingly, external sensory cues and sensory neurons have been shown to alter the lifespan of both *C. elegans* and *D. melanogaster* (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004; Libert et al., 2007; Lee and Kenyon, 2009).

In *C. elegans*, the laser ablation of a specific subset of gustatory or olfactory neurons extends lifespan, whereas ablation of a different subset of gustatory neurons shortens lifespan (Alcedo and Kenyon, 2004). Interestingly, at least part of this sensory influence on lifespan has also been observed in other animals. In *Drosophila*, impairment of its olfaction through a mutation in the gene *Or83b*, which encodes a broadly expressed atypical odorant receptor (Larsson et al., 2004), leads to increases in lifespan (Libert et al., 2007). In addition, exposure of calorically-restricted flies to food odors, like live yeast, can partly suppress their long-life phenotype (Libert et al., 2007). Since these studies show that the olfactory influence on lifespan is conserved, it raises the likelihood that gustatory inputs will also bidirectionally alter both the lifespan of *C. elegans* and *D. melanogaster*.

The effects of sensory neurons on *C. elegans* lifespan have been shown to be partly mediated by insulin/IGF signaling (Apfeld and Kenyon, 1999; Lin et al., 2001; Alcedo and Kenyon, 2004). The insulin/IGF pathway also affects fly lifespan: downregulation of the activities of the insulin receptor InR and the receptor substrate, CHICO, extend lifespan (Clancy et al., 2001; Tatar et al., 2001). Moreover, an increase in activity of the downstream

transcription factor dFOXO, which is negatively regulated by both InR and CHICO, increases fly lifespan (Giannakou et al., 2004; Hwangbo et al., 2004). Consistent with these observations, mutations in several of the *Drosophila* insulin like peptide (*dilp*) genes (Grönke et al., 2010), which are expressed in the median neurosecretory cells (mNSCs) in the fly brain (Brogiolo et al., 2001; Cao and Brown, 2001; Ikeya et al., 2002; Rulifson et al., 2002), or ablation of the mNSCs (Broughton et al., 2005) extends lifespan. Because these mNSCs send projections to the subesophageal ganglion (SOG) (Ikeya et al., 2002; Rulifson et al., 2002), a group of interneurons involved in processing gustatory information in the fly brain (Scott et al., 2001; Melcher and Pankratz, 2005), it raises the intriguing possibility that, like in worms, the effects of the insulin/IGF pathway on fly lifespan are also subject to gustatory cues.

Thus, in this study, we tested whether the gustatory influence on lifespan is present in flies, and whether its effects are mediated by insulin/IGF signaling. *Drosophila* has on its labellum (mouthpart), legs and wings many taste sensilla that have bristle-like structures, which are innervated by two to four gustatory neurons and a mechanosensory neuron (Gerber et al., 2009). Using genetic tools that eliminate a subset or most of the fly's taste bristles and the corresponding gustatory neurons that innervate them, we demonstrate that, like in *C. elegans*, there are taste inputs that lengthen *Drosophila* lifespan and other taste inputs that shorten it. We also show that the gustatory influence on fly lifespan is only partly dependent on (i) the activity of the *dFOXO* transcription factor, which acts downstream of insulin signaling, and (ii) the effects of yeast in the food source.

4.3. Results

4.3.1. Taste Inputs Affect Drosophila Lifespan

To test the hypothesis whether taste inputs affect fly lifespan, we compared two classes of taste-impaired flies to control flies with wild-type taste perception. Accordingly, we used the *Pox neuro* (*Poxn*) null mutant $Poxn^{\Delta M22\text{-}B5}$, whose taste bristles are either missing or are transformed into bristles that lack gustatory innervations but retained the mechanosensory innervation (Awasaki and Kimura, 1997). However, *Poxn* is a gene with pleiotropic activities, which also include functions in the central nervous system and the development of antenna, legs and male genitalia (Bopp et al., 1989; Dambly-Chaudière et al., 1992; Takeshi and Ken-ichi, 1997; Awasaki and Kimura, 2001; Boll and Noll, 2002). Hence, for our studies, we compared $Poxn^{\Delta M22\text{-}B5}$ mutants that carry the complete rescue construct to $Poxn^{\Delta M22\text{-}B5}$ mutants that carry rescue constructs that lack enhancer elements required for the formation of either a subset of (labellar) or most taste bristles [Figure S1; (Boll and Noll, 2002; Krstic et al., 2009)].

We analyzed different combinations of independent transgenic lines that were extensively backcrossed to the same background and found that flies missing a subset of taste inputs, *i.e.*, labellar taste bristles, live longer than flies with wild-type taste inputs (Figures 1A, 1B and 1E; Table S1). We observed that this effect is more robust in females than in males (Figures 1A, 1B and 1E; Table S1). Interestingly, we also found that the removal of additional taste bristles on the legs and wings completely or partially suppresses the long-life phenotype of both labellar taste-impaired male and female flies (Figures 1C-1E; Table S1). Thus, these studies show that both the positive and negative influences of taste inputs on lifespan are conserved in *Drosophila*.

4.3.2. *The Physiology of Taste-impaired Flies Do Not Resemble Those of Calorically-Restricted Flies*

It is possible that loss of taste inputs might lead to decreases in feeding rates, which in turn would alter *Drosophila* lifespan. Indeed, a reduction in the level of food intake that does not result in malnutrition, which is known as calorie restriction (CR), extends lifespan, whereas a further reduction in feeding, which presumably mimics a state of starvation, causes a shorter lifespan (McCay et al., 1935). Surprisingly, however, we observed that both classes of taste-impaired flies have increased food intake compared to control flies (Figures 2A and 2B), which suggests that the lifespan phenotypes of these flies do not correlate with their feeding rates. In males the differences in food intake are already visible after the fourth day of adulthood, while in females the differences become more apparent only after ten days of adulthood. Consistent with these increases in food intake, we found that taste-impaired flies have higher body weights (Figures 2C and 2D). Since calorie-restricted flies are reported to have lower body weights (Bross et al., 2005), our observations suggest that the taste-impaired flies are not necessarily subject to CR.

Other hallmarks of CR are decreased fat storage (Wang et al., 2009) and reduced reproductive output (Chapman and Partridge, 1996). To show further that taste-impaired flies are not eating less food due to the lack of hedonic stimuli from food components, we measured their triglyceride (TAG) levels in terms of fat content, their rates of reproduction and total fecundity. We saw no correlation between the lifespan and TAG levels of these flies: long-lived flies with no labellar taste bristles have similar TAG content as control flies (Figures 2E and 2F). Indeed, we also found that female flies missing most taste bristles actually have increased TAG levels compared to flies with wild-type taste function (Figure 2F). In addition, although we detected some differences between the taste mutants and

control flies in the number of eggs laid per day (Figure 2G), we observed that the various groups of flies laid a similar cumulative number of eggs within a period of ten days (Figure 2H). Thus, our data together suggest that the lifespan alterations we observe in flies lacking taste bristles are not simply due to the general restriction of food levels.

4.3.3. Some Taste Inputs Act Independent, while Others Modulate the Lifespan Effects of Yeast in the Food Source

Besides food levels, the nature of the food source has also been shown to influence an animal's lifespan (Garsin et al., 2003; Mair et al., 2005; Maier et al., 2010), such as live yeast as a food source for flies (Libert et al., 2007). Since yeast has been shown to shorten fly lifespan partly through an olfaction-mediated mechanism (Libert et al., 2007), we asked whether the effect of yeast on lifespan also acts via taste inputs. We found that the absence of a yeast supplement in the standard diet (see Experimental Procedures) can still extend the lifespan of all taste-impaired flies and control flies (Figures 3A-3D; Table S1). Yet, we also observed that the lifespan extension conferred by defects in labellar taste inputs depends on the presence of this food source—completely in males but only partly in females (Figures 3A and 3B; Table S1). In addition, we found that the absence of this supplement lengthens the lifespan of male flies missing most taste bristles (51%; Table S1) more so than control flies (29%; Figure 3C; Table S1). On the other hand, the lack of this food source increases the lifespan of female flies that have all taste bristles and of female flies missing most taste bristles to the same extent (28% vs 30%, respectively; Figure 3D; Table S1). Together these observations suggest the existence of two classes of taste inputs: (i) those that modulate the lifespan-shortening effect of yeast; and (ii) inputs that act independent of this food source, which can come from the labella and/or legs and wings.

The existence of yeast-dependent and yeast-independent taste inputs support the hypothesis that the taste influence on lifespan is mediated by several mechanisms, which include those that do not involve the restriction of calories but depend on specific food sources. Interestingly, we also note that the sexual dimorphism of the taste influence on lifespan appears to be subject to the yeast-dependence of the gender-specific taste inputs: males have more inputs that modulate the effects of yeast, while females have more yeast-independent inputs. Accordingly, this may reflect the differences in the nutrient requirements of unmated male and female flies.

4.3.4. Some Taste Inputs Require the Activity of the Insulin Pathway Effector dFOXO

In worms, the taste influence on lifespan can act in parallel to CR but is mediated by the insulin signaling pathway (Alcedo and Kenyon, 2004). In flies, mutations in the insulin receptor (*InR*) (Tatar et al., 2001) or the receptor substrate *chico* (Clancy et al., 2001) have been shown similarly to extend lifespan, which require the activity of the transcription factor *dFOXO* (Giannakou et al., 2004; Hwangbo et al., 2004). In contrast, *dFOXO* activity is not required for the lifespan increase caused by CR (Giannakou et al., 2008). Thus, these observations led us to ask whether *dFOXO* also mediates the lifespan extension observed in flies lacking a subset of taste inputs.

We found that removal of *dFOXO* suppressed the long-life phenotype of labellar taste-impaired flies (Figures 3E and 3F; Table S1). However, we also observed that labellar taste-impaired females lacking *dFOXO* activity live shorter than *dFOXO* mutant females with wild-type taste bristles (Figure 3F; Table S1). This suggests that the female labella receive not only taste inputs that shorten lifespan in a *dFOXO*-dependent manner, but also inputs that lengthen lifespan in parallel to *dFOXO*. Moreover, we found that loss of additional taste

bristles from the legs and wings of female *dFOXO* mutants further shortened their lifespan (Figure 3H; Table S1), which suggests that leg and wing taste inputs affect lifespan independent of *dFOXO*. It should be noted that the sexual dimorphism of the taste influence on lifespan again extends to these *dFOXO*-independent inputs, *i.e.*, they are largely present in females rather than in males (Figures 3E-3H; Table S1).

4.3.5. Taste-impaired Flies Have Altered Expression of dFOXO Target Genes

Since the taste influence on lifespan appears to require dFOXO activity only partly, we measured the steady-state transcript levels of several dFOXO targets—*dilp3*, *dilp6*, the small heat shock protein *l(2)efl* and the translational inhibitor *4E-BP* (Broughton et al., 2008; Flatt et al., 2008; Slaidina et al., 2009; Grönke et al., 2010)—in both classes of taste mutants. We detected no significant change in *dilp3*, but we found that the expression of some of the dFOXO target genes are elevated in both classes of ten-day-old taste-impaired flies: *dilp6* in the female heads, *l(2)efl* in both male and female bodies, and *4E-BP* in female bodies (Figure 4). The lack of correlation between these expression levels and the lifespan of taste-impaired flies is again consistent with taste inputs affecting lifespan in a *dFOXO*-independent manner. At the same time, the combined observations that loss of *dFOXO* does suppress the long-life phenotype of labellar taste-impaired flies (Figures 3E and 3F; Table S1) and that certain dFOXO targets are upregulated in these flies (Figure 4) suggest that there are other taste inputs that also modulate dFOXO activity in some tissues.

The increase in dFOXO target gene expression, like *dilp6* and *4E-BP*, have been reported in flies lacking the mNSC-expressed *dilp2*, *dilp3* and *dilp5* (Grönke et al., 2010). However, we observed no significant effect on *dilp2*, *dilp3* and *dilp5* transcripts, with the exception of *dilp5* in ten-day-old males (Figure 4). Interestingly, unlike *dilp2*, *dilp3* and

dilp5, we found that *dilp1* expression, which is also present in the mNSCs (Rulifson et al., 2002) but so far has not been implicated in regulating lifespan (Grönke et al., 2010), is significantly higher in ten-day-old labellar taste-impaired female flies than in control flies (Figure 4G). Thus, our findings raise the possibility that taste inputs do not modulate all *dilp* transcription but perhaps the translation and/or secretion of some, *e.g.*, through the SOG interneurons that can act as a relay center between gustatory neurons and the *dilp*-expressing mNSCs (Scott et al., 2001; Rulifson et al., 2002; Melcher and Pankratz, 2005).

dilp6 expression is induced in the fat body upon a pause in feeding during the transition from the larval to pupal stages (Okamoto et al., 2009; Slaidina et al., 2009) and in the adult abdominal fat body upon loss of *dilp2*, *dilp3* and *dilp5* (Grönke et al., 2010). The induction in *dilp6* expression in the late larval and pupal fat body cells has been proposed to promote growth at the expense of nutrient storage in the absence of food uptake (Slaidina et al., 2009). This is supported by the observation that *dilp6* loss- or reduction-of-function mutants have a smaller body size and are starvation resistant, while *dilp6*-overexpressing flies have a larger body size and decreased resistance to starvation (Okamoto et al., 2009; Slaidina et al., 2009). In contrast, our observed increase in *dilp6* in the head fat body is not accompanied by a change in the abdominal fat body, except in one-day-old female flies missing most taste bristles (Figure 4). In addition, this *dilp6* increase is not associated with lower, but higher, resistance to starvation in both classes of female taste mutants (Figure S2). Although the significance of this increase remains unclear, dFOXO, which is required for *dilp6* activity (Slaidina et al., 2009), has been shown to have systemic effects on fly physiology by acting in the head fat body (Hwangbo et al., 2004), a possible mechanism through which taste inputs could influence lifespan.

4.4. Discussion

Many biological processes are conserved between *C. elegans*, *Drosophila* and higher organisms. For example, the insulin/IGF-1 pathway regulates the physiology, and consequently the longevity, of worms, flies and mice (Clancy et al., 2001; Tatar et al., 2001; Blüher et al., 2003; Holzenberger et al., 2003; Kenyon, 2010). Similarly, the sensory influence on lifespan is conserved in both worms (Alcedo and Kenyon, 2004) and flies at the level of olfaction (Libert et al., 2007; Poon et al., 2010) and gustation (this study).

Like in *C. elegans* (Alcedo and Kenyon, 2004), our study also shows that the sensory system can affect *Drosophila* lifespan bidirectionally (Figure 1; Table S1), independent of the level of food intake (Figure 2). Indeed, both longer-lived and shorter-lived taste-impaired flies have higher levels of food intake, body weights and, in one case, even fat storage (Figure 2), which is reminiscent of human studies that demonstrated a negative correlation between food intake (or body mass index) and taste sensitivity to certain food components (Simchen et al., 2006; Stewart et al., 2010).

In contrast to its independence of overall food intake levels, we find that the gustatory influence on fly lifespan does depend partly on the type of food source, *e.g.*, a yeast-enriched diet versus a standard diet (Figures 3A-3D; Table S1). This suggests the possibility that this sensory influence, as in worms, depends on the recognition of food types, which can have different effects on lifespan (Garsin et al., 2003; Mair et al., 2005; Maier et al., 2010). In the fly, the food-type effect on lifespan has been demonstrated through alterations in the protein composition of its food source: *i.e.*, yeast restriction or an imbalance in dietary amino acids can extend lifespan (Mair et al., 2005; Skorupa et al., 2008; Grandison et al., 2009). However, yeast restriction alone does not always increase fly lifespan under all conditions that do not cause malnutrition (Skorupa et al., 2008), which suggests that other lifespan-influencing food-derived factors are also involved. Since the gustatory system senses many

different food-derived cues, which could elicit different physiological outcomes, it is not surprising that the taste influence on lifespan would only be partly dependent on the effects of yeast.

Consistent with the idea that the gustatory system will affect lifespan in response to a variety of food-derived cues, we show that this influence requires both *dFOXO*-dependent and *dFOXO*-independent pathways (Figures 3E-3H; Table S1). It is likely that different cues will modulate the activities of distinct pathways. Moreover, our findings that subsets of dFOXO targets in subsets of tissues are altered in either one or both classes of taste-impaired flies (Figure 4) might suggest that specific gustatory cues modulate the activities of discrete sets of *dilps*, either in the mNSCs or the fat body cells in the head or abdomen.

Finally, the observation that the gustatory and olfactory systems influence the lifespan of both worms and flies [(Alcedo and Kenyon, 2004; Libert et al., 2007; Poon et al., 2010); this study] raises the intriguing possibility that the mammalian sensory system also affects its lifespan. In mammals, both gustatory and olfactory information are relayed to the hypothalamus, a region in the brain that controls behavior and physiology [see review by (Alcedo et al., 2010)]. Thus, it is conceivable that the processing of such sensory information by the hypothalamus may lead to physiological changes, which in turn may have bidirectional effects on mammalian lifespan.

4.5. Experimental Procedures

4.5.1. Fly Stocks

The genotypes of the transgenic flies are:

wild-type *Poxn* line 1 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$ *SuperA-158*],

wild-type *Poxn* line 2 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *SuperA-207-1*],

wild-type *Poxn* line 1 / line 2 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$ *SuperA-158* / $Poxn^{\Delta M22\text{-}B5}$; *SuperA-207-1* / +],

wild-type *Poxn* line 1 / line 2; *dFOXO* [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$ *SuperA-158* / $Poxn^{\Delta M22\text{-}B5}$; *SuperA-207-1* $dFOXO^{21}$ / $dFOXO^{25}$,

no labellar taste bristles line 1 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full1*],

no labellar taste bristles line 2 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full115*],

no labellar taste bristles line 3 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full152*],

no labellar taste bristles line 1 / line 2 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full1* / *Full115*],

no labellar taste bristles line 1 / line 3 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full1* / *Full152*],

no labellar taste bristles line 2 / line 3 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full115* / *Full152*],

no labellar taste bristles line 1 / line 2; *dFOXO* [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full1* $dFOXO^{25}$ / *Full115* $dFOXO^{21}$],

missing most taste bristles line 1 [*ΔXBs* w^{1118}; $Poxn^{\Delta M22\text{-}B5}$],

missing most taste bristles line 1 / line 2 [*ΔXBs* w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *ΔPBs* / +],

missing most taste bristles line 1 / line 3 [*ΔXBs* w^{1118}; $Poxn^{\Delta M22\text{-}B5}$ / $Poxn^{\Delta M22\text{-}B5}$ *ΔPBs*], and

missing most taste bristles line 1 / line 3; *dFOXO* [*ΔXBs* w^{1118}; $Poxn^{\Delta M22\text{-}B5}$ / $Poxn^{\Delta M22\text{-}B5}$ *ΔPBs*; $dFOXO^{21}$ / $dFOXO^{25}$].

All transgenic rescue constructs, *SuperA-158*, *SuperA-207-1*, *Full1*, *Full115*, *Full152*, *ΔXBs* and *ΔPBs*, were as formerly described (Boll and Noll, 2002; Krstic et al., 2009). The *dFOXO* null alleles, $dFOXO^{21}$ and $dFOXO^{25}$ (gift of Ernst Hafen), were also as described previously (Jünger et al., 2003). To minimize the effect of the genetic background on the experimental results, all flies were backcrossed at least seven times to the w^{1118} background before any analyses was conducted. All flies were maintained at 25°C.

4.5.2. *Lifespan Assays*

The lifespan of the progeny of 3- to 5-day-old males and females were measured at 25^{o}C under constant humidity (60%) with a 12h/12h light/dark cycle. To minimize stress-induced mortality in very young adults, freshly eclosed flies (within a 2h time window) were transferred to new bottles, where they were allowed to age for 2h. Subsequently, these flies were collected under mild CO_2 anesthesia for the lifespan assays, in which adult virgin males and virgin females were separated. The lifespan measurements were done by placing approximately 10 flies per vial (10 vials per trial), which contains standard Zurich fly food (10% yeast, 7.5% dextrose, 5.5% corn meal, 1% flour, 0.8% agar, 0.1% nipasol and 0.05% nipagin) supplemented with a drop of yeast paste on top of the food, unless stated otherwise. Flies were transferred to fresh tubes and scored for survival thrice a week. The JMP 5.1 (SAS) software was used to determine the Kaplan-Meier survival probabilities, mean lifespans and statistical comparisons among the different assay conditions, according to the logrank or Wilcoxon tests where appropriate. If the ratio of hazard functions (ratio of mortality rates) between two groups of animals stays approximately constant over time, the logrank test serves as the appropriate test; otherwise, the Wilcoxon test is more appropriate (Lee and Go, 1997). We reported here both test results for comparison purposes.

4.5.3. *Feeding Assays*

Flies were collected as described above and transferred regularly to fresh food until 4- or 10-days of adulthood, upon which 3 to 4 biological replicates, each of which consists of 7 to 10 flies, were transferred to vials containing food sources (standard Zurich fly food and yeast supplement) that have been dyed with 0.5% FD&C Blue #1 (Brilliant Blue FCF, Sigma). This dye is metabolically neutral and thermostable (Libert et al., 2007). The flies were allowed to

feed on the dyed food sources for 24h at 25°C, after which they were decapitated and the bodies were collected in Eppendorf tubes. Each replicate of 7 to 10 fly bodies was then homogenized in 10 µl phosphate-buffered saline (PBS) solution, which was subsequently centrifuged at 13,000g for 15min. The absorbance of the resulting supernates was measured at 630nm. The amount of food consumed was estimated from a standard curve of the same dye solution. The differences between the groups were assessed using a one-way ANOVA test.

4.5.4. Body Weight and Triglyceride Measurements

Newly eclosed flies were collected and kept in groups of 10 per vial as above, with regular transfers onto fresh fly food at 25°C. For determinations of body weight, 30 individual male or female flies per age group per genotype were measured. For determinations of TAG levels, flies were aged for 10 days, after which five replicates (9-10 flies per replicate) per genotype were snap-frozen in liquid nitrogen. Then, at 4°C, the wet weights of the flies were measured prior to homogenization in 0.05% PBS/Triton-X buffer. Subsequently, TAG levels were determined with a Serum Triglyceride Determination Kit (Sigma, cat. no. TR0100), according to the manufacturer's instructions. The protein levels of the same flies were also determined using the standard BioRad protein assay (BioRad, Switzerland), and the resulting TAG levels were normalized per mg protein, per mg wet weight or per fly. Statistically significant differences were only observed when TAG levels were normalized per mg protein. To test the differences between groups in all our measurements, we performed a one-way ANOVA test.

4.5.5. *Fecundity Assays*

We measured fecundity using 6-10 single adult females per genotype at 25°C. All females were aged for one day prior to the start of the experiments. Then, we placed a single female per genotype with two wild-type *Poxn* males per vial, which contained the same fly food as above, including the yeast supplement. Flies were then transferred onto fresh food daily and fecundity was measured every day for 10 days. Statistical differences were determined according to a one-way ANOVA test.

4.5.6. *Quantitative Measurement of mRNA Levels*

We measured the relative abundance of specific mRNAs through quantitative RT-PCR (qRT-PCR). Ten-day-old male and female flies were collected separately in 1.5-ml Eppendorf tubes and snap-frozen in liquid nitrogen. Afterwards, the heads were disconnected from the bodies by vigorous shaking of the tubes, after which the heads and bodies were funneled through a fine mesh for further separation. Total RNAs were then extracted from 30 heads or bodies per replicate per condition (3 to 4 replicates per condition) with a Nucleospin RNA II kit (Macherey-Nagel, Germany). Upon RNA isolation, the samples were treated with DNase and inhibitors of RNases. Then, mRNAs in the samples were reversely transcribed using a Transcriptor HiFi cDNA Synthesis Kit (Roche, Switzerland). All steps prior to RNAse inactivation were performed in liquid nitrogen or on dry ice in a 4°C cold room.

qRT-PCR reactions were carried out in triplicates using the Applied Biosystems SYBR Green kit and the ABI Prism 7900HT System (Applied Biosystems, California). All results were normalized to the *actin5C*, *tubulin-1α*, and *GAPDH2* mRNA levels. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method (see Applied Biosystems user bulletin #2, updated version 04/2001). The resulting data was log2 transformed prior to statistical testing.

To test for differences among the different groups, we performed a randomized complete block design (RCBD) ANOVA test (Faraway, 2002) by using the R statistical software package (R Development Core Team, 2009), where each plate was treated as an experimental block.

To determine the mRNA levels of dFOXO targets and all *dilps*, qRT-PCRs were performed in both head and body tissues. The primers for *dilp1* to *dilp7*, *l(2)efl*, *4E-BP* and *GAPDH2* were as described (Flatt et al., 2008; Kim et al., 2008). The other primers used are: *actin5C*, forward primer, GCC CAT CTA CGA GGG TTA TGC; *actin5c* reverse primer, AAT CGC GAC CAG CCA GAT C; *tubulin-1α* forward primer, GCC AGA TGC CGT CTG ACA A; and *tubulin-1a* reverse primer, AGT CTC GCT GAA GAA GGT GTT GA. We found that *dilp1* is expressed at a much lower level than other neural *dilps*.

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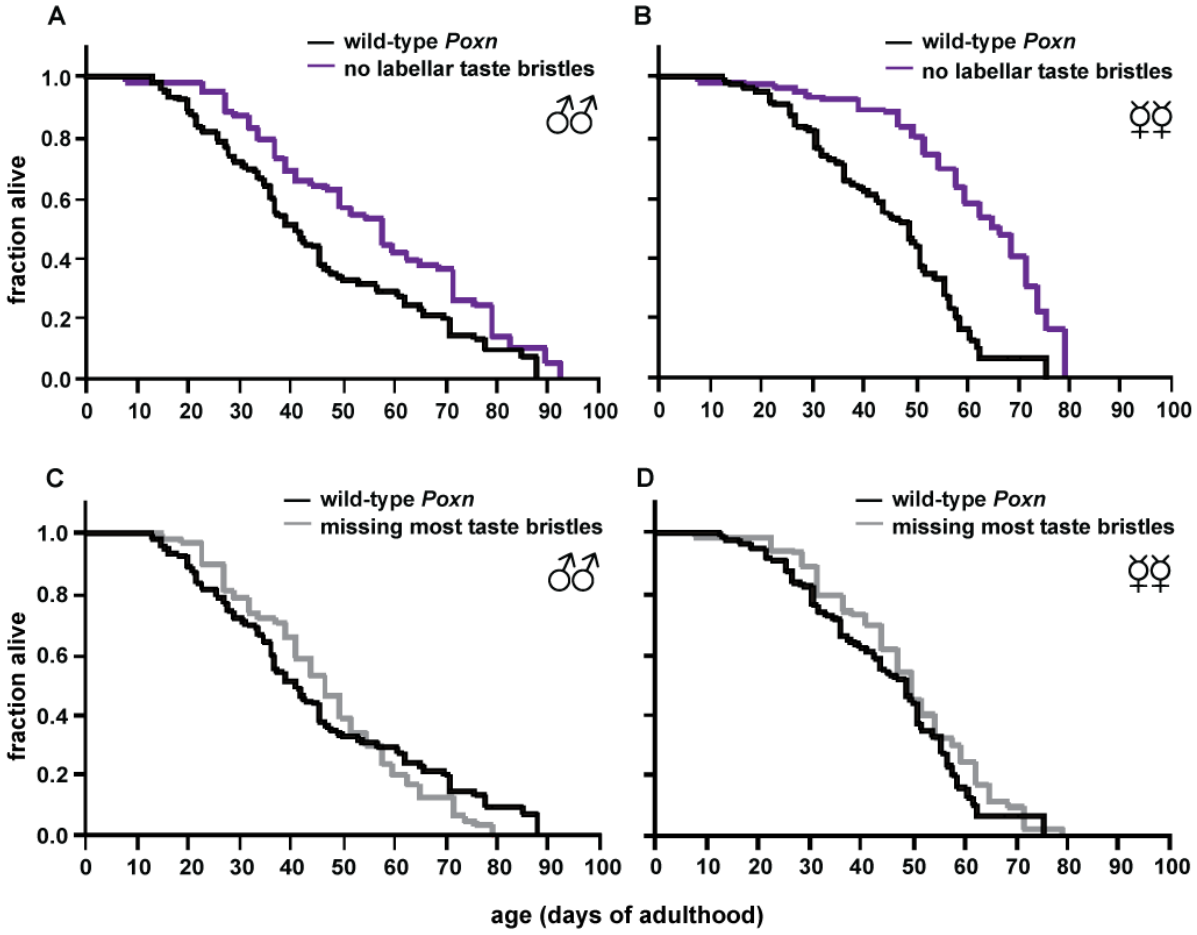
4.7. Figure Legends

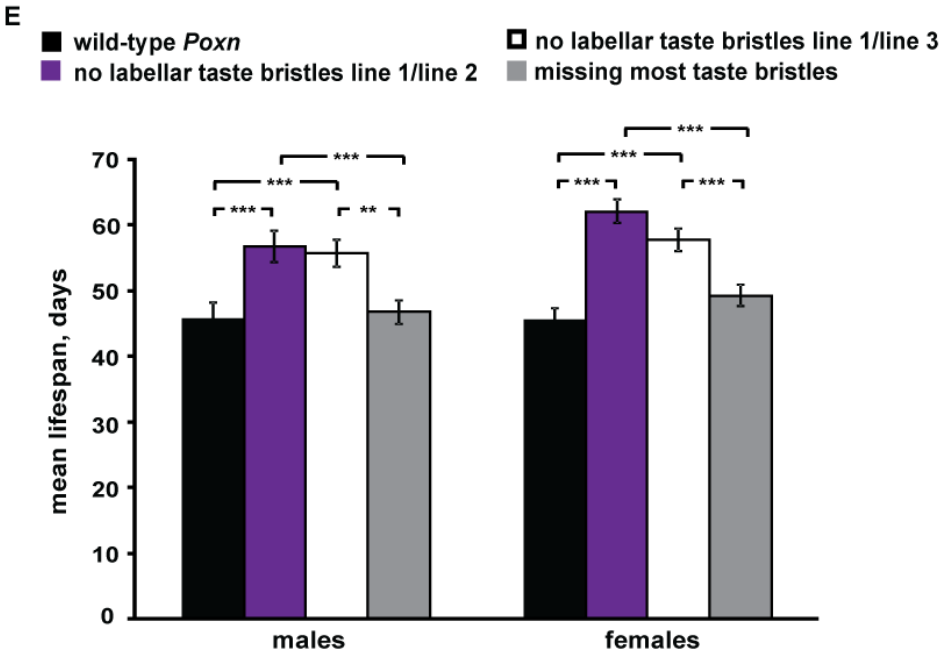
Figure 1. Taste Inputs Affect Fly Lifespan Bidirectionally. (A-B) Unmated males and females lacking labellar taste bristles (purple curve) live longer than wild-type *Poxn* control flies (black curve). The detailed statistical data on these (trial 1) and subsequent survival analyses are shown in Table S1. (C-D) Loss of additional taste bristles (gray curve; trial 1 in Table S1) suppresses the long-life phenotype of labellar taste-impaired flies. (E) For comparison of the different genotypes, the mean lifespans are shown as a bar graph. All error bars represent ± S.E.M. ** indicates $p \leq 0.01$, while ***, $p \leq 0.001$, according to the Wilcoxon test.

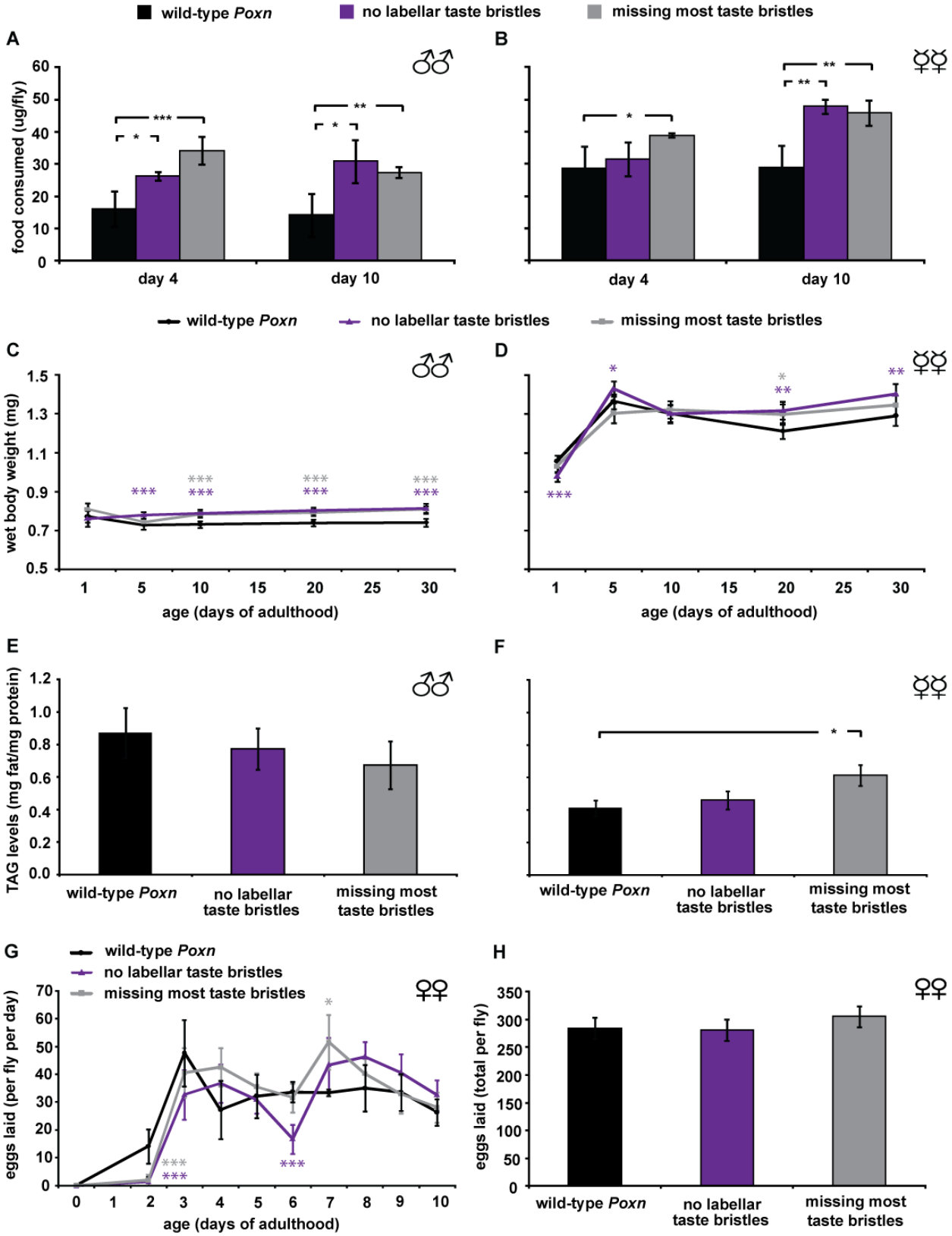
Figure 2. Taste-impaired Flies Do Not Resemble Calorically Restricted Flies. (A-B) Taste-impaired adult mutant (purple and gray bars) males and females are compared to control flies (black bars) at two different ages. The food consumption values are normalized per fly and each mean is derived from 3-4 biological replicates of 7-10 pooled flies. The error bars in these and subsequent panels within this figure represent 95% confidence intervals. (C-D) The body weights of taste mutants (purple and gray lines) are compared to control flies (black line) at different ages. Each data point represents the mean from at least three measurements of 10 individual flies. (E-F) TAG levels of 10-day-old adult taste mutant and control flies are compared. Each mean represents 5 biological replicates of 9-10 pooled flies. (G-H) The fecundity of taste-impaired flies is compared to that of control flies. The number of eggs laid per fly per day (G) or the total number of eggs laid per fly over a period of 10 days (H) is shown. Each time point represents data from 6-10 adult females, which carry transheterozygous insertions of the relevant transgenes in their genomes. * indicates $p \leq 0.05$; **, $p \leq 0.01$; and ***, $p \leq 0.001$.

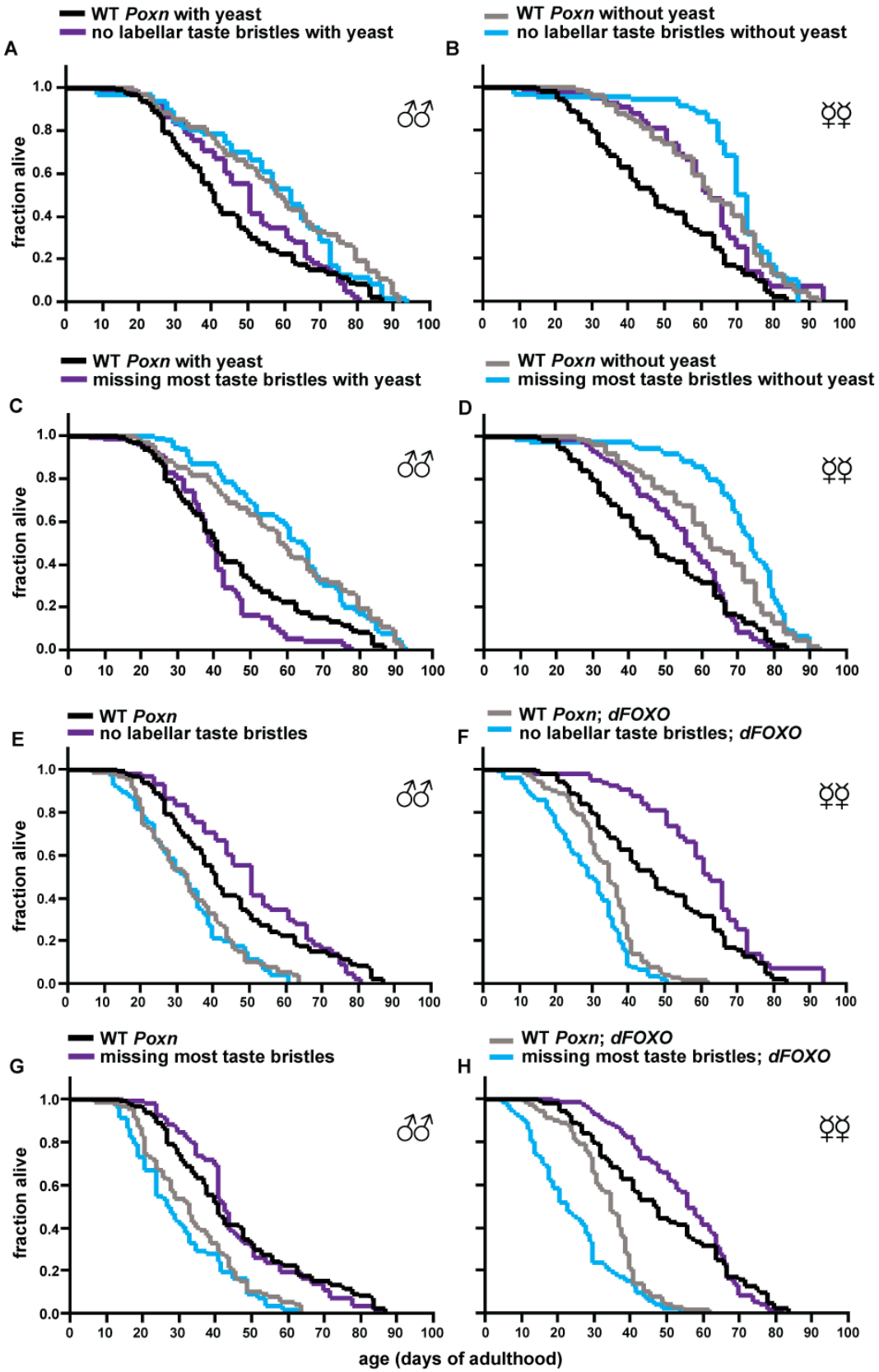
Figure 3. The Taste Influence on Lifespan Is Partly Dependent on Yeast and dFOXO. (A-B) The lifespan on yeast-enriched food of labellar taste-impaired (purple) and control (black) flies (Table S1, trial 3) compared to those of the corresponding taste mutant (light blue) and control (gray) flies on standard food (Table S1, group D). (C-D) The lifespan on yeast-enriched food of control flies (black) and flies missing additional taste bristles (purple; Table S1, trial 3) compared to those of the corresponding taste mutant (light blue) and control (gray) flies on standard food (Table S1, group D). (E-F) Survival curves of labellar taste mutant (purple) and control (black) flies that have wild-type *dFOXO* (Table S1, trial 3) versus those of the corresponding taste mutant (light blue) and control (gray) flies that carry mutations in *dFOXO* (Table S1, group E). (G-H) Survival curves of control flies (black) and flies missing additional taste bristles (purple), in the presence of *dFOXO* (Table S1, trial 3), versus those of the corresponding taste mutant (light blue) and control (gray) flies in the absence of *dFOXO* (Table S1, group E).

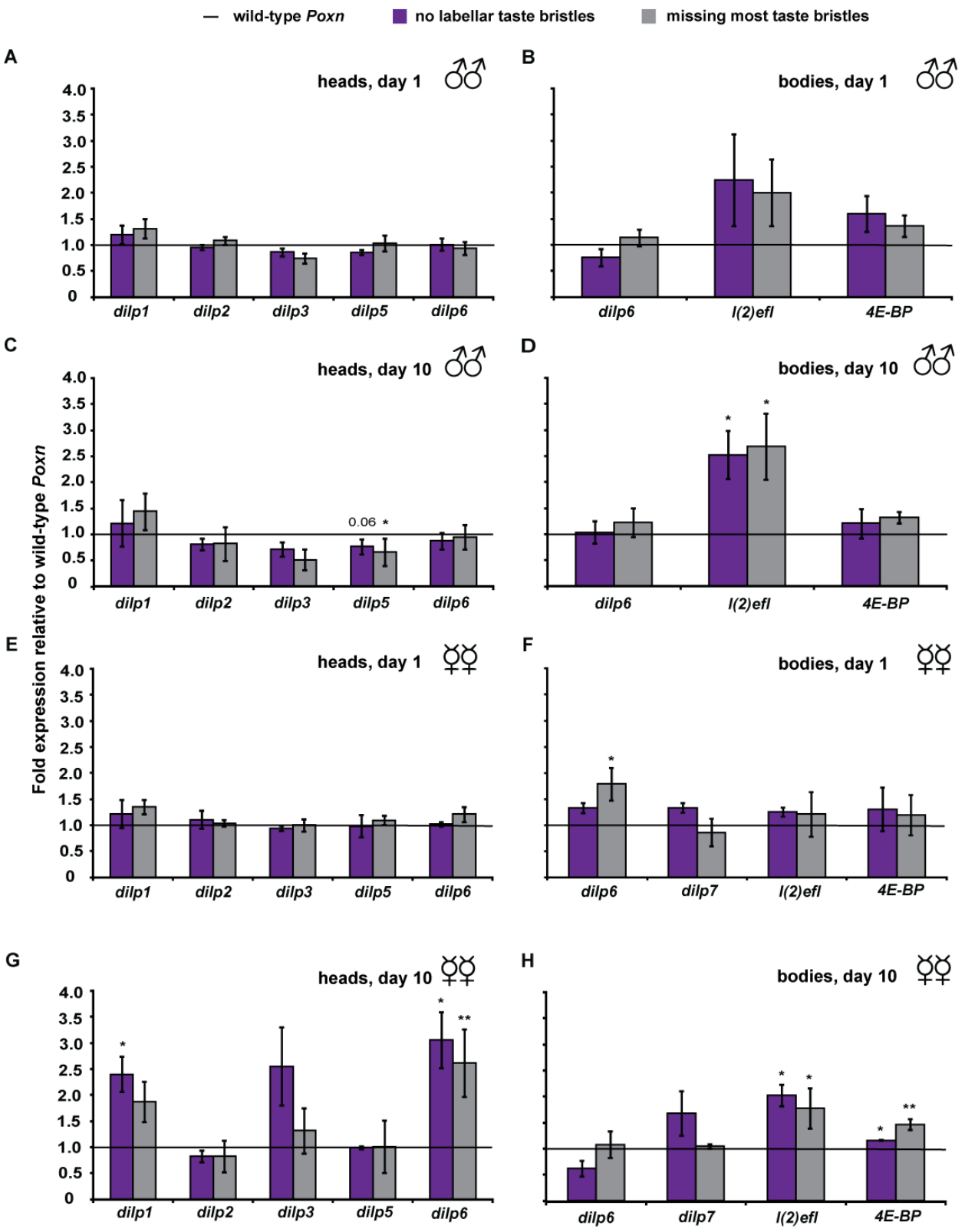
Figure 4. Taste Mutants Have Altered mRNA Levels of dFOXO Targets. The expression levels of dFOXO targets and *dilps* in the heads (A, C, E, G) and/or bodies (B, D, F, H) of adult male (A-D) and female (E-F) taste mutants (purple and gray bars) at two different ages are shown relative to control levels, which are set as 1.0 and depicted as a line across each bar graph. Each mean value shown represents 3-4 biological replicates of 30 pooled flies, which carry transheterozygous insertions of the relevant transgenes in their genomes. All error bars represent ± S.E.M. * indicates $p < 0.05$; **, $p < 0.01$; 0.06, the p value for *dilp5* levels in 10-day-old labellar taste mutant males compared to control.


A
wild-type Poxn
no labellar taste bristles
B
wild-type Poxn
no labellar taste bristles
C
wild-type Poxn
missing most taste bristles
D
wild-type Poxn
missing most taste bristles
fraction alive
age (days of adulthood)


E
wild-type Poxn
no labellar taste bristles line 1/line 2
no labellar taste bristles line 1/line 3
missing most taste bristles
mean lifespan, days
males
females


wild-type Poxn
no labellar taste bristles
missing most taste bristles
A
B
food consumed (ug/fly)
day 4
day 10
C
D
wet body weight (mg)
age (days of adulthood)
E
F
TAG levels (mg fat/mg protein)
wild-type Poxn
no labellar taste bristles
missing most taste bristles
G
H
eggs laid (per fly per day)
eggs laid (total per fly)


A
WT Poxn with yeast
no labellar taste bristles with yeast
B
WT Poxn without yeast
no labellar taste bristles without yeast
C
WT Poxn with yeast
missing most taste bristles with yeast
D
WT Poxn without yeast
missing most taste bristles without yeast
E
WT Poxn
no labellar taste bristles
F
WT Poxn; dFOXO
no labellar taste bristles; dFOXO
G
WT Poxn
missing most taste bristles
H
WT Poxn; dFOXO
missing most taste bristles; dFOXO
fraction alive
0.0
0.2
0.4
0.6
0.8
1.0
0 10 20 30 40 50 60 70 80 90 100
♂♂
♀♀
age (days of adulthood)


wild-type Poxn
no labellar taste bristles
missing most taste bristles
A
heads, day 1
B
bodies, day 1
C
heads, day 10
D
bodies, day 10
E
heads, day 1
F
bodies, day 1
G
heads, day 10
H
bodies, day 10
Fold expression relative to wild-type Poxn
dilp1
dilp2
dilp3
dilp5
dilp6
dilp7
l(2)efl
4E-BP
0.06 *

4.8. Supplementary table and figures

Table S1. Adult Lifespans of Taste-impaired Mutant Flies at 25°C. We assayed control and mutant flies in parallel in independent trials. The % difference between wild-type *Poxn* flies and taste mutant flies under different conditions is indicated in the fourth column. The differences that are significant ($P \leq 0.05$) according to the Wilcoxon test, which in most cases is also significant according to the logrank test, are in boldface type. The differences that are significant only according to the logrank test are italicized. The % difference between certain groups of flies as specified by the superscripted symbols is shown in the seventh column.

Figure S1. Description of the Different *Poxn* Rescue Constructs (Boll and Noll, 2002; Krstic et al., 2009). (A) Genetic map of the *Poxn* locus, which shows unique restriction sites. The *Poxn* translation start site and its stop codon (*) are shown. The entire coding region is depicted in black bars, the introns in hatched bars, the 5' and 3' UTRs in gray and the upstream and downstream *cis* regulatory regions in white. The extent of the $Poxn^{\Delta M22\text{-}B5}$ deletion is indicated. In addition, the structures of the different *Poxn* rescue constructs are depicted: the complete rescuing transgene; the transgene that lacks the enhancers required for the formation of labellar taste bristles, in which introns 3 and 4 are also missing; and the transgenes that lack the enhancers for most taste bristles, which are also either missing all introns and a *Pst*I-*BstX*I fragment in the 5' *cis* regulatory region (*ΔPBs*) or missing only introns 3 and 4 and an *Xba*I-*BstX*I fragment in the 5' *cis* regulatory region (*ΔXBs*). (B) The phenotypic defects of the *Poxn* deletion rescued by the different transgenes are listed. Since the *ΔXBs* transgene only partly rescues the leg/antenna segmentation phenotype of $Poxn^{\Delta M22\text{-}B5}$ null mutants, it was combined with one copy of the *ΔPBs* transgene, which completely rescues this phenotype (Krstic et al., 2009).

Figure S2. Taste-impaired Flies Have Increased Resistance to Starvation. (A) The mean survival of 10-day-old taste-impaired flies upon starvation is compared to control flies. (B) The statistics for the starvation response data. The analyses performed here are as described in the legend of Table S1. To determine the starvation response of flies, virgin males and females, which carry transheterozygous insertions of the relevant transgenes in their genomes, were collected and aged for 10 days on the yeast-enriched food source, as described in the Experimental Procedures. On day 10 of adulthood, the flies were transferred to fresh vials, which contain only 1% agar, and scored for survival at least 3-4 times a day. The flies were kept at 25°C, 60% humidity and a 12h/12h light/dark cycle for the duration of the experiment. * indicates $p \leq 0.05$, while ***, $p \leq 0.001$, according to the Wilcoxon test.

Supplemental References

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Supplementary Table 1. Adult lifespans

Strain/Treatment	Mean Survival ± SEM (Days)	No. of Animals Observed	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)	% Specified group	*P* Value Against Specified Groups (Logrank)	*P* Value Against Specified Groups (Wilcoxon)
Male transheterozygotes – trial 1								
A.1. wild type *Poxn* line 1 / line 2	45.7 ± 2.4	77				- 2 vs A.4	0.84 vs A.4	0.20 vs A.4
A.2. no labellar taste bristles line 1 / line 2	56.7 ± 2.4	70	+ **24**	0.001	0.0007	+ **21** vs A.4	< 0.0001 vs A.4	< 0.0001 vs A.4
A.3. no labellar taste bristles line 1 / line 3	55.6 ± 2.0	76	+ **22**	0.007	0.0002	+ **19** vs A.4	0.0008 vs A.4	0.004 vs A.4
A.4. missing most taste bristles line 1/ line 2	46.8 ± 1.8	81	+ 2	0.84	0.20			
Female transheterozygotes – trial 1								
A.5. wild type *Poxn* line 1 / line 2	45.6 ± 1.8	70				- 7 vs A.8	0.11 vs A.8	0.12 vs A.8
A.6. no labellar taste bristles line 1 / line 2	62.1 ± 1.8	67	+ **36**	< 0.0001	< 0.0001	+ **26** vs A.8	< 0.0001 vs A.8	< 0.0001 vs A.8
A.7. no labellar taste bristles line 1 / line 3	57.8 ± 1.7	74	+ **27**	< 0.0001	< 0.0001	+ **17** vs A.8	< 0.0001 vs A.8	0.0003 vs A.8
A.8. missing most taste bristles line 1/ line 2	49.2 ± 1.6	77	+ 8	0.11	0.12			
Male transheterozygotes – trial 2								
B.1. wild type *Poxn* line 1 / line 2	45.1 ± 2.4	82				+ 13 vs B.5	0.08 vs B.5	0.64 vs B.5
B.2. no labellar taste bristles line 1 / line 2	52.4 ± 2.1	61	+ **16**	0.19	0.005	+ **31** vs B.5	0.0002 vs B.5	< 0.0001 vs B.5
B.3. no labellar taste bristles line 1 / line 3	47.0 ± 1.9	73	+ 4	0.82	0.16	+ **17** vs B.5	0.03 vs B.5	0.01 vs B.5
B.4. no labellar taste bristles line 2 / line 3	50.6 ± 2.1	75	+ **12**	0.45	0.03	+ **26** vs B.5	0.001 vs B.5	0.001 vs B.5
B.5. missing most taste bristles line 1/ line 2	40.1 ± 1.9	75	- 11	0.08	0.42			
B.6. missing most taste bristles line 1/ line 3	39.8 ± 1.7	74	- *12*	0.03	0.64	- 0.7 vs B.5	0.76 vs B.5	0.84 vs B.5
Female transheterozygotes – trial 2								
B.7. wild type *Poxn* line 1 / line 2	43.7 ± 1.8	81				**-14** vs B.11	0.03 vs B.11	0.005 vs B.11
B.8. no labellar taste bristles line 1 / line 2	63.8 ± 1.4	73	+ **46**	< 0.0001	< 0.0001	+ **26** vs B.11	< 0.0001 vs B.11	< 0.0001 vs B.11
B.9. no labellar taste bristles line 1 / line 3	60.7 ± 1.6	74	+ **39**	< 0.0001	< 0.0001	+ **20** vs B.11	0.0003 vs B.11	< 0.0001 vs B.11
B.10. no labellar taste bristles line 2 / line 3	62.3 ± 1.6	84	+ **43**	< 0.0001	< 0.0001	+ **23** vs B.11	< 0.0001 vs B.11	< 0.0001 vs B.11
B.11. missing most taste bristles line 1/ line 2	50.6 ± 1.9	70	+ **16**	0.03	0.005			
B.12. missing most taste bristles line 1/ line 3	51.9 ± 1.3	71	+ **19**	0.04	< 0.0001	+ 3 vs B.11	0.94 vs B.11	0.33 vs B.11

Supplementary Table 1. Continued

Strain/Treatment	Mean Survival ± SEM (Days)	No. of Animals Observed	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)	% Specified group	*P* Value Against Specified Groups (Logrank)	*P* Value Against Specified Groups (Wilcoxon)
Male transheterozygotes – trial 3								
C.1. wild type *Poxn* line 1 / line 2	45.4 ± 1.8	109				+ *12* vs C.3 - 2 vs C.4	0.02 vs C.3 0.83 vs C.4	0.52 vs C.3 0.16 vs C.4
C.2. no labellar taste bristles line 1 / line 2	50.5 ± 1.9	71	**+ 11**	0.17	0.01	**+ 24** vs C.3 **+ 9** vs C.4	< 0.0001 vs C.3 0.08 vs C.4	0.0001 vs C.3 0.05 vs C.4
C.3. missing most taste bristles line 1/ line 2	40.7 ± 1.2	110	- *10*	0.02	0.52	**- 12** vs C.4	0.002 vs C.4	0.008 vs C.4
C.4. missing most taste bristles line 1/ line 3	46.4 ±1 .5	101	+ 2	0.83	0.16	**+ 14** vs C.3	0.002 vs C.3	0.008 vs C.3
Female transheterozygotes – trial 3								
C.5. wild type *Poxn* line 1 / line 2	48.7 ± 1.8	99				**- 11** vs C.7 **- 12** vs C.8	0.16 vs C.7 0.12 vs C.8	0.0008 vs C.7 0.002 vs C.8
C.6. no labellar taste bristles line 1 / line 2	61.2 ± 1.8	67	**+ 26**	< 0.0001	< 0.0001	**+ 12** vs C.7 **+ 11** vs C.8	< 0.0001 vs C.7 0.01 vs C.8	< 0.0001 vs C.7 0.03 vs C.8
C.7. missing most taste bristles line 1/ line 2	54.8 ± 1.2	131	**+ 12**	0.16	0.0008	- 0.5 vs C.8	0.64 vs C.8	0.68 vs C.8
C.8. missing most taste bristles line 1/ line 3	55.1 ± 1.4	94	**+ 13**	0.12	0.002	- 0.5 vs C.7	0.64 vs C.7	0.68 vs C.7
Male transheterozygotes – without yeast supplement								
D.1. wild type *Poxn* line 1 / line 2	58.7 ± 2.3	85				- 4 vs D.3 **+ 29** vs C.1	0.81 vs D.3 < 0.0001 vs C.1	0.35 vs D.3 < 0.0001 vs C.1
D.2. no labellar taste bristles line 1 / line 2	55.7 ± 2.1	84	- 2	0.38	0.90	- 6 vs D.3 **+ 14** vs C.2	0.34 vs D.3 < 0.0001 vs C.2	0.28 vs D.3 < 0.0001 vs C.2
D.3. missing most taste bristles line 1/ line 2	61.3 ± 1.9	81	+ 5	0.81	0.35	**+ 51** vs C.3	< 0.0001 vs C.3	<0.0001 vs C.3
Female transheterozygotes – without yeast supplement								
D.4. wild type *Poxn* line 1 / line 2	62.4 ± 1.7	90				**- 12** vs D.6 **+ 28** vs C.5	0.006 vs D.6 < 0.0001 vs C.5	0.0002 vs D.6 < 0.0001 vs C.5
D.5. no labellar taste bristles line 1 / line 2	69.4 ± 1.6	78	**+ 11**	0.04	0.0004	- 3 vs D.6 **+ 13** vs C.6	0.18 vs D.6 0.004 vs C.6	0.24 vs D.6 0.01 vs C.6
D.6. missing most taste bristles line 1/ line 2	71.2 ± 1.6	70	**+ 14**	0.006	0.0002	**+ 30** vs C.7	< 0.0001 vs C.7	< 0.0001 vs C.7

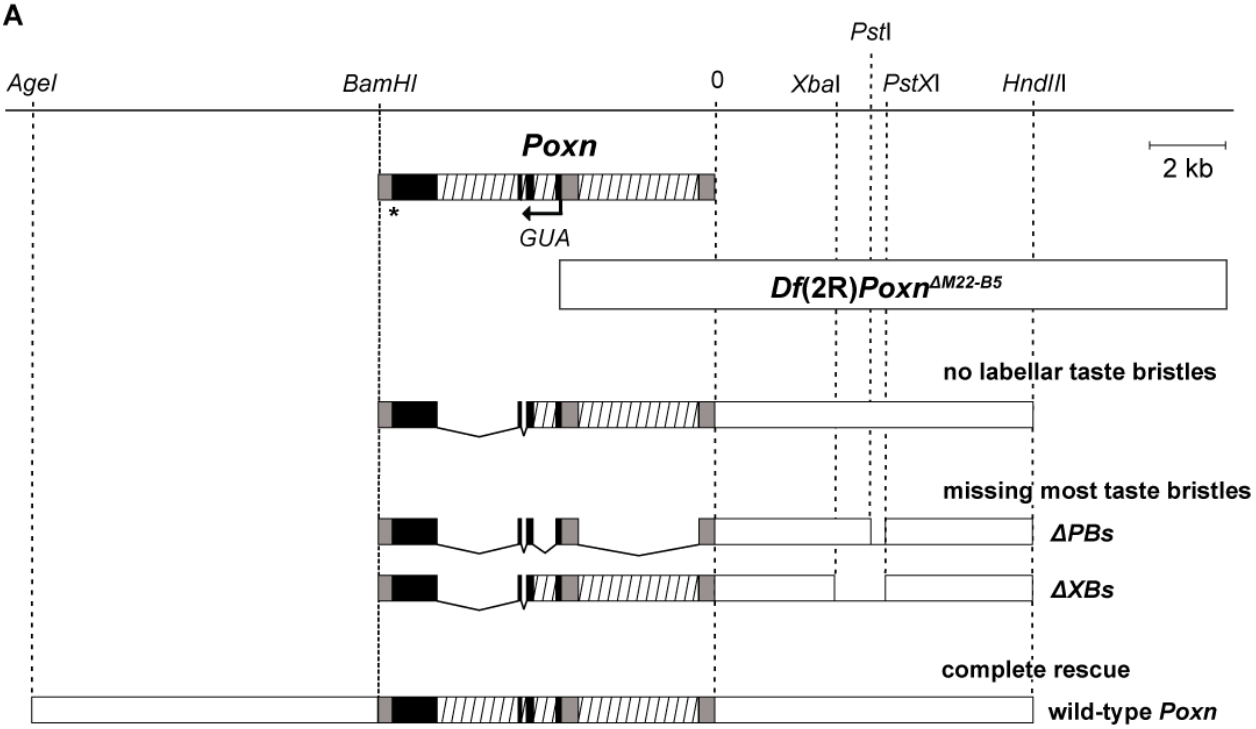
Supplementary Table 1. Continued

Strain/Treatment	Mean Survival ± SEM (Days)	No. of Animals Observed	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)	% Specified group	*P* Value Against Specified Groups (Logrank)	*P* Value Against Specified Groups (Wilcoxon)
Male transheterozygotes – without dFoxo								
E.1. wild type *Poxn* line 1 / line 2; *dFoxo*$^{21/25}$	33.7 ± 1.4	87				+ 11 vs E.3 **- 26** vs C.1	0.19 vs E.3 < 0.0001 vs C.1	0.08 vs E.3 < 0.0001 vs C.1
E.2. no labellar taste bristles line 1 / line 2; *dFoxo*$^{21/25}$	32.8 ± 1.3	89	- 3	0.57	0.79	+ 8 vs E.3 **- 35** vs C.2	0.31 vs E.3 < 0.0001 vs C.2	0.17 vs E.3 <0.0001 vs C.2
E.3. missing most taste bristles line 1 / line 3; *dFoxo*$^{21/25}$	30.4 ± 1.7	63	- 10	0.18	0.08	**- 34** vs C.4	< 0.0001 vs C.4	< 0.0001 vs C.4
Female transheterozygotes – without dFoxo								
E.4. wild type *Poxn* line 1 / line 2; *dFoxo*$^{21/25}$	34.1 ± 1.0	85				**+ 38** vs E.6 **- 30** vs C.5	< 0.0001 vs E.6 < 0.0001 vs C.5	< 0.0001 vs E.6 <0.0001 vs C.5
E.5. no labellar taste bristles line 1 / line 2; *dFoxo*$^{21/25}$	28.8 ± 1.1	101	**- 16**	0.003	0.001	**+ 17** vs E.6 **- 53** vs C.6	0.13 vs E.6 < 0.0001 vs C.6	0.007 vs E.6 < 0.0001 vs C.6
E.6. missing most taste bristles line 1/ line 3; *dFoxo*$^{21/25}$	24.7 ± 1.4	73	**- 28**	< 0.0001	< 0.0001	**- 55** vs C.8	< 0.0001 vs C.8	< 0.0001 vs C.8
Male homozygotes – trial 1								
F.1. wild type *Poxn* line 1	46.0 ± 1.9	75				*+ 11* vs F.5	0.02 vs F.5	0.07 vs F.5
F.2. no labellar taste bristles line 1	53.2 ± 2.1	68	**+ 16**	0.006	0.02	**+ 28** vs F.5	< 0.0001 vs F.5	< 0.0001 vs F.5
F.3. no labellar taste bristles line 2	44.0 ± 2.0	70	- 4	0.59	0.51	+ 6 vs F.5	0.16 vs F.5	0.53 vs F.5
F.4. no labellar taste bristles line 3	48.1 ± 2.1	70	+ 5	0.31	0.78	*+ 16* vs F.5	0.003 vs F.5	0.08 vs F.5
F.5. missing most taste bristles line 1	41.5 ± 1.4	99	*- 10*	0.02	0.07			
Female homozygotes – trial 1								
F.6. wild type *Poxn* line 2	32.3 ± 1.2	80				**- 30** vs F.10	< 0.0001 vs F.10	< 0.0001 vs F.10
F.7. no labellar taste bristles line 1	56.8 ± 1.7	74	**+ 76**	< 0.0001	< 0.0001	**+ 24** vs F.10	< 0.0001 vs F.10	< 0.0001 vs F.10
F.8. no labellar taste bristles line 2	53.8 ± 1.6	67	**+ 66**	< 0.0001	< 0.0001	**+ 17** vs F.10	< 0.0001 vs F.10	< 0.0001 vs F.10
F.9. no labellar taste bristles line 3	56.4 ± 1.7	80	**+ 75**	< 0.0001	< 0.0001	**+ 23** vs F.10	< 0.0001 vs F.10	< 0.0001 vs F.10
F. 10. missing most taste bristles line 1	45.9 ± 1.2	95	**+ 42**	< 0.0001	< 0.0001			

Supplementary Table 1. Continued

Strain/Treatment	Mean Survival ± SEM (Days)	No. of Animals Observed	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)	% Specified group	*P* Value Against Specified Groups (Logrank)	*P* Value Against Specified Groups (Wilcoxon)
Male homozygotes – trial 2								
G.1. wild type *Poxn* line 1	43.3 ± 2.3	79				+ *7* vs G.2	0.05 vs G.2	0.83 vs G.2
						- 1 vs G.6	0.49 vs G.6	0.39 vs G.6
G.2. wild type *Poxn* line 2	40.3 ± 1.6	77	- *7*	0.05	0.83	- 8 vs G.6	0.10 vs G.6	0.10 vs G.6
G.3. no labellar taste bristles line 1	50.9 ± 2.0	67	**+ 18**	0.09	0.005	**+ 26** vs G.2	< 0.0001 vs G.2	0.0002 vs G.2
						+ 16 vs G.6	0.002 vs G.6	0.02 vs G.6
G.4. no labellar taste bristles line 2	45.4 ± 2.1	73	+ 5	0.65	0.28	+ *13* vs G.2	0.009 vs G.2	0.15 vs G.2
						+ 4 vs G.6	0.26 vs G.6	0.96 vs G.6
G.5. no labellar taste bristles line 3	42.6 ± 1.9	67	- 2	0.50	0.59	+ 6 vs G.2	0.18 vs G.2	0.42 vs G.2
						- 3 vs G.6	0.83 vs G.6	0.36 vs G.6
G.6. missing most taste bristles line 1	43.8 ± 1.7	70	+ 1	0.49	0.39	+ 9 vs G.2	0.10 vs G.2	0.10 vs G.2
Female homozygotes – trial 2								
G.7. wild type *Poxn* line 1	39.2 ± 1.6	76				+ 11 vs G.8	0.18 vs G.8	0.10 vs G.8
						- 15 vs G.12	0.007 vs G.12	0.0002 vs G.12
G.8. wild type *Poxn* line 2	35.4 ± 1.7	64	- 10	0.18	0.10	**- 23** vs G.12	< 0.0001 vs G.12	< 0.0001 vs G.12
G.9. no labellar taste bristles line 1	55.2 ± 1.4	75	**+ 41**	< 0.0001	< 0.0001	**+ 56** vs G.8	< 0.0001 vs G.8	< 0.0001 vs G.8
						+ 20 vs G.12	< 0.0001 vs G.12	< 0.0001 vs G.12
G.10. no labellar taste bristles line 2	60.2 ± 1.4	83	**+ 54**	< 0.0001	< 0.0001	**+ 70** vs G.8	< 0.0001 vs G.8	< 0.0001 vs G.8
						+ 31 vs G.12	< 0.0001 vs G.12	< 0.0001 vs G.12
G.11. no labellar taste bristles line 3	56.9 ± 1.6	79	**+ 45**	< 0.0001	< 0.0001	**+ 61** vs G.8	< 0.0001 vs G.8	< 0.0001 vs G.8
						+ 24 vs G.12	< 0.0001 vs G.12	< 0.0001 vs G.12
G.12. missing most taste bristles line 1	46.0 ± 1.3	78	**+ 17**	0.007	0.0002	**+ 30** vs G.8	< 0.0001 vs G.8	< 0.0001 vs G.8

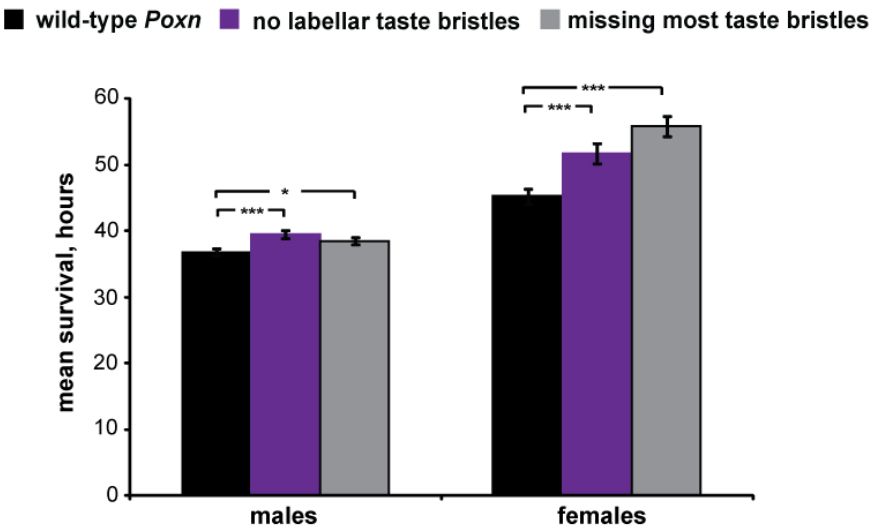
A



B

Mutation	***Poxn*** $^{\Delta M22\text{-}B5}$				
Rescue	**wild-type *Poxn***	**∆*XBs***	**∆*PBs***	**∆*XBs* / ∆*PBs***	**no labellar taste bristles**
Taste: leg and wing	+	-	-	-	+
Taste: labellum	+	-	-	-	-
Leg and antennae segmentation	+	(+)	+	+	+
Male posterior lobe	+	+	-	+	+
Penis	+	+	+	+	+
Ventral ganglion expression	+	+	+	+	+
Brain expression	+	+	+	+	+

A



B

Starvation response of taste-impaired flies

Strain/Treatment	Mean Survival ± SEM (hours)	No. of Animals Observed	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)
Cumulative					
Males					
wild-type *Poxn*	36.8 ± 0.5	111 (2)			
no labellar taste bristles	39.5 ± 0.6	114 (2)	+ **7**	< 0.0001	0.0001
missing most taste bristles	38.5 ± 0.6	120 (2)	+ **5**	0.005	0.03
Females					
wild-type *Poxn*	45.3 ± 1.1	119 (2)			
no labellar taste bristles	51.7 ± 1.5	117 (2)	+ **14**	0.002	0.001
missing most taste bristles	55.8 ± 1.5	110 (2)	+ **23**	< 0.0001	< 0.0001

5. Discussion

The neurobiology of the taste response has been studied in some detail: a wide range of experiments has shown how gustatory information in general is registered and transmitted by gustatory neurons. However, the neurophysiology of the taste response has received less attention. The following remains unclear: (i) how taste affects physiology; (ii) the nature of the underlying molecular and neuronal networks that governs this process; and (iii) how these physiological changes consequently affect adult lifespan.

The research conducted in *C. elegans*, as well as in other animals, has shed some light on this question. We now know some of the signaling pathways that respond to environmental cues via the sensory system to regulate animal physiology [reviewed in (Alcedo et al., 2010)]. An example that perfectly illustrates the sensory-mediated interaction between the environment and an animal's physiology is the regulation of the *C. elegans* developmental programs [reviewed by (Riddle and Albert, 1997)]. Under good environmental conditions, the worm undergoes reproductive development (Riddle and Albert, 1997). On the other hand, cues that signal harsh environments trigger very young larvae to enter an alternative developmental program that produces long-lived and highly stress-resistant dauer larvae, which can exit into reproductive development once environmental conditions improve (Riddle and Albert, 1997). This process is genetically regulated and involves the IIS pathway (Riddle and Albert, 1997). Interestingly, this switch between developmental programs is also mediated by a specific subset of gustatory neurons (Bargmann and Horvitz, 1991; Schackwitz et al., 1996). Other examples that demonstrate the taste influence on physiology are the bidirectional effects of gustatory neurons on *C. elegans* adult lifespan, which again are mediated by IIS activity, and a specific subset of neurons that only partly overlaps with the neurons that regulate the dauer program (Alcedo and Kenyon, 2004). Thus, these findings

show how perception of environmental cues by specific subsets of taste neurons can have different effects on animal physiology through the modulation of internal signaling activities in a temporal (during development and post-development), and perhaps spatial, manner.

For my thesis, I have asked whether the gustatory influence on lifespan also exists in other animals, like *Drosophila*. Many biological processes are found to be conserved between *C. elegans* and higher organisms. For example, two forms of environmental manipulations have been shown to affect lifespan consistently across many species, such as food-level restriction (a change in total food caloric value that does not lead to malnutrition) or a change in the type of food sources that has little or no effect on caloric value (Garsin et al., 2003; Mair et al., 2005; Mobbs et al., 2006; Skorupa et al., 2008; Maier et al., 2010). The effects of these dietary manipulations on lifespan in principle could be mediated by the sensory system. This hypothesis is confirmed by the observation that exposure of calorically-restricted, long-lived flies to live yeast odors alone shortens their lifespan (Libert et al., 2007). In addition, this finding is the first evidence that the sensory effect on lifespan is found in other animals. Indeed, this is further confirmed by the long-life phenotype observed in flies that exhibit olfactory defects due to a mutation in the widely expressed olfactory co-receptor *Or83b* (Libert et al., 2007).

Understanding how the sensory system in general affects lifespan should not only yield a new perspective within the field of aging studies, but could also provide additional insight into the effects of nutrition and feeding behavior on human health. Gustatory function in humans has been shown to decline with age and this can have severe consequences on the diet regimen and overall health of elderly people (Kaneda et al., 2000). Thus, elucidating the processes that link taste, feeding, physiology and lifespan in model organisms might provide us with an opportunity to prevent the pathological states that arise due to defects in any of

these processes. Accordingly, in this study, I show how taste affects *Drosophila* lifespan in response to specific food-derived signals and the signaling activities involved in this process.

5.1. The taste influence on lifespan is conserved in Drosophila

To test the hypothesis whether the taste influence on lifespan is evolutionarily conserved, I have studied the physiology of *D. melanogaster* mutants that lack a subset (labellar) or most of their taste inputs. I have found that many taste inputs from the fly labella shorten lifespan, whereas other taste inputs from the legs and wings lengthen lifespan (Chapter 4, Figure 1 and Table S1). This is similar to the gustatory influence on *C. elegans* lifespan, which involves both lifespan-lengthening and lifespan-shortening gustatory neurons (Alcedo and Kenyon, 2004). Interestingly, I have also found that the taste-mediated influence on lifespan is more robust in females than in males, which might reflect the sexual dimorphism in the organization and function of the fly gustatory system (Bray and Amrein, 2003; Park et al., 2006; Vosshall and Stocker, 2007). These findings might also suggest that the mechanisms through which taste affects lifespan differ between male and female flies.

5.2. Taste inputs affect lifespan independent of food levels but dependent on food type

One possible mechanism through which taste affects lifespan is through a reduction in food intake due to the lack of hedonistic stimuli that otherwise would have been perceived by the taste neurons. It is possible that flies with impaired labellar taste inputs live long because they eat less food and are thus food level-restricted, while flies lacking additional taste inputs live shorter because they eat much lower amounts of food to the point of malnourishment. Food level-restricted or malnourished flies also have reduced body weights (Bross et al., 2005), fat content (Wang et al., 2009) and fecundity (Chapman and Partridge, 1996). However, in contrast to what might be expected, I find the opposite: both groups of taste-

impaired flies eat more and have increased body weights and food intake, whereas their fat content and fecundity remain largely unchanged (Chapter 4, Figure 2).

Interestingly, several observations are present in the scientific literature that link taste, feeding and body weight in other organisms. For example, in the medical literature, it has been demonstrated that negative correlations between body mass index (BMI) and taste sensitivity to certain food components exist (Simchen et al., 2006; Stewart et al., 2010), which suggests that persons with lowered sensitivity to certain food components have a higher chance of developing obesity. In addition, it has been shown that in diabetic patients there is a lowering of taste sensitivity, which again correlates with increased BMI (Simchen et al., 2006). At the same time, diabetic obese *db/db* mice, which carry a defect in the leptin receptor (Chen et al., 1996), also display an enhanced response to sweet stimuli, more so than normal (Kawai et al., 2000). This would suggest that the leptin pathway, which is an important regulator of mammalian satiety, is also a modulator of sweet taste. This further suggests another possibility that other hormones, like insulin, will also modulate taste perception in mammals.

In *Drosophila*, the motor neurons required for food intake send processes predominantly to the ventral region of the SOG and partly overlap with sensory projections from taste neurons (Rajashekhar and Singh, 1994). Moreover, the neuropeptide *hugin* expressed in the fly SOG interneurons has been found to regulate the initiation of *Drosophila* feeding processes and communicate with labellar gustatory neurons (Melcher and Pankratz, 2005). Furthermore, the blowfly labellar taste sensilla, together with internal satiety signals, play an important role in feeding regulation (Pollack, 1977). Thus, these findings, together with my own observations, are consistent with the idea that taste neurons are important in the regulation of feeding and satiety-related signals. Indeed, it has recently been shown that loss of the atypically expressed gustatory receptor Gr28a leads to anorexia in flies, which suggests

that different types of gustatory cues also have different effects on feeding (Ayres and Schneider, 2009). Since little is known about the molecular and cellular networks underlying these processes, additional work would be needed to elucidate the interplay between taste perception on the one hand and appetite and obesity on the other. Given my results, as well as others (Melcher and Pankratz, 2005), these suggest that *Drosophila* serves as an ideal genetic model system to explore this link further.

Although my data suggest that the long-lived, taste-impaired flies are not food-level restricted, I do find that the taste influence on the lifespan of these flies is food type-dependent. This is consistent with the idea that the inability to sense cues from particular food types leads to changes in feeding behavior and physiology, and consequently lifespan. For example, odors derived from live yeast shorten fly lifespan (Libert et al., 2007). My studies demonstrate that taste inputs that are distributed on the labella, legs and/or wings also modulate the response to yeast, more so in male than in female flies (Chapter 4, Figure 3 and Table S1). In addition, my data suggest that the gustatory effects on lifespan can act in parallel to the effects of yeast, since female flies lacking most taste bristles exhibit the same degree of lifespan extension compared to controls, either on a yeast-enriched or a standard diet (Chapter 4, Figure 3 and Table S1). This would suggest that taste inputs affect lifespan in response to many food cues, of which yeast is only one. Consistent with this idea, yeast restriction alone does not increase fly lifespan under all conditions that do not promote malnutrition (Skorupa et al., 2008).

5.3. Taste affects lifespan through insulin-dependent and insulin-independent pathways

The altered lifespan responses due to the presence or absence of yeast enrichment in taste-impaired flies suggest that IIS might be involved in mediating these effects. It has been

shown that flies with increased dFOXO activity, compared to control flies, have different lifespan profiles in response to a reduction in the yeast to sugar ratios of their diet (Giannakou et al., 2008). Thus, it is possible that long-lived, labellar taste-impaired flies have decreased IIS levels, which would be reflected by an increase in dFOXO activity.

Drosophila insulin-like peptides (*dilps*), of which there are seven (Brogiolo et al., 2001; Ikeya et al., 2002; Rulifson et al., 2002), have important effects on feeding behavior, at least in larvae: overexpression of some of the DILPs in the nervous system of fasted *Drosophila* larvae can suppress the hunger-driven appetitive behavior toward less palatable food (Wu et al., 2005b). In addition, it has recently been shown that *dilp6* mRNA levels increase in nonfeeding pupae, while a reduction in *dilp6* decreases body size and promotes starvation resistance in adults, which led to the proposal that *dilp6* promotes growth at the expense of food storage upon nutritional deprivation (Slaidina et al., 2009). DILPs also affect lifespan: the deletion of *dilp2*, *dilp3* and *dilp5* increases lifespan (Grönke et al., 2010). These *dilps* are expressed in the brain mNSCs and genetic ablation of the mNSCs has been found to extend lifespan (Broughton et al., 2005). Interestingly, mNSCs communicate with SOG interneurons, which receive axonal projections from gustatory neurons and accordingly process taste-related information (Brogiolo et al., 2001; Scott et al., 2001; Ikeya et al., 2002; Rulifson et al., 2002; Melcher and Pankratz, 2005; Agrawal et al., 2009). Thus, it is possible that taste neurons indirectly communicate with mNSCs and thus regulate the synthesis or release of DILPs, which in turn would modulate the activity of the IIS pathway to affect lifespan.

5.3.1. Taste influence on dilp mRNA levels

My measurements of the mRNA abundance of different *dilp*s produced in the fly heads reveal complex and sexually dimorphic interactions between taste inputs and IIS (Chapter 4, Figure 4). I find that the levels of the nutrient-responsive *dilp5* (Ikeya et al., 2002; Grönke et al., 2010) in 10-day-old males, and not females, are reduced. Interestingly, DILP5 has previously been shown to respond to changing yeast concentrations and has been proposed to mediate the yeast-restricted lifespan response, albeit possibly independent of *dFOXO* (Grönke et al., 2010). Considering that a large number of yeast-dependent taste inputs appear to influence male lifespan more so than female lifespan, these results suggest the possibility that the taste perception of yeast by males is sufficient to alter their levels of *dilp5* mRNA.

In females, the situation is different and appears to be more complex. On the tenth day of adulthood, I see a significant increase in *dilp6* levels in the head but not in the body of age-matched flies (Chapter 4, Figure 4). DILP6 is an IGF-like peptide that is produced in the fat body cells found in the fly head and abdomen (Okamoto et al., 2009; Slaidina et al., 2009), which has been suggested to mediate the systemic effects of dFOXO, since its expression is induced upon dFOXO activation (Grönke et al., 2010). In addition, compensatory increases in *dilp6* mRNA levels in the abdominal fat body are observed in *dilp2 dilp3 dilp5* triple mutants, which suggest that *dilp6* is part of a complex regulatory system between the central nervous system and peripheral tissues (Grönke et al., 2010). At the same time, the observation that reduced *dilp6* activity increases starvation resistance, while increased *dilp6* does the opposite (Slaidina et al., 2009), might suggest that taste-impaired female flies are starvation-sensitive, despite being long-lived. However, this is not the case, since I find that both classes of taste-impaired female flies are starvation-resistant (Chapter 4, Figure S2). One possible explanation for the observed increase in *dilp6* in females might be that female taste inputs

regulate *dilp6* transcription, but that additional layers of regulation exist at the post-transcriptional level that would be required to promote full *dilp6* activity. Indeed, the lack of change in *dilp2, dilp3* and *dilp5* mRNA levels also does not preclude the possibility that taste inputs could still modulate the activities of these other *dilps* at the postranscriptional level. This latter possibility would be consistent with the hypothesis where *dilp2*, *dilp3* and/or *dilp5* increase *dilp6* expression in the fat body cells via dFOXO activity (Grönke et al., 2010).

The differential regulation of *dilps* in males and females might explain gender-specific differences in taste inputs, processing of taste-related information or different nutritional requirements. Indeed, fly head fat body cells display sex-specific differences (Bownes and Hames, 1977; Lazareva et al., 2007), which could explain the female-specific changes in head *dilp6* levels.

I would also like to note that these changes in *dilp* levels, with the exception of the mNSC-expressed *dilp1*, occur in both long-lived and shorter-lived taste mutant flies (Chapter 4, Figure 4), which might suggest that the gustatory effects on lifespan have both IIS-dependent and IIS-independent components.

5.3.2. The taste influence on lifespan is partly dFOXO-dependent

Consistent with the above observations on the taste influence on *dilp* expression, I find that *dFOXO* is required for the lifespan extension due to the loss of labellar taste inputs in both males and females (Chapter 4, Figure 3 and Table S1). This would suggest that the wild-type function of labellar taste inputs is to shorten lifespan by inhibiting *dFOXO*, presumably by stimulating IIS activity. Interestingly, I find that female labellar taste inputs also appear to have a second effect on lifespan, i.e., they also lengthen lifespan by acting in parallel to *dFOXO*, since labellar taste-impaired females without *dFOXO* activity actually

live shorter than *dFOXO* single mutant female flies (Chapter 4, Figure 3 and Table S1). I also show that female taste inputs from the legs and wings lengthen lifespan in parallel to *dFOXO* (Chapter 4, Figure 3 and Table S1). This is because females lacking additional taste inputs from these appendages, as well as *dFOXO*, live even shorter than either *dFOXO* single mutant females or labellar taste-impaired females with no *dFOXO* activity (Chapter 4, Figure 3 and Table S1).

At the same time, the observed genetic interactions between taste inputs and *dFOXO* could also suggest the alternative model: (i) labellar taste inputs only have lifespan-shortening, *dFOXO*–dependent activities; and (ii) leg and wing taste inputs have two different effects on lifespan—a major effect that is lifespan-lengthening and a minor effect that is lifespan-shortening—both of which act at least partly in parallel to *dFOXO*. These complex effects coming from the leg and wing taste inputs would presumably be masked by the activation of *dFOXO*, but removal of *dFOXO* and the different inputs would then reveal these different influences on lifespan. On the other hand, in males, I observe no *dFOXO*-independent lifespan effects, which could suggest that all gustatory effects in males are mediated via the IIS pathway. These two alternative models are summarised in Figure 12.

Similar to the modulation of *dilp6*, a dFOXO target, in the head fat body, I also show that taste inputs affect the expression of two other dFOXO targets in the fly body (Chapter 4, Figure 4), the small heat shock protein *l(2)efl* and the translational inhibitor *4E-BP* (Flatt et al., 2008). On the tenth day of adulthood, both taste-impaired male and female flies have elevated *l(2)efl* transcripts and female taste mutants have increased *4E-BP* levels. These data are consistent with these flies having reduced IIS and increased dFOXO activities. Further support for the role of IIS in the gustatory influence on lifespan is the modulation of lifespan in response to yeast in labellar taste-impaired flies. Consistent with the idea that high dFOXO levels modulate the lifespan response to yeast concentrations (Giannakou et al., 2008), both

males and females lacking labellar taste inputs, but have high dFOXO activities, also exhibit, compared to controls, a modulated lifespan response to yeast (Chapter 4, Figure 3 and Table S1). Taken together these observations suggest that the role of IIS in mediating the effects of taste on lifespan is conserved in flies.

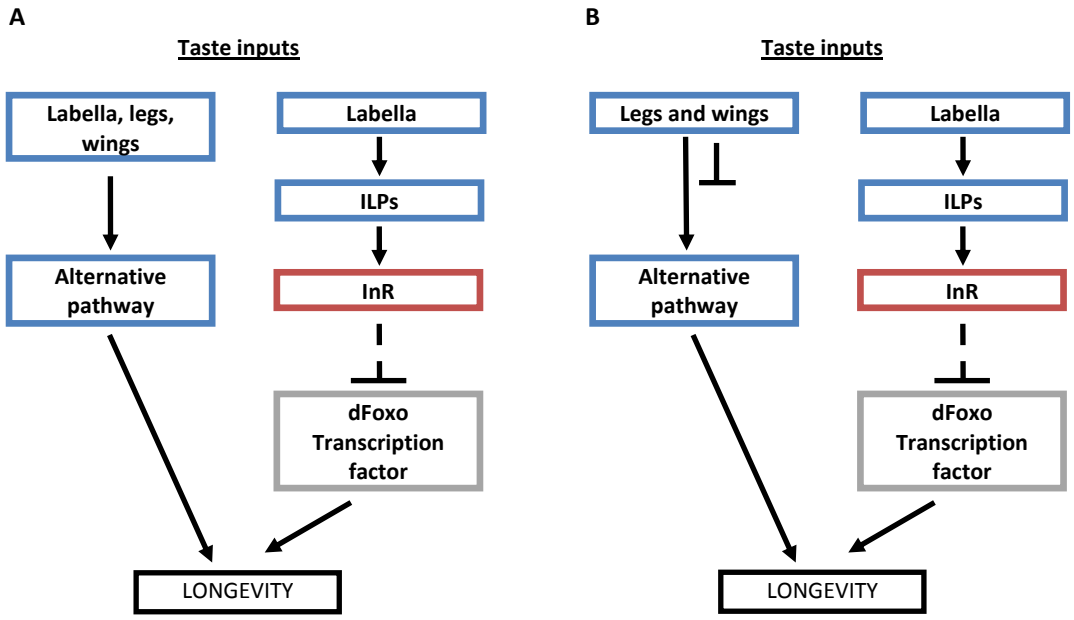


Figure 12. Two alternative models that explain the influence of fly taste bristles on longevity. A subset of taste inputs from the labella inhibits longevity by stimulating the release of Insulin-like peptides (ILPs) that in turn inhibit the dFoxo transcription factor through activation of the Insulin-like receptor. According to model A, taste inputs from the labella, legs and wings promote longevity through an unidentified alternative pathway, while according to panel B, the taste inputs from the legs and wings have both longevity-promoting and longevity-inhibiting effects. For more details, see the text above.

However, the lack of correlation between the changes in these transcripts and the lifespan phenotypes again suggest that the IIS pathway alone cannot explain all the taste-dependent effects on lifespan. These data might suggest that dFOXO activity is increased in both classes of taste mutants, but that there is alternative pathway acting in parallel to dFOXO, in response to leg and wing taste inputs, which would antagonize the dFOXO effects.

Further work is needed to map precisely the underlying neuronal and molecular networks involved in the gustatory influence on lifespan, e.g., the nature of the alternative molecular pathway(s) acting at least partly in parallel to dFOXO. Interestingly, a receptor for

a neuropeptide neuromedin U (NMU)-like signal has recently been reported to act with sensory neurons to modulate the food type-dependent effects on *C. elegans* lifespan in a *daf-16/FOXO*-independent manner (Maier et al., 2010). In flies, *Drosophila hugin*, which encodes an NMU-like peptide (Melcher et al., 2006), is expressed in SOG interneurons that not only receive labellar gustatory inputs but also send projections to or near *dilp*-expressing mNSCs (Melcher and Pankratz, 2005). This raises an intriguing possibility that an NMU-like pathway in flies [*hugin* or any of the ligands for the four predicted NMU-like receptors within the animal (Park et al., 2002)] might also mediate the gustatory influence on lifespan in a food type- and/or *dFOXO*-independent manner.

Another candidate pathway acting downstream of the taste influence on lifespan is the nutrient-sensing TOR pathway (Wullschleger et al., 2006). Activated TOR signaling phosphorylates 4E-BP, the eukaryotic initiation factor eIF4E binding protein, to inhibit its activity (Wullschleger et al., 2006). Conversely, downregulation of TOR signaling leads to hypophosphorylation of 4E-BP, which activates this protein to inhibit the activity of the eIF4E complex and growth-dependent protein synthesis (Wullschleger et al., 2006). 4E-BP has been suggested to be an important mediator of the effects of yeast restriction in a *dFOXO*-independent manner (Zid et al., 2009). Thus, it is an intriguing possibility that the gustatory information from longevity-promoting neurons modulates TOR pathway activity in response to yeast, in parallel to dFOXO signaling.

The existence of a sensory influence on lifespan, whether in flies or worms, provides a mechanism that allows the animal to fine-tune its physiology and optimize its survival in response to different environmental cues. The integration of this information can occur at the interneuron or non-neuronal level. For example, in the fly, this can occur at the SOG interneurons or even within the neuroendocrine mNSCs that express *dilp1*, *dilp2*, *dilp3* and *dilp5* (Brogiolo et al., 2001; Ikeya et al., 2002; Rulifson et al., 2002). The mNSCs have been

shown to be involved in regulating the response to food (Ikeya et al., 2002; Broughton et al., 2010). Indeed, DILP5 levels respond to yeast concentrations, i.e., they increase with increasing yeast (Broughton et al., 2010); and DILP5, together with DILP2 and DILP3, are proposed to signal to peripheral tissues, like the fat body cells, to promote the effects of IIS (Grönke et al., 2010). Intriguingly, male taste inputs, some of which are yeast-dependent, also regulate *dilp5* levels (Chapter 4, Figure 4), an observation consistent with the idea that mNSCs are involved in integrating gustatory inputs and mediating their systemic effects.

The presence of such a circuit might also include peripheral tissues regulating the activities of relevant neuronal cells, e.g., the neuroendocrine mNSCs, the SOG interneurons or even the sensory neurons, perhaps through a feedback mechanism. In *C. elegans*, the gustatory neuron ASI has been shown to shorten the lifespan of well-fed worms (Alcedo and Kenyon, 2004). However, ASI has also been found to lengthen lifespan, but of food level-restricted worms, and this requires the activity of the transcription factor SKN-1 (Bishop and Guarente, 2007). Upon restricting the level of food intake, SKN-1 is activated in ASI and this event signals to the periphery to adjust the worm's metabolism (Bishop and Guarente, 2007). At the same time, it seems that this ASI SKN-1-dependent lifespan increase in food-restricted worms is at least partly independent of gustatory function (Bishop and Guarente, 2007). Thus, these data are consistent with the idea that internal signals also modulate sensory neuron activities and that sensory neurons perceive both external and internal cues to affect lifespan.

5.4. Taste effects on physiology and lifespan might be conserved in other organisms

Throughout this discussion, I have argued in favor of the model where external and internal food-derived cues are integrated in the neuronal tissues of worms and flies, and that

this consequently controls the physiology and lifespan of the animal through cell non-autonomous neuroendocrine signals. I also presented evidence that the sensory system is an important part of this regulatory axis in these animals.

In mammals, a similar regulatory axis could exist. Immediately after food intake, chemosensory and olfactory cues induce the secretion of hormones from the stomach and pancreas [reviewed by (Squire et al., 2003; Zafra et al., 2006)]. After a mock feeding, which only involved tasting without ingestion of the food, there is also an increase in the secretion of the leptin and insulin hormones independent of blood glucose levels in rodents, dogs and humans (Berthoud et al., 1980; Secchi et al., 1995). In addition, mammalian anticipatory responses that optimize digestion are initiated by gustatory and olfactory cues [reviewed by (Zafra et al., 2006)]. These observations are not surprising, since both gustatory and olfactory information can be relayed to the hypothalamus [reviewed by (Squire et al., 2003; Lundy Jr. and Norgren, 2004)]. For example, mammalian gustatory information from the tongue taste receptors are conveyed through three cranial nerves to the nucleus of solitary tract (NST), the parabrachial nuclei of the pons (PBN) and the parvocellular component of the ventrobasal complex of the thalamus (VPMpc) (Squire et al., 2003; Lundy Jr. and Norgren, 2004). At the same time, one of the ascending projection targets of PBN is the hypothalamus, which links the mammalian nervous system to its endocrine system (Squire et al., 2003; Lundy Jr. and Norgren, 2004). Thus, these findings on gustatory and olfactory-induced endocrine changes raise the possibility that gustatory and olfactory cues can also influence the lifespan of mammals via hypothalamic processing of these information.

Finally, the influence of taste inputs on lifespan through the insulin/IGF-1 pathway is consistent with the complex adaptive system view of animal physiology. In this sense, specific taste inputs could act as modulators of the control parameter, namely the insulin/IGF-1 pathway, and accordingly affect the physiological homeodynamics. This newly discovered

network link between taste and insulin/IGF-1 signaling could both increase network stability and enable the organism to respond timely and pro-actively to changes in the external environment.

5.5. Conclusion and outlook

The primary goal of my thesis was to examine whether the taste influence on lifespan is conserved in *Drosophila*. Accordingly, I find that flies missing labellar taste inputs live long and that loss of additional taste inputs from the legs and wings suppresses this phenotype. These suggest that there are longevity-inhibiting and longevity-promoting taste neurons in flies that regulate the animal's physiological response to the environment, and consequently its lifespan.

The second aim of my research was to attempt to reveal the pieces of the neuroendocrinal network that governs this process. After excluding the possibility that these flies are living longer simply because they eat less food, I do show that the lifespan phenotypes of these taste mutants are food type-dependent, a dietary influence on lifespan that has been shown to be distinct from that of food-level restriction (Maier et al., 2010). I also show that the loss of labellar taste inputs, regardless of the presence or absence of leg and wing taste inputs, increase the activity of dFOXO and levels of subsets of dFOXO target genes. This is an important finding, since it suggests a genetic link between taste and insulin signaling in flies: taste perception affects IIS pathway activity. Thus, this thesis presents a first attempt in explaining the underlying molecular machinery in *Drosophila* that regulates an animal's physiology and lifespan in response to perception of environmental stimuli.

Since taste perception is important in modulating the food-derived hedonistic stimuli, it is possible that the fly insulin-like peptides are also some of the internal signals that modulate this process. Indeed, *dilp* overexpression changes the appetitive response of starved

flies to unpalatable food (Wu et al., 2005a). This link could be explored further by performing a few crucial experiments. For example, it is possible that overexpression of some *dilps* in adult wild-type flies would modulate their food-choice responses, similar to larvae. Overexpression of *dilps* in taste-impaired flies could show whether taste perception is required for this potential switch in food choice.

Studying the link between taste, food cues, IIS and aging could have important biomedical implications. If this process is conserved in humans, these studies could make it possible to control feeding behavior and prevent or treat obesity and diabetes, through therapeutic interventions at the level of taste perception. The palatability of different diets could be designed to improve the health conditions of different patients.

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Abbreviations

4EBP	4E binding protein
5'UTR	5’ untranslated region
age-1	C. elegans ortholog of the phosphoinositide 3-kinase (PI3K) p110 catalytic subunit
akt-1	*C.elegans* ortholog of the serine/threonine kinase Akt/PKB
akt-2	*C.elegans* homolog of the serine/threonine kinase Akt/PKB
AL	Antennal lobe, part of the fly brain
BMI	Body Mass index
chico	*Drosophila* insulin receptor substrate protein
daf-15	*C.elegans o*rtholog of RAPTOR (the regulatory associated protein of mTOR)
daf-16	*C. elegans* FOXO transcription factor ortholog
daf-18	encode lipid phosphatase homologous to the human PTEN tumor suppresor
daf-2	*C. elegans* insulin/IGF-1-like receptor
db/db	Mouse mutant in diabetes gene, model for diabetes
DCSO	Dorsal cibarial sense organs, part of the fly’s taste system
dilp1-7	*Drosophila* insulin like peptide (from 1 to 7)
DR	Dietary restriction
eat-1 and *2*	encodes gene *EATing;* mutant has abnormal pharyngeal pumping
eIF4E	Eukaryotic translation initiation factor 4E
FOXO	Forkhead box O transcription factor (dFOXO in *Drosophila*)
GR	Gustatory receptor

GRN	Gustatory receptor neuron
IGF-1	Insulin growth factor -1
IIS	Insulin/IGF-1 signaling
InR	*Drosophila* Insulin receptor
Irs2	Mammalian Insulin receptor substrate protein 2
let-363	*C.elegans* orthologous to S. cerevisiae Tor1p and Tor2p and human FRAP1
LSO	*Drosophila* labral sense organ
mNSCs	Median neurosecretory cells located in the fly brain
NST	Nucleus of solitary tract
OR	Olfactory receptor
Or83b	*Drosophila* gene Odorant receptor 83b
ORN	Olfactory receptor neuron
PBN	Parabrachial nuclei of the pons
pdk-1	*C. elegans* 3-phosphoinositide-dependent kinase 1 ortholog
PI-3	Phosphatidylinositol - 3
PI-3K	Phosphatidylinositol – 3 kinase
PIP-2	Phosphatidylinositol 4,5-bisphosphate
PIP-3	Phosphatidylinositol (3,4,5)-trisphosphate
Poxn	*Drosophila* gene Pox neuro, PAX family transcription factor
sgk-1	Orthologous to the mammalian serum- and glucocorticoid-inducible kinases (SGKs)
skn-1	*C.elegans* SKiNhead transcription factor
SOG	Subesophageal ganglion
TOR	Target of rapamycin gene
TORC1 and 2	Target of rapamycin comple 1 and 2
TSC1 and 2	Tuberous sclerosis protein 1 and 2

VCSO	Ventral cibarial sense organs
VPMpc	Pravocellular component of the ventrobasal complex of the thalamus