Positive and negative gustatory inputs affect *Drosophila* lifespan partly in parallel to dFOXO signaling

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In *Caenorhabditis elegans*, a subset of gustatory neurons, as well as olfactory neurons, shortens lifespan, whereas a different subset of gustatory neurons lengthens it. Recently, the lifespan-shortening effect of olfactory neurons has been reported to be conserved in *Drosophila*. Here we 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. We also note that the gustatory influence on lifespan does not necessarily depend on food intake levels. Finally, we identify the nature of some of the taste inputs that could shorten versus lengthen lifespan. Together our data suggest 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.

sensory system | insulin signaling | physiology | gustatory receptors | aging

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 neuron activities have been shown to alter the lifespan of both *Caenorhabditis elegans* and *Drosophila melanogaster* (1–6).

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 it (1). Interestingly, at least part of this sensory influence on lifespan has also been observed in other animals. In *Drosophila*, impairment of olfaction through a mutation in *Or83b*, which encodes a broadly expressed atypical odorant receptor (7), increases lifespan (3). In addition, exposure of dietary-restricted flies to food odors, like live yeast, can partly suppress their long-life phenotype (3). The conservation of the olfactory influence on lifespan is thus consistent with the possibility that gustatory inputs will also bidirectionally alter the lifespan of both *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 (1, 2, 8). The insulin/IGF pathway also affects fly lifespan: down-regulation of the activities of the insulin receptor InR and the receptor substrate, CHICO, extends lifespan (9, 10). Moreover, an increase in activity of the downstream transcription factor dFOXO, which is negatively regulated by both InR and CHICO, increases fly lifespan (11, 12). Consistent with these observations, mutations in several of the *Drosophila* insulin-like peptide (*dilp*) genes (13), which are expressed in the median neurosecretory cells (mNSCs) in the fly brain (14–17), or ablation of the mNSCs (18) also extends lifespan. Because these mNSCs send projections to the subesophageal ganglion (SOG) (14, 17), a group of interneurons involved in processing gustatory information in the fly brain (19, 20), 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 (refs. 21 and 22; reviewed in ref. 23). 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 partly dependent on the activity of the dFOXO transcription factor, which acts downstream of insulin signaling. Through a screen of taste receptor mutants, we additionally uncover the possible nature of the gustatory cues that can lengthen versus shorten lifespan.

Results

Taste Inputs Affect *Drosophila* Lifespan. To test the hypothesis that taste inputs affect fly lifespan, we first compared two classes of taste-impaired flies to control flies that have wild-type taste

Significance

The ability of the worm *Caenorhabditis elegans* to taste or smell can influence its lifespan, and the effect of odors on lifespan has also been shown to exist in the fruit fly *Drosophila*. Now we provide evidence that fly lifespan is also affected by its ability to taste: some taste inputs shorten lifespan, whereas others increase it. In flies, the lifespan-shortening taste inputs act via an insulin-like signaling pathway and its downstream transcription factor dFOXO, whereas the lifespan-lengthening taste inputs can act independently of this pathway. The taste influence on lifespan is also unlikely linked to changes in food intake levels. Thus, different taste inputs will affect lifespan through more than one mechanism.

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The authors declare no conflict of interest.

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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 retain the mechanosensory innervation (24). 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 (24–28). Hence, for our studies, we compared $Poxn^{\Delta M22\text{-}B5}$ mutants that carry the complete rescue construct with $Poxn^{\Delta M22\text{-}B5}$ mutants that carry rescue constructs that lack enhancer elements required for the formation of either a subset of (labellar) or most taste bristles (Fig. S1) (27, 29).

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 (Fig. 1 *A*, *B*, and *E* and Table S1). We observed that this effect is more robust in females than in males (Fig. 1 *A*, *B*, and *E* and Table S1). Interestingly, we also found that the removal of additional taste bristles from the legs and wings suppresses the long-life phenotype of both labellar taste-impaired male and female flies (Fig. 1 *C*–*E* and Table S1). Because the difference between the two classes of taste-impaired flies resides in the number and location of the missing taste bristles (Fig. S2) (27, 29), this sug-gests that the suppression was due to other taste deficiencies rather than general deleterious effects elsewhere. Notably, this suppression could also be complete (Fig. 1 *C*–*E*) or partial (see Fig. 3 *A* and *B*), which may arise from the variability of gustatory cues present in the environment. Accordingly, taste sensory organs that perceive a specific set of cues would presumably affect lifespan only in the presence of such cues, an idea that has been illustrated in *C. elegans* by sensory mutants that exhibit lifespan phenotypes only on certain food sources (30). Thus, the complete or partial antagonism between labellar versus leg and wing taste inputs could be explained, for example, by the variable quality of the yeast present in the *Drosophila*'s diet from experiment to experiment. Together these studies suggest that both the positive and negative influences of taste inputs on lifespan are conserved in *Drosophila*.

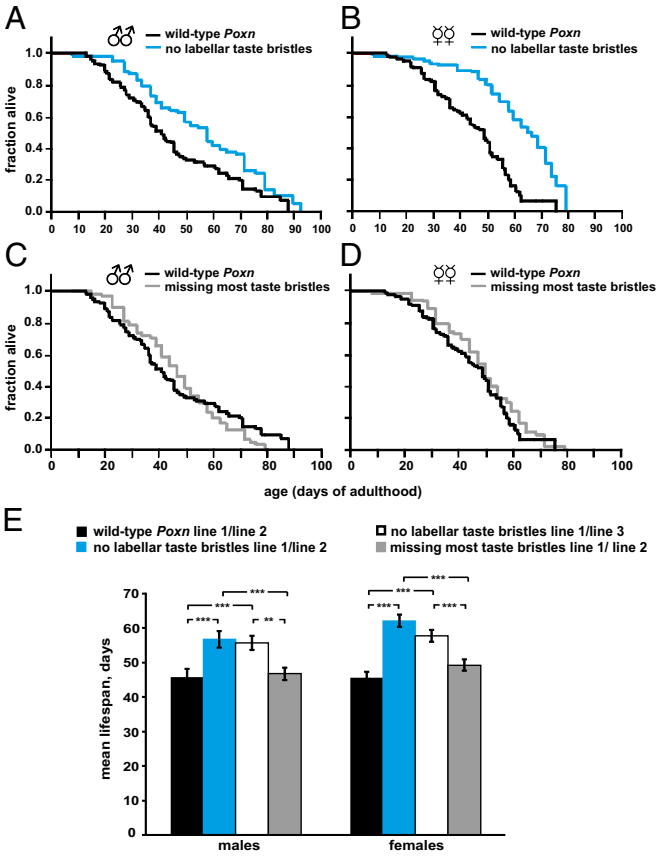

Fig. 1. Taste inputs affect fly lifespan bidirectionally. (*A* and *B*) Unmated males and females lacking labellar taste bristles (blue curve) live longer than wild-type *Poxn* control flies (black curve). The detailed statistical data on the survival analyses in this figure (trial 1) are shown in Table S1. (*C* and *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 ±SEM. $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$, according to the Wilcoxon test.

The Physiology of Taste-Impaired Flies Does Not Resemble That of Food Level-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 commonly known as dietary restriction (DR), extends lifespan, whereas a further reduction in feeding, which presumably leads to a state of starvation, causes a shorter lifespan (31). Surprisingly, however, we observed a lack of correlation between food intake and lifespan of control and labellar taste-impaired flies (Fig. S2*A* and Table S1). Indeed, in most cases, both classes of taste-impaired flies exhibit increased food intake compared with control flies (Fig. 2 *A* and *B* and Fig. S2*A*), which shows that the lifespan phenotypes of these flies (Table S1) are not necessarily due to a restriction in food intake. Moreover, we found that taste-impaired flies weigh heavier as they get older (Fig. 2 *C* and *D*), which is again unlike the lower body weights observed in flies with restricted food intake levels (32).

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 triacylglyceride (TAG) levels. Again, we saw no correlation between the lifespan and TAG levels of these flies: taste-impaired flies have similar TAG content as control flies (Fig. 2 *E* and *F* and Fig. S2*B*).

Because reduced reproductive output is also a hallmark of DR (33), we compared the rates of reproduction and total fecundity in control and taste-mutant flies. Although we detected some differences between these flies in the number of eggs laid per day (Fig. 2*G*), we observed that the various groups of flies laid a similar cumulative number of eggs within a period of 10 d (Fig. 2*H*). 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.

Besides food levels, the nature of the food source has also been shown to influence an animal's lifespan (30, 34–37). Because yeast is a component of fly food that can shorten fly lifespan (36) partly through an olfaction-mediated mechanism (3), we asked whether the effect of yeast on lifespan also acts via taste inputs. However, we found that the absence of a yeast supplement in the diet (*Materials and Methods*) can still extend the lifespan of all taste-impaired and control flies, and that the taste-impaired female flies can still live longer than control flies on the non–yeast-enriched diet (Fig. 3*A* and Table S1).

Some Taste Inputs Require the Activity of the Insulin Pathway Effector dFOXO. In worms, the taste influence on lifespan can act in parallel to DR but is mediated by the insulin signaling pathway (1). In flies, reduction-of-function mutations in the insulin receptor *InR* (10) or the receptor substrate *chico* (9) have been shown similarly to extend lifespan, which require the activity of the transcription factor dFOXO (11, 12). In contrast, dFOXO activity is not required for the lifespan increase caused by DR (38). Thus, these observations led us to ask whether

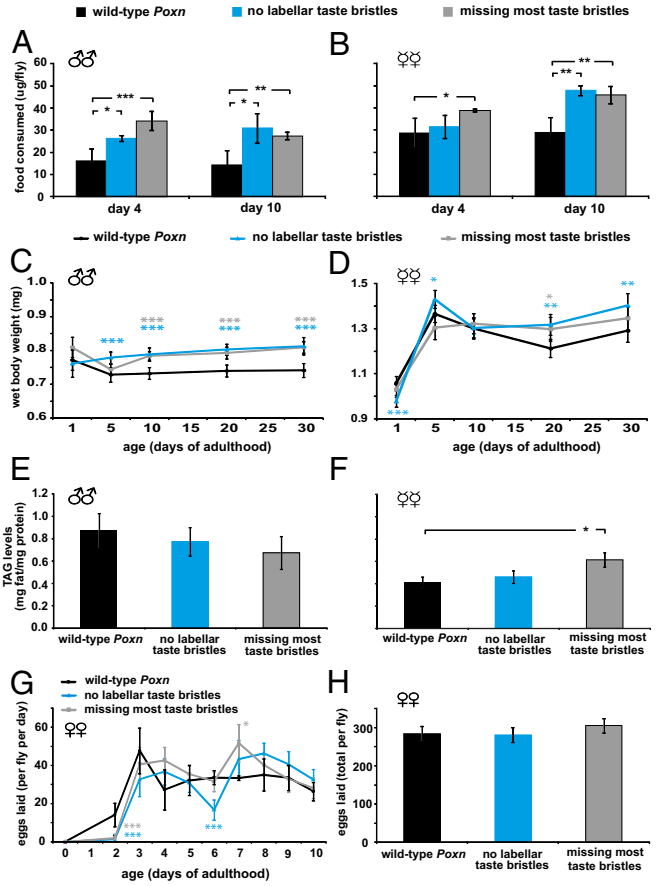

Fig. 2. Taste-impaired flies do not resemble food level-restricted flies. (*A* and *B*) Taste-impaired adult mutant (blue and gray bars) males and females are compared with controls (black bars) at two different ages after a 24-h feeding regimen. The food consumption values are normalized per fly and each mean is derived from 3 to 4 biological replicates of 7 to 10 pooled flies. The error bars in *A*–*H* represent 95% confidence intervals. (*C* and *D*) The body weight of taste mutants (blue and gray lines) are compared with controls (black line) at different ages. Each data point represents the mean from at least three measurements of 10 individual flies. (*E* and *F*) TAG levels of 10-d-old adult taste-mutant and control flies are compared. Each mean represents five biological replicates of 9 to 10 pooled flies. (*G* and *H*) The fecundity of taste-impaired flies is compared with that of controls. The number of eggs laid per fly per day (*G*) or the total number of eggs laid per fly over a period of 10 d (*H*) is shown. Each time point represents data from 6 to 10 adult females. All flies in *A*–*H* carry transheterozygous insertions of the relevant transgenes in their genomes. $*P \leq 0.05$, $**P \leq 0.01$, and $***P \leq 0.001$.

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 (Fig. 3 *B* and *C* and Table S1), which suggests that the labella receive taste inputs that shorten lifespan in a *dFOXO*-dependent manner. Moreover, we observed that loss of additional taste bristles from the legs and wings of female *dFOXO* mutants further shortened their lifespan (Fig. 3 *B* and *D* and Table S1), which suggests that leg and wing taste inputs also affect lifespan independent of *dFOXO*.

Because the taste influence on lifespan appears to partially require dFOXO activity, we measured the steady-state transcript levels of several *dilps*, some of which are known dFOXO targets—e.g., *dilp3* and *dilp6* (13, 39–41)—in both classes of taste mutants. We detected significant changes in only some of the *dilps* of the taste-impaired flies (Fig. 3*E*). In females, *dilp1*, *dilp3*, and *dilp6* are elevated in both classes of taste mutants (Fig. 3*E*), and these taste-dependent increases are suppressed by loss of *dFOXO* activity (Fig. S3). To determine whether *dFOXO* targets other than *dilp3* and *dilp6* are also affected by taste inputs, we compared the levels of *l(2)efl*, which encodes a small heat shock protein whose expression requires *dFOXO* (42), in the bodies of taste-mutant and control flies. Similar to *dilp1*, *dilp3*, and *dilp6*, we found that *l(2)efl* is increased in both taste mutants in the presence of *dFOXO*, but not in the absence of *dFOXO* (Fig. 3*F*). Together our findings suggest that loss of labellar taste inputs increase *dFOXO* activity to lengthen lifespan (Fig. 3 *B*, *C*, *E*, and *F* and Table S1). Interestingly, because we observed that the *dilp* changes are similar in both classes of taste-impaired flies (Fig. 3*E*

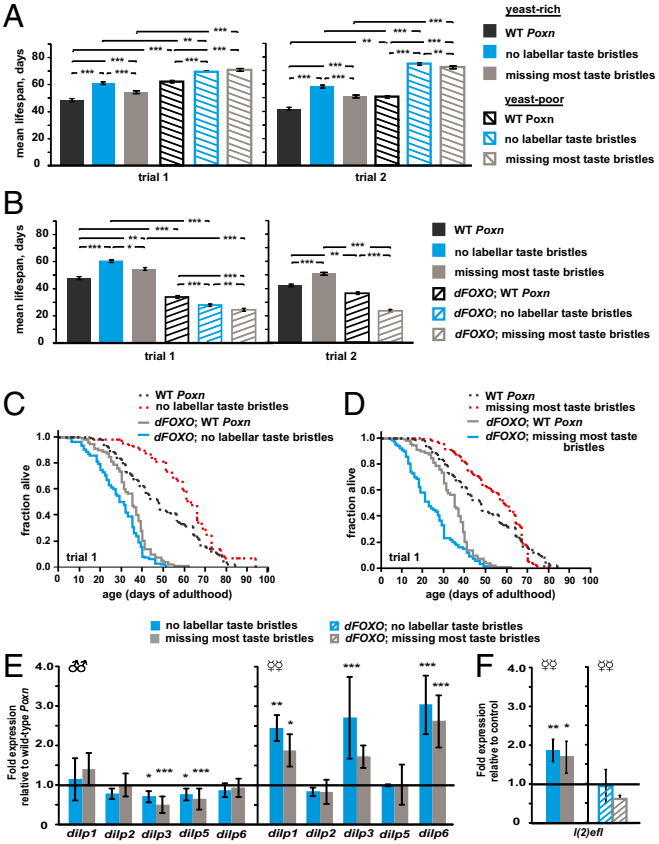

Fig. 3. The effects of yeast and *dFOXO* on the taste influence on lifespan. (*A*) The mean lifespan (±SEM) of control and taste-mutant flies on yeast-enriched versus nonenriched Zurich fly food are depicted as bar graphs. (*B*) The mean lifespan (±SEM) of control and taste mutants that have wild-type (WT) *dFOXO* versus those of flies that carry mutations in *dFOXO* are shown as bar graphs. (*C* and *D*) Survival curves of taste-mutant (red) and control (black) flies that have wild-type *dFOXO* versus those of the corresponding taste-mutant (blue) and control (gray) flies that carry mutations in *dFOXO*. (*E* and *F*) The transcript levels of *dilps* in the heads (*E*) and of *l(2)efl* in the bodies (*F*) of adult male and/or female taste mutants (blue and gray bars) are shown normalized to control levels (horizontal line across the graph). The relative expression levels in the heads of controls for males are 0.72 ± 0.14 (*dilp1*), 1.26 ± 0.41 (*dilp2*), 1.96 ± 0.71 (*dilp3*), 1.63 ± 0.48 (*dilp5*), and 1.32 ± 0.29 (*dilp6*); and females 0.45 ± 0.08 (*dilp1*), 1.12 ± 0.08 (*dilp2*), 0.83 ± 0.10 (*dilp3*), 1.15 ± 0.09 (*dilp5*), and 0.57 ± 0.03 (*dilp6*). The relative expression levels of *l(2)efl* in the bodies of control female flies are 0.76 ± 0.25 for wild-type *dFOXO* and 0.32 ± 0.08 for mutant *dFOXO*. Each mean value represents three biological replicates of 30 pooled flies. All flies in *A*–*F* again carry transheterozygous insertions of the relevant transgenes in their genomes. All error bars represent ±SEM. $*P \leq 0.01$, $**P \leq 0.01$, and $***P \leq 0.001$ (Wilcoxon test for *A*, and *B*; randomized complete block design ANOVA for *E* and *F*). The detailed statistical data on the above lifespan analyses can be found in Table S1.

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and Fig. S3), these data also suggest that the *dFOXO*-independent leg and wing taste inputs antagonize, and/or act completely parallel to, DILP function.

The sexual dimorphism of the taste influence on lifespan, i.e., its more robust effects in females than in males (Fig. 1 and Table S1), was also apparent at the level of *dilp* expression (Fig. 3*E*). In contrast to the changes in *dilp* expression in females, we found a decrease in *dilp3* and *dilp5* transcripts of male taste-impaired flies (Fig. 3*E*). These differences in *dilp* expression patterns in response to taste inputs may reflect the differences in the perceived nutrient requirements of unmated male and female flies.

Together these data show that the taste influence on lifespan involves distinct mechanisms that act in parallel: a *dFOXO*-dependent versus a *dFOXO*-independent pathway (Fig. 3 *B–D* and Table S1). This is also reflected in the differences in the taste influence on the expression of specific *dilps* (Fig. 3*E*), each of which may promote a particular set of physiological changes that ultimately affect lifespan (13, 40).

Different Gustatory Receptors Have Distinct Effects on Lifespan. To confirm the bidirectional effects of taste on lifespan, we assayed the lifespan of some known gustatory receptor mutants. We found that some taste receptor mutants have a short lifespan, e.g., the sweet taste receptor *Gr5a* (43), whereas other taste receptor mutants have a long lifespan (Table 1) (44), like the water receptor *ppk28* (45). Interestingly, mutations that disrupt the *Gr5a* locus have previously been shown to decrease lifespan (46), consistent with our observation with a mutation that specifically deletes this gene [*ΔGr5a* (43); Table 1]. Moreover, the companion article (44) in PNAS details the mechanisms through which the water receptor *ppk28* affects lifespan. Thus, our data together suggest the nature of some taste inputs that can modulate *Drosophila* longevity.

Discussion

Many biological processes are conserved between *C. elegans*, *Drosophila*, and higher organisms. For example, the insulin/IGF1 pathway regulates the physiology, and consequently the longevity, of worms, flies, and mice (9, 10, 47–49). Similarly, the sensory influence on lifespan is conserved in both worms (1) and flies at the level of olfaction (3, 5). This study, as well as that of Waterson et al. (44), shows that the conserved sensory effects on lifespan can also be extended to gustation.

As in *C. elegans* (1), our study illustrates that the gustatory system can also affect *Drosophila* lifespan bidirectionally (Figs. 1 and 3 *A* and *B*, Table 1, and Table S1), which shows no correlation with and is thus likely independent of the animal's level of food intake (Fig. 2 *A* and *B* and Fig. S2*A*). Indeed, both longer- and shorter-lived taste-impaired flies usually have higher food intake levels and body weights (Fig. 2 *A–D* and Fig. S2*A*), 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 (50, 51).

Although it is independent of food intake levels, it remains possible that the gustatory influence on fly lifespan could depend on the type of food source. In *C. elegans*, the sensory influence depends on the recognition of food types, which can have different effects on lifespan (30, 34–37). In *Drosophila*, the food-type effect on lifespan has been demonstrated through alterations in the protein composition of its food source. For example, yeast restriction or an imbalance in dietary amino acids can extend lifespan (36, 52, 53). However, yeast restriction alone does not always increase fly lifespan under all conditions that do not cause malnutrition (52), which suggests that other lifespan-influencing food-derived factors are also involved. In fact, the perception of water is one of the cues, among many, that can affect lifespan (Table 1) (44, 54). Thus, because the gustatory system senses many different types of food-derived cues, which

Table 1. Specific gustatory receptor mutants exhibit short- versus long-lived phenotypes

Genotype			Sample size		Lifespan, d			
Mutant	Control [G]	Food	N_{mut}	N_{ctrl}	$Mean_{mut}$	$Mean_{ctrl}$	Δ lifespan, %	*P* value
				Male				
w; dpr^{1}	w^{1118} [6]	ZRH	92	94	43.62	47.76	−8.67	0.0615
w; ΔGr5a	w^{1118} [8]	SY10	246	248	60.38	64.21	−5.96	0.0285
w; ΔGr5a	w^{1118} [8]	SY10	294	284	57.79	66.60	−13.23	0
w; ΔGr33a	w^{1118} [6]	ZRH	92	84	49.88	55.23	−9.69	0.0038
w; ΔGr33a	w^{1118} [7]	SY10	241	250	65.04	62.32	−4.36	0.4320
w; ΔGr66a	w^{1118} [4]	ZRH	78	94	62.00	47.76	29.82	0
w; ΔGr66a	w^{1118} [6]	SY10	237	250	69.11	62.32	10.90	0.0060
w; ΔGr93a	w^{1118} [6]	ZRH	77	94	46.52	47.76	−2.60	0.3384
w; Δppk28	w^{1118} [6]	ZRH	91	84	61.58	55.23	11.50	0.0165
w; Δppk28	w^{1118} [8]	SY10	245	243	72.09	61.72	16.80	2.45×10^{-5}
				Female				
w; dpr^{1}	w^{1118} [6]	ZRH	188	91	41.18	47.28	−12.90	0.0002
w; ΔGr5a	w^{1118} [8]	SY10	225	251	52.74	64.93	−18.93	0
w; ΔGr5a	w^{1118} [8]	SY10	297	280	51.55	70.52	−26.90	0
w; ΔGr33a	w^{1118} [6]	ZRH	93	96	51.42	48.05	7.01	0.0741
w; ΔGr33a	w^{1118} [7]	SY10	247	250	69.11	58.23	18.71	0
w; ΔGr64	w^{1118} [8]	S30Y5	295	272	36.72	45.90	−20.00	0
w; ΔGr66a	w^{1118} [4]	ZRH	86	91	58.90	47.28	24.58	0
w; ΔGr66a	w^{1118} [6]	SY10	251	250	75.78	58.23	30.14	0
w; ΔGr93a	w^{1118} [6]	ZRH	98	91	44.95	47.28	4.93	0.0090
w; Δppk28	w^{1118} [6]	ZRH	96	96	54.31	48.05	13.03	0.0003
w; Δppk28	w^{1118} [8]	SY10	241	246	74.79	55.97	33.63	0

We measured the lifespan of control (ctrl) and mutant (mut) flies in parallel in independent trials. The number of backcrosses to w^{1118} (*w*) per taste mutant is indicated in column 2 by "[G]." Each assay was carried out according to the Zurich (ZRH) protocol and diet (supplemented with yeast paste) or to the Ann Arbor protocol and diet (SY10 or S30Y5). See *Materials and Methods* for the exact genotype of each receptor mutant assayed. *P* values are according to the log-rank test.

could elicit different physiological outcomes, it should not be surprising that the taste influence on lifespan will involve more than one mechanism.

Consistent with this idea, we show that this influence requires both *dFOXO*-dependent and -independent pathways that act in parallel (Fig. 3 *B–D* and Table S1). Labellar taste inputs inhibit longevity through the dFOXO pathway (Fig. 3 *B* and *C* and Table S1), an effect that is either completely (Fig. 1) or partly (Fig. 3 *A* and *B* and Table S1) antagonized by parallel taste inputs from the legs and wings. Because flies missing most taste inputs show a similar change in the expression of *dilps* and the dFOXO target *l(2)efl* compared with flies that are only impaired in labellar taste (Fig. 3 *E* and *F*), this suggests that (*i*) the *dFOXO*-dependent lifespan-lengthening signal is present in both classes of taste mutants and (*ii*) the *dFOXO*-independent taste inputs from the legs and wings counteract this signal. However, the degree of antagonism between these two pathways presumably depends on the presence or absence of specific gustatory cues in the animal's environment. This would be analogous to *C. elegans* sensory mutants that exhibit food source-dependent lifespan phenotypes (30).

Our findings that only some *dilps* are altered in taste-impaired flies (Fig. 3*E*) suggest that specific gustatory cues modulate the activities of discrete sets of *dilps*, which are expressed either in the mNSCs or the fat bodies of the fly (13–17, 40, 55). This could occur through the SOG interneurons that can act as a relay center between gustatory neurons and the *dilp*-expressing mNSCs (14, 19, 20) or fat bodies (13, 40). Moreover, the nature of the *dilps* that have altered expression in female versus male taste-impaired flies could yield insight into the sexual dimorphism of the taste influence on lifespan. In females, *dilp1*, *dilp3*, and *dilp6* are highly expressed in the heads of taste-impaired flies (Fig. 3*E*). Although high *dilp1* or *dilp3* expression has not been shown to affect lifespan, increased expression of *dilp6* in the head fat body through overexpression of *dFOXO* has previously been shown to extend lifespan (41). Thus, it is tempting to speculate that labellar taste-impaired female flies live longer due to *dilp6* overexpression. In males, on the other hand, taste impairment does not change *dilp6* levels, but slightly down-regulates *dilp3* and *dilp5* (Fig. 3*E*). Loss of these *dilps*, together with that of *dilp2*, has been found to mediate the DR effects on lifespan (13). However, it is currently unclear how down-regulation of *dilp3* and *dilp5* would promote longevity in male taste-mutant flies.

Finally, the observation that the gustatory and olfactory systems influence the lifespan of both worms and flies (this study and refs. 1, 3, 5, 44, and 54) raises the intriguing possibility that the sensory system also affects mammalian lifespan. In mammals, both gustatory and olfactory information are relayed to the hypothalamus, a region in the brain that controls behavior and physiology (reviewed in ref. 56). 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.

Materials and Methods

Fly Stocks. All transgenic rescue constructs (*SuperA-158*, *SuperA-207-1*, *Full1*, *Full115*, *Full152*, *ΔXBs*, and *ΔPBs*) were as formerly described (27, 29). The *dFOXO* null alleles ($dFOXO^{21}$ and $dFOXO^{25}$) (57), and the taste receptor mutants [dpr^1 (58), *ΔGr5a* (43), $Gr33a^1$ (*ΔGr33a*) (59), *ΔGr64* (60), $Gr66a^{ex83}$ (*ΔGr66a*) (61), $Gr93a^2$ (*ΔGr93a*) (62), and *Δppk28* (45)] were also as described previously. All flies were backcrossed at least seven times to the w^{1118} background (*SI Materials and Methods*), with the exception of some of the taste receptor mutants, which were backcrossed four or six times to w^{1118} before the lifespan screen. To further minimize the effect of the genetic background on the experimental results, transheterozygous combinations of the relevant *Poxn* rescue constructs and *dFOXO* alleles were compared, which should also ensure that the disruption of insertion sites is heterozygous in the transgenic animals analyzed. The full genotypes of the transgenic flies used in this study are listed in *SI Materials and Methods*. All flies were maintained at 25 °C.

Lifespan Assays. ***Zurich.*** The lifespan of the progeny of 3- to 5-d-old male and female wild-type *Poxn* and taste bristle mutant flies in the presence or absence of *dFOXO*, as well as some of the taste receptor mutants, were measured at 25 °C under constant humidity (60%) with a 12:12 h light:dark cycle. To minimize stress-induced mortality in very young adults, freshly eclosed flies (within a 2-h time window) were transferred to new bottles, where they were aged for 2 h. These flies were then collected under mild CO_2 anesthesia for the lifespan assays, in which adult virgin males and virgin females were separated. The lifespan measurements, which were started on the first day of adulthood, were done by placing 8–12 flies per vial, which contained standard Zurich fly food [10% (wt/vol) yeast, 7.5% (wt/vol) dextrose, 5.5% (wt/vol) corn meal, 1% (wt/vol) 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 lifespan, and statistical comparisons among the different assay conditions by applying the log-rank or Wilcoxon test where appropriate. If the ratio of hazard functions (ratio of mortality rates) between two groups of animals stays approximately constant over time, the log-rank test serves as the appropriate test; otherwise, the Wilcoxon test is more appropriate (63). ***Ann Arbor.*** The lifespan of some of the taste receptor mutants were measured as described in ref. 44, where flies were fed a 10% (wt/vol) sucrose and yeast diet (SY10) or a 30% (wt/vol) sucrose–5% (wt/vol) yeast diet (S30Y5).

Feeding Assays, Body Weight, and TAG Measurements and Quantitative Measurement of mRNA Levels. Control and mutant flies were collected as described above (Zurich protocol and diet) and transferred regularly to fresh food until the specified days of adulthood, upon which food intake, body weight, TAG levels, and *dilp* and *l(2)efl* expression were measured. See *SI Materials and Methods* for a description of the different assays.

Fecundity Assays. See *SI Materials and Methods* for a description of the assays.

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