Sensory perception of dead conspecifics induces aversive cues and modulates lifespan through serotonin in *Drosophila*

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Sensory perception modulates health and aging across taxa. Understanding the nature of relevant cues and the mechanisms underlying their action may lead to novel interventions that improve the length and quality of life. We found that in the vinegar fly, *Drosophila melanogaster*, exposure to dead conspecifics in the environment induced cues that were aversive to other flies, modulated physiology, and impaired longevity. The effects of exposure to dead conspecifics on aversiveness and lifespan required visual and olfactory function in the exposed flies. Furthermore, the sight of dead flies was sufficient to produce aversive cues and to induce changes in the head metabolome. Genetic and pharmacologic attenuation of serotonergic signaling eliminated the effects of exposure on aversiveness and lifespan. Our results indicate that *Drosophila* have an ability to perceive dead conspecifics in their environment and suggest conserved mechanistic links between neural state, health, and aging; the roots of which might be unearthed using invertebrate model systems.
Sensory perception influences energy homeostasis, tissue physiology, and organism aging through neuronal circuits that emanate from sensory tissues and that interface with deeper regions of the central nervous system. The molecular nature of these relationships was first described in the nematode, *Caenorhabditis elegans*, and sensory effects on aging have been observed across the phylogeny of vertebrate and invertebrate animals. Sensory inputs relate information about nutrient availability and reproductive opportunity to rapidly initiate physiological changes that occur in coordination with known behavioral outcomes, suggesting similarities in the underlying circuitry. Conserved neuromodulators, including biogenic amines and neuropeptides, that influence responses to food and mates are known to also modulate lifespan in a state-dependent manner.

The ability to perceive dead individuals is not exceptional in the animal kingdom, as individuals from a range of species respond to dead conspecifics with a variety of different effects. Social insects, including ants and honey bees, exhibit necrophoresis, in which dead colony members are systematically removed from the nest to promote hygienic conditions. Dead zebrafish scents provoke defensive behavior in live individuals, and the sight of a dead conspecific induces alarm calling in scrub-jays, suggesting that dead individuals may indicate danger. Elephants and nonhuman primates exhibit stereotypical behaviors toward dead individuals associated with permanent loss of a group member. In humans, the effects of experiences with death include emotional dysregulation and depression, as well as physical effects such as headaches, fatigue, and cardiovascular disease.

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We tested whether the effects of exposure to dead animals would be influenced by the evolutionary relatedness between the dead and live animals by exposing *Drosophila melanogaster* to dead individuals from one of three related species (*Drosophila virilis, Drosophila simulans*, and *Drosophila erecta*). We found...
that exposure to dead animals from the two closely related species (D. simulans and D. erecta) were able to induce aversive cues in D. melanogaster to a similar extent as did exposure to their conspecifics, while exposure to the evolutionarily more distant D. virilis did not (Fig. 2a).

**Exposure to dead conspecifics alters physiology and lifespan.**

Having observed that exposure of healthy flies to dead conspecifics consistently resulted in the production of aversive cues that repelled naive choosers, we next sought to investigate whether this treatment affected physiology and longevity in the exposed flies. We found that short-term exposure of D. melanogaster to dead conspecifics compromised starvation survival and reduced levels of triacylglycerol (TAG), which is the primary storage lipid in flies (Fig. 2b, c). It also resulted in a moderate but significant reduction in CO₂ production, indicative of an altered metabolic rate (Fig. 2d). Exposed flies were capable of normal levels of spontaneous activity and exploration (Fig. 2e), but they showed impaired motivated climbing ability (Fig. 2f). Finally, chronic exposure to dead animals significantly reduced lifespan (Fig. 2g), which was robust to experimental strain (Supplementary Fig. 3a), was sex-specific in its magnitude (Supplementary Fig. 3b), was reduced in isolation (Supplementary Fig. 3c), and was not caused by population density...
Fig. 1 Flies become aversive after exposure to dead conspecifics. a Cartoon representing the exposure protocol and binary T-maze apparatus used in our choice behavior assays. Pl = preference index calculated as (number of flies in the exposed arm (N_e) − number of flies in the unexposed arm (N_u))/total (N_c + N_u). b Flies of two different laboratory strains (Canton-S and w1118) that were exposed to dead conspecifics for 48 h were aversive to naive Canton-S choosing females (N = 9 for Canton-S and N = 7 for w1118, P < 0.001 for Canton-S and P = 0.001 for w1118). c Flies exposed to dead conspecifics retained their aversive characteristics to naive choosing flies for up to 9 min after the dead flies were removed (N = 9 for each treatment, P = 0.01 for 6 min, and P = 0.22 for 9 min, group analysis of variance (ANOVA) P < 0.001). d, e When flies were exposed to dead conspecifics, they evoked avoidance behavior in naive choosing females that was intensified with d longer periods of exposure (N = 19 for 24 h, N = 9 for 48 h, and N = 14 for 72 h, group ANOVA P = 0.027) and e the number of dead animals used during the exposure treatment (N = 6 for each treatment, P = 0.043 for 5 flies, P = 0.01 for 10 flies, group ANOVA P < 0.001). f Flies exposed to animals that died of natural or starvation-induced death, but not freezing death, evoked avoidance behaviors in naive flies (N = 8 for natural and starvation-induced death and N = 10 for frozen induced death, group ANOVA P = 0.04). g Newly dead flies effectively induced the aversive cues in exposed animals, but long dead flies did not (N = 6 for each treatment, P = 0.17 for 11 days dead and P = 0.14 for 46 days dead flies, group ANOVA P < 0.001). Except where noted in b, all naive choosing female flies were from the Canton-S strain. Each T-maze sample tests 20 flies. Error bars represent standard error of the mean (SEM). All P values were determined by non-parametric randomization (see Methods for details).

(Supplementary Fig. 3d) or by environmental structure (Supplementary Fig. 3e).

Sight of dead is necessary and sufficient for aversive cues. We hypothesized that the effects of exposure to dead animals relied on one or more sensory modalities in the healthy exposed flies. This is supported by the fact that gustatory and olfactory circuits have previously been shown to influence aging and physiology in *Drosophila*.

We therefore asked which sensory modalities were necessary for aversive cues to be triggered upon exposure to dead animals. We found that naive choosers exhibited no behavioral preference in the T-maze when the exposure to dead flies had taken place in the dark (Fig. 3a). To further demonstrate that the response is visually mediated and to assess potential interaction between light and dead flies, we repeated these experiments in our standard 12:12 h light–dark conditions using *norpA* mutant flies, which are blind. We observed no evidence of aversive cues from *norpA* mutants following exposure to dead flies (Fig. 3b).

*Orco* mutant flies are broadly anosmic, and they exhibited a significant, but not complete, loss of the aversive cues (Fig. 3c). On the other hand, flies lacking the ionotropic receptor 76b (*Ir76b*)

which is involved in chemosensory detection of amino acids and salt, exhibited normal aversion following dead exposure, as did flies lacking both *Ir8a* and *Ir25a* ionotropic receptors, which are required for multiple sensory functions

(Supplementary Fig. 4a and b). Flies carrying a *poxn* mutant allele, which have impairment in taste perception, exhibited a similar response to death exposure as genetically homogenous control flies (Fig. 3d).

We next asked whether different sensory properties of the dead flies as stimuli were sufficient to induce aversiveness in healthy flies. Using a chamber that was designed to allow flies to see dead flies but remain physically separated from them (Supplementary Fig. 5a), we found that the sight of starvation-killed flies was sufficient to induced aversive cues to the same extent as direct exposure (Fig. 3e). Using this chamber, the sight of flies that had been killed by immersion in liquid nitrogen had no effect (Fig. 3e), thus replicating our earlier results that the type of death is important for the induction of aversive cues (Fig. 1f; Supplementary Fig. 6a), and that flies killed by freezing lack one or more key visual characteristics that are present in flies that died from starvation. The ability of flies to distinguish differences by sight may also explain why live *D. melanogaster* do not convey aversive signals when exposed to dead *D. virilis*, as these flies are darker and larger than *D. melanogaster* themselves (Supplementary Fig. 6b). Interestingly, however, repeated exposure to flies killed by freezing over a 20-day (but not 10-day) period was sufficient to induce aversiveness cues, indicating that flies may eventually learn to recognize these as dead or to respond to alternative cues (Supplementary Fig. 5b, c). A second specialized chamber was used to investigate if olfactory cues (Supplementary Fig. 5d) were sufficient to induce aversion. An aversive effect was not seen in flies that were exposed to volatile odors from flies that died of starvation, indicating that olfactory cues alone were not sufficient to induce aversion (Fig. 3f). Olfactory cues from starved animals were also incapable of gating otherwise insufficient visual cues from animals that had died by freezing (Fig. 3g). Finally, aversiveness cues were not induced in flies by direct exposure to extracts from homogenized flies that had died by starvation, suggesting that gustatory cues are also not sufficient to induce aversiveness (Fig. 3h).

Vision is necessary for impaired lifespan in exposed flies. The significant reduction in lifespan that resulted from chronic exposure to dead animals was absent when flies were aged in constant darkness (Fig. 4a; Supplementary Fig. 7a). Unexposed control flies were longer-lived in constant darkness, which is consistent with an effect of death perception in cohorts aging normally in light–dark cycles. Blind *norpA* mutants also exhibited significantly reduced effects of exposure to dead flies on lifespan (Fig. 4b; Supplementary Fig. 7c). Long-term exposure to flies killed by freezing reduced lifespan (Supplementary Fig. 7b). Largely anosmic *Orco* mutant flies exhibited a partial decrease of the effect of exposure to dead flies on lifespan (Fig. 4c), while flies lacking *Ir76b* or flies lacking both *Ir8a* and *Ir25a* exhibited a decreased lifespan following exposure to dead flies to the same extent as genetically homogenous control animals (Supplementary Fig. 4c and d). Finally, *poxn* mutants also exhibited a similar decrease in lifespan in response to exposure to dead flies as control flies (Fig. 4d; Supplementary Fig. 7c).

Our findings suggest the effects of exposure to dead animals are not caused by infection, bacterial proliferation, or changes in the gut microbiota in either dead or exposed animals. Similar levels of aversion were induced when axenic flies were used as both exposed and dead flies, establishing that these factors do not play a significant role in the aversive response (Supplementary Fig. 8). It remains possible that the effects of death exposure on aversion and lifespan are, at least in part, mechanistically distinct, and we were unable to formally test the latter because lifespan exposure experiments comprised entirely of axenic animals is technically unfeasible. To date, we know of no evidence that the disparate environmental and genetic manipulations used here to reduce or eliminate the effects of exposure to dead conspecifics on lifespan affect the microbiota, but nonetheless we cannot rule this out as a potential confound.

Together, these data indicate that there is an essential perceptual component associated with the physiological and health effects of exposure to dead conspecifics. Sight of naturally dead conspecifics is both necessary and sufficient to induce
physiological effects, suggesting a model in which visual cues serve as the primary way in which D. melanogaster distinguish dead flies. While gustatory cues are not involved in the effects that we observe, the role of olfaction is less clear. Smell-deficient flies respond less strongly to dead conspecifics, but odors from dead flies are not sufficient to induce changes in aversiveness. The aversive cues emitted by flies following exposure to dead individuals do have a significant olfactory component: when Orco^2 mutants were used as naive choosers in the T-maze assay (e.g., described in Fig. 1a), they assorted randomly between the two arms (Supplementary Fig. 9). Similar results were observed when naive choosers carried a loss of function mutation for Gr63a, an essential component of the Drosophila CO2 receptor (Supplementary Fig. 9). We therefore currently favor a model in which olfaction mediates a social cue among exposed animals as a result of visual perception of dead flies.

**Changes in the head metabolome follow exposure to dead flies.**

We next asked whether we might identify a signature of this putative perceptive event by comparing the metabolome of the
homogenized heads of experimental flies following 48 h of dead exposure to that of unexposed animals. norpA mutant flies, which were treated identically but which lacked the ability to see dead flies, were analyzed simultaneously to account for temporal effects and to isolate potential causal metabolites. Targeted metabolite analyses identified 119 metabolites present in treatments for positive and negative modes (see Supplementary file for raw data). Using a randomization procedure together with principal component analysis (PCA), we identified a single principal component (PC10) that significantly distinguished the neuro-metabolomes of exposed and unexposed flies but was unchanged by exposure in norpA mutant flies (Fig. 5a; Supplementary Fig. 10a). The multivariate analysis revealed that five of the top ten metabolites associated with the effects of exposure to dead flies (i.e., those strongly loaded on PC10) have been implicated in models of anxiety, depression, and/or mood disorders in mammals (specifically lactate31, quinolinate32, sorbitol33, 3-hydroxybutyric acid34, and sarcosine35). In addition to our systems biology analysis, we also asked whether any individual metabolites were statistically correlated with exposure to dead individuals in experimental but not norpA mutant flies (i.e., exhibited a statistically significant interaction between
Fig. 3 Sensory perception is required for the induction of aversive cues following exposure to dead flies. a When flies were exposed to dead flies in the dark, they failed to evoke avoidance behavior in naive choosing females (N = 19 for light exposure and N = 20 for dark exposure). b Blind, norpA mutant flies exposed under lighted conditions also failed to induce aversive cues following exposure to dead flies (N = 8 for each treatment). c Orco\textsuperscript{2} mutant flies, which have impaired olfaction, evoked a small, but significant, avoidance behavior in choosing females following death exposure (N = 9 for Orco\textsuperscript{2} and N = 8 for control). d Flies carrying the PoxnM22-85∆X8 mutation, which have impaired taste function, exhibited a similar induction of aversive cues in response to death exposure as did control flies (N = 9 for each treatment, P = 0.002 for control and P < 0.001 for poxn). e The sight of starvation-killed flies was sufficient to induce aversive cues to the same extent as direct exposure, while the sight of flies killed by immersion in liquid nitrogen had no effect (N = 19 for direct exposure, N = 20 for vision only). f The smell of starvation-killed flies, which was provided by isolating dead animals behind a fine mesh screen, failed to induce aversive cues (N = 10 for each treatment). g The sight of flies killed by freezing in liquid nitrogen failed to induce aversive cues (frozen; vision only), and this was not affected by simultaneous smell of starvation-killed animals (N = 8 for frozen only, N = 13 forstarved andstarved + frozen). h Homogenized dead flies failed to evoke avoidance behavior in naive flies (N = 10 for ground up and control treatments). For binary choice assays, all exposed flies and naive choosing flies were from the Canton-S strain. Each T-maze sample tests 20 flies. Error bars represent standard error of the mean (SEM). P values for binary choice were determined by non-parametric randomization.

| Exposure | Light | Dark |
|----------|------|------|
| Unexposed | 0.6 | 0.6 |
| Dead exposed | 0.4 | 0.4 |

| Exposure | Control | Orco \textsuperscript{2} |
|----------|---------|----------------|
| Unexposed | 0.6 | 0.6 |
| Dead exposed | 0.4 | 0.4 |

Serotonin signaling mediates the effects of exposure to dead. Following the metabolomic analysis, we focused on pharmaco-logic compounds with anti-depressant or anti-anxiety effects with the goal of identifying molecular targets that are required for the health consequences of exposure to dead flies (Supplementary Table 1). Pirenperone is a putative antagonist of the serotonin 5-HT2 receptor in mammals, although it may interact with other biogenic amine receptors at high concentrations.\textsuperscript{36} We therefore tested whether loss of the 5-HT2A receptor recapitulated the effects of pirenperone and abrogated the effects of death perception on aversion and lifespan in Drosophila. We found that it did (Fig. 5e, f; Supplementary Fig. 10b, c), suggesting that serotonin signaling through the 5-HT2A branch is required to modulate health and lifespan in response to this perceptive experience.

Finally, we asked whether activation of 5-HT2A\textsuperscript{+} neurons was sufficient to recapitulate the aversion and lifespan phenotypes we observed following death perception. We ectopically expressed...
Fig. 5 Death perception elicits acute changes in the neuro-metabolome, and its effects on health are mitigated by manipulations that attenuate serotonin signaling. **a** Principal component plot showing the distribution of samples for each treatment. Neuro-metabolites weighted heavily in PC1 distinguish the effect of death exposure, while those favored in PC1 distinguish genotype. Plots represent mass spectrometry analysis of metabolites identified under positive mode (N = 8 biological replicates, with 40 fly heads per replicate). **b** Glyceraldehyde abundance was significantly increased in flies following death perception, but it was unchanged in blind norpA mutant flies similarly treated. **c, d** Pharmacologic treatment of Canton-S females with the serotonin receptor 5-HT2A antagonist, pirenperone, during exposure to dead conspecifics effectively protected them from the consequences of death perception on aversive cues detected by naïve choosing flies (N = 10 for each treatment) and d lifespan (N = 75 for pirenperone-fed dead exposed, N = 76 for pirenperone-fed unexposed, N = 75 for vehicle-fed dead exposed, and N = 77 for vehicle-fed unexposed, P = 0.001 for vehicle-fed and P = 0.86 for pirenperone-fed, P = 0.007 for the interaction between drug and exposure via analysis of variance (ANOVA)). **e, f** Null mutation of serotonin receptor 5-HT2A-protected flies from the consequences of death perception on e aversive cues detected by naive choosing flies (N = 15 for each treatment) and f lifespan (N = 63 for 5-HT2A+/− dead exposed, N = 78 for 5-HT2A−/− unexposed, N = 75 for Canton-S dead exposed, and N = 72 for Canton-S unexposed, P < 0.001 for Canton-S control flies and P = 0.06 for 5-HT2A−/− mutants). A replicate lifespan experiment revealed the same results (see Supplementary Fig. 5c), and P = 0.05 for the combined interaction between genotype and exposure via ANOVA. P values for principal component analysis and for binary comparisons were determined by non-parametric randomization. Each T-maze sample tests 20 flies. Error bars represent standard error of the mean (SEM). Comparison of survival curves was via log-rank test, and individual metabolites were evaluated for significance by t-test (see Methods for details).
lifespan through neural circuits that utilize conserved neuropeptides to establish motivation and reward\(^9,10,13\). Loss of specific olfactory and gustatory neurons modulates lifespan and physiology and influences measures of healthy aging, including sleep and daily activity patterns\(^28,39,40\). Our data are consistent with an additional perceptive influence that affects longevity in Dro sophila, exposure to dead conspecifics, which depends on visual and olfactory function. Not all dead flies induced such effects (flies that died by freezing or flies that were long dead or were of a distantly related species failed to do so), suggesting that flies have the perceptive ability to distinguish differences in these carcasses. These results are consistent with reports documenting sufficient visual acuity in flies to distinguish different ecologically relevant cues in their environment, such as parasites and competitors\(^41,42\). Additional experiments designed to identify the precise cues that convey information about dead conspecifics to exposed flies, and onto non-exposed counterparts, will be required.

This report adds to growing evidence that serotonin is an important component of how different sensory experiences modulate aging and aging-related disease across taxa. Serotonin modulates sensory integration in mammals\(^43\) and has been linked to the longevity effects associated with sensory perception of food\(^11,44\) and hypoxia\(^46-48\). In C. elegans, a global, cell non-autonomous response to heat is triggered by thermo-sensory neurons in a serotonin-dependent manner, which is also capable of extending lifespan\(^49\). Lifespan extension by activation of the 5-HT2A neurons via expression of UAS-TrpA1 relative to S-HT2A-GAL4;+ control flies results in a increased aversiveness (\(N = 19, P < 0.001\)) and b significantly decreased lifespan (\(N = 194\) for S-HT2A>TtpA1 and \(N = 195\) for S-HT2A-GAL4;+, \(P < 0.001\)). Each T-maze sample tests 20 flies. Error bars represent standard error of the mean (SEM). Comparison of survival curves was via log-rank test.

**Methods**

**Contact for reagent and resource sharing.** Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Scott D. Fletcher (splech@umich.edu).

**Experimental model and subject details.** The laboratory stocks w\(^{118}\) Canton-S, UAS-dTrpA1, norpA, and Ir766\(^{–/–}\) [BL51309] Drosophila lines were obtained from the Bloomington Stock Center. Poxr\(^{1M22}\)–Bd5X and Poxr\(^{Full}\) were provided by J. Alcedo\(^{56}\). Ore\(^{0}\) mutant flies were a generous gift from L. Vosshall\(^{49}\). Gr6a\(^1\) mutant flies were a gift from A. Ray. S-HT2A\(^{L00952}\) and 5-HT2A-GAL4 (3299-GAL4) flies were graciously provided by H. Dierick. Ir8a\(^{−/−}\)/Ir25a\(^{−/−}\) mutant flies were kindly provided by R. Benton. Three species of Drosophila (D. simulans, D. erecta, and D. virilis) were generously provided by P. Wittkopp. All of these strains were maintained on standard food at 25°C and 60% relative humidity in a 12:12 h light–dark cycle.

**Generation of dead flies.** Unless otherwise noted, dead flies were generated by starvation. One- to two-week-old Canton-S female flies were separated using CO\(_2\) anesthesia and transferred to vials containing 2% agar. Flies were transferred to fresh agar vials every ~3 days and dead flies were collected within 3 days of death. Vials in which dead flies stuck to the agar were not used. For the data in Fig. 1f, flies died from natural causes or were killed by rapid freezing in liquid nitrogen. Age-matched Canton-S females were used for rapid freezing.

**Short-term exposure to dead flies.** Twenty-two-week-old mated female flies were collected under light CO\(_2\) anesthesia and exposed for 48 h to 14 freshly dead female flies in standard food, where they freely interacted with the dead flies. During the 48-hour exposure period, flies were maintained in a 12:12 h light–dark cycle, except for those involving exposure in 100% darkness, which took place in a closed incubator. In both cases, flies were maintained at 25°C and 60% relative humidity. To test species specificity, experimental female flies (D. melanogaster) were exposed to each of three different species of dead Drosophila: we used 14 freshly dead female flies of D. melanogaster, D. simulans, and D. erecta, and 8 freshly dead D. virilis due to their larger size.

**Behavioral preference assays.** To generate naive choosers for preference assays, newly eclosed virgin female flies (<7 h old) were collected and transferred to standard food vials with 3 male flies per 20 females. Flies were kept at 25°C and 60% humidity, with a 12:12 h light–dark cycle for 2 days, after which they were briefly anesthetized to
remove the male flies. Mated female flies were then transferred to fresh vials for one day to recover. On day 4 post eclosion, the flies were placed into vials containing moist tissue paper for 4 h prior to their introduction into the T-maze for behavioral monitoring. Choice was measured using binary traps made from commercially available T connectors (McMaster-Carr Part Number 3532K615) with 200 µl pipette tips, which were trimmed, attached to opposite ends of the T connectors to form one-way doors that end in small collection chambers. Experimental flies that were pre-exposed to dead flies were loaded into a collection chamber with moist tissue paper in one arm of the T-connector, while unexposed flies were loaded into the opposite chamber that also contained moist tissue paper. Unless otherwise noted, 20 live flies were exposed to 14 dead animals. Modifications were made to the apparatus to test specific sensory modalities.

Vision only: Canton-S mated female flies were exposed to flies that were dead either due to starvation or to liquid nitrogen immersion where the dead were kept beneath an acrylic floor on a thin layer of agar. This apparatus ensures that only visual cues are transferred. As a control treatment, a group of flies were directly exposed to starvation-induced dead for 48 h in the absence of the acrylic barrier.

Gustatory only: Starvation-induced dead flies were homogenized and spread on top of the food prior to exposure. Mated Canton-S female flies were then exposed to either intact or ground dead flies for 2 days. Twenty naive choosing flies were introduced into the central arm of the maze, and the number of flies trapped in each arm was recorded at regular intervals. Behavioral preference was measured in a dark room under dim 660 nm red light at 24 °C with a humidifier on and in an incubator at 3.6 °C. A preference for a specific arm was calculated as follows: (number of flies in exposed arm - number of flies in unexposed arm) / (number of flies in unexposed arm + number of flies in exposed arm). The fraction of flies that participated in the experiment was calculated as: (number of flies + number of flies) / 20. Average PI values are weighted mean values among replicates with weights proportional to the number of flies in each arm. P values for all of the T-maze assays were >0.05. Experiments were replicated at least two independent times. Beads used for mock dead flies were obtained from Cospheric Innovation in Microtechnology (Catalog number: CAS-BK 1.5 mm).

TAG assays. Four-day-old, adult Canton-S female flies were collected and subsequently handled using our standard short-term exposure protocol (see above). Following the 48 h exposure to dead animals, live experimental flies and their corresponding unexposed controls were removed and transferred to chambers by aspiration. Negative geotaxis was measured using DDrop, an automated machine developed in the Pletcher laboratory that drops chambers by aspiration. Negative geotaxis was measured using DDrop, an automated machine developed in the Pletcher laboratory that drops chambers by aspiration. The sterility of the axenic media. Flies were aged for 2 weeks, during which time fresh sterile media were provided every 2–3 days. After flies were split into two groups, with half of the group given fresh, sterile agar (to generate sterile dead flies) and the other half given fresh, sterile standard fly media. Sterile dead exposures occurred in the sterile hood using sterile tools and sterile technique. Control, conventionally reared flies (non-sterile) were handled in an identical manner without the exception of washing. The sterility of the media was verified by plating the supernatant from total fly extracts onto brain heart infusion agar plates; colonies grew from the extracts of traditionally reared flies but not from the extracts of sterile flies.

CO₂ measurement. Four-day-old, adult Canton-S female flies were collected and subsequently subjected to our standard short-term exposure protocol (see above). Following the 48–3 h pre-exposure, the flies were collected immediately. The amount of CO₂ in each sample was measured using the Infinity Triglyceride reagent (Thermo Electron Corp.) according to the manufacturer’s instructions. Eight independent biological replicates (of 10 flies each) were obtained for treatment and control cohorts.

CO₂ production per group. CO₂ production values were obtained using the EXPDATA software from Sable Systems, following adjustment using a proportional baseline.

Video tracking. Mated Canton-S female flies were starved for 4 h in a vial with moist tissue paper prior to the experiment to encourage movement within the chambers. Each chamber contained two dumbbell-shaped arenas comprised of two circles (internal diameter = 1.0 in.) separated by a narrow corridor connecting them. A thin 2% layer of agar served as the floor of the chambers. Dead flies were weighed and placed into the agar of each arena to secure them. The positions of these stimuli were randomized within each experiment. Five minutes prior to recording, single exposed flies were loaded into the chambers by aspiration. Movement in each arena was recorded for 2 h in a 25 °C incubator under white light. Recordings were analyzed using the DTrack Software, developed in the Pletcher laboratory. From the tracking data, we calculated the speed, total distance traveled, and amount of time each fly spent in each side of the arena.

Feeding analysis. Feeding behavior was measured using the fly liquid interaction counter (FLIC) as described previously. Following our standard 48 h exposure treatment, individual Canton-S female flies were placed into a single FLIC chamber with two food wells, each containing a 10% sucrose solution. Two independent experimental blocks were conducted using 15 dead exposed and 15 unexposed flies per experiment, providing a total of 30 flies per treatment. The experiments were performed at constant temperature (25 °C) with 12:12 h light–dark cycle. Throughout the experiment, three dead flies were kept in the chambers assigned to the dead exposed condition. Feeding interactions with the food were measured for 24 h continuously using the FLIC reservoir system (see http://www.wiki.lifc.com). Data were analyzed using the FLIC Analysis R Source Code (available from wiki.lifc.com). Relevant feeding measures included the number of total interactions with the food, the total time spent interacting with the food, mean duration of each putative feeding event, and mean time between feeding events. These data were determined to be normally distributed, and a t test was used to determine significance. No significant differences were observed after noting the absence of significant block effects.

Axenic fly culturing. Canton-S flies were placed into cages with purple grape agar and yeast paste for approximately 18 h. Embryos were then collected with 10 ml PBS and moved into a sterile hood, where they were treated with 10 ml 1:10 sterile bleach solution (3✕ washes) and then washed with sterile water. Using sterile technique, 8 μl of embryos were aliquoted into sterile 50 ml Falcon tubes that contained 8 ml sterile standard fly media. Embryos were allowed to develop in a humidified incubator at 25 °C with 12:12 h light–dark cycles. After 12 days, axenic flies were collected in a sterile hood into fresh, sterile 50 ml Falcon tubes containing 8 ml sterile standard fly media. Flies were aged for 2 weeks, during which time fresh sterile media were provided every 2–3 days. After flies were split into two groups, with half of the group given fresh, sterile agar (to generate sterile dead flies) and the other half given fresh, sterile standard fly media. Sterile dead exposures occurred in the sterile hood using sterile tools and sterile technique. Control, conventionally reared flies (non-sterile) were handled in an identical manner without the exception of washing. The sterility of the media was verified by plating the supernatant from total fly extracts onto brain heart infusion agar plates; colonies grew from the extracts of traditionally reared flies but not from the extracts of sterile flies.

Metabolic analysis. Following 48 h of dead fly exposure, experimental flies were quickly frozen in a dry ice bath, and stored at −80 °C overnight. Heads were removed via vortexing and manually separated from the body parts. Forty heads were then homogenized for 20 s in 200 μl of a 1:4 (vv) water–MeOH solvent mixture using the Fast Prep 24 (MP Biomedicals). Following the addition of 800 μl of methanol, the samples were incubated for 30 min on dry ice, and then homogenized again. The mixture was spun at 13,000 RPM for 5 min at 4 °C, and the soluble extract was collected into vials. This extract was then dried in a speedvac at 30 °C for approximately 3 h. Using a LC-QQQ-MS machine in the MRM mode, we targeted ~200 metabolites in 25 important metabolic pathways, in both positive and negative MS modes. After removing any metabolites missing from more than 5 out of 32 samples (15%), we were left with 119 metabolites. Metabolite abundance was determined by plating the supernatant from total fly extracts onto brain heart infusion agar plates; colonies grew from the extracts of traditionally reared flies but not from the extracts of sterile flies.
the number of groups and $n_i$ indicates the number of samples in group $i$. The distribution of $Z$-score was obtained from 10,000 randomized datasets. $P$-values that were significantly deviated from this randomized distribution were considered as a significant separation of groups.

To identify individual metabolites of interest that are likely to be associated with death exposure, we sorted them per loading on PC10 and selected the top 10. For these candidates, we looked for metabolites that were ($i$) significantly different between control flies (dead vs. live, no legs) and flies with no exposure and were not (ii) significantly different in norpA mutant flies. We found glyceraldehyde significantly up-regulated upon death exposure in Canton-S flies (one-sided Student’s $t$ test, $P = 0.007$), whereas such differences were not significant in norpA flies (one-sided Student’s $t$ test, $P = 0.07$).

Survival experiments. For lifespan experiments, experimental and control flies were reared under controlled larval density and collected as adults within 24 h of emergence onto standard food where they were allowed to mate freely for 2–3 days. At 3 days post eclosion, female flies were transferred to standard food vials and kept in the absence of dead flies. Canton-S flies were pretreated with 1 mM pirenperone or an equivalent dilution of DMSO for 2 weeks to up-regulate 5-HT2A receptors. All drugs were purchased from Sigma-Aldrich. Each drug was dissolved in water to a final concentration. A similar dilution of DMSO alone was made in water as a control. Then, 100 µl of the diluted drug or vehicle control was added to each vial, coating the top of the food surface. After the liquid evaporated (~2 h), the vials were loaded with 10 fully grown flies. For the behavior assay, flies were pre-treated with 1 mM pirenperone or an equivalent dilution of DMSO for 2 weeks prior to exposure, in the absence of dead flies.

5-HT2A neuron activation methods. 5-HT2A-GAL4 (3299-GAL4, H. Dietrich, BCM) flies were crossed to UAS-dTrpA1 to generate 5-HT2A-dTrpA1 flies. UAS-dTrpA1 was backcrossed for at least 10 generations to w1118 and then 5-HT2A-GAL4;~ was used as a genetic control strain. For behavioral assays, progeny from both crosses were maintained at 18 °C throughout development. Following eclosion, females were mated for 3 days, separated by gender, and placed at 29 °C to begin lifespan measurement.

Bacterial infection with P. aeruginosa. The PA14 psc strain used in this study was obtained from L. Rahe (Harvard Medical School). For each experiment, a glycerol culture reached logarithmic phase. Subsequently, the culture was diluted in 25 ml of LB/gentamicin. A single colony was picked and grown in 1 ml of LB/gentamicin until this seed culture reached logarithmic phase. Subsequently, the culture was diluted in 25 ml of LB/gentamicin and grown until the desired Abio concentration was reached. Finally, the bacterial culture was centrifuged and the pellet resuspended in LB media to obtain an Abio reading of 100. The culture was kept on ice during infection. Needles were directly placed in the concentrated bacterial solution and then poked into the fly abdomen. After infection, flies were transferred to standard food vials and kept in the incubator at 25 °C and 60% humidity. Flies were collected 24 h post infection for behavioral experiments. Infected flies were loaded in one arm of the T connectors and control flies (not infected with P. aeruginosa) were loaded into the opposite arm. Twenty naive choosing flies were introduced into the central arm of the maze and the number of flies in each arm of the trap was counted at regular intervals.

Quantification and statistical analysis. For all preference assays, $P$ values comparing the PI among treatments was obtained using a randomization procedure and the statistical software R. Briefly, the null distribution of no difference among treatments was obtained by randomizing individual PIs obtained from groups of 20 flies among all measurements maintaining the block structure when appropriate) and 100,000 $t$-statistics (or $F$-statistics for multiple comparisons). $P$ values (one-sided or two-sided as appropriate) were determined by computing the fraction of null values that were equal or more extreme to the observed $t$-statistic (or $F$-statistic). Mean preference values were plotted and weighted by the number of choosing flies in each trial, with the error bars representing either the standard error of the mean (SEM). Experiment-wise error rates for experiments comparing three or more treatments were protected by presentation of treatment $P$ value from non-parametric, randomization ANOVA, which are reported in the figure legends when appropriate. For lifespan and starvation assays, we employed survival analysis. Unless otherwise indicated, groupwise and pairwise comparisons among survivorship curves (both lifespan and starvation) were performed using the DLIfe computer software and the statistical software R. $P$ values were obtained using the log-rank analysis (select pairwise comparisons and group comparisons or interaction studies) as noted. Interaction $P$ values were calculated using Cox regression when the survival data satisfied the assumption of proportional hazards. In other cases (as noted in the figure legends), we used ANOVA to calculate $P$ values for the interaction term for age at death. For all box plots, the box represents SEM (centered on the mean), and whiskers represent 10%/90%. For CO2, TAG, and negative geotaxis measures, $P$ values were obtained by standard two-sided t test after verifying normality and equality of variances. Details of the metabolomics analysis are presented above.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Metabolomics data and analyses are provided as Supplementary File 1. All additional data and analysis scripts that support the findings of this study are available from the corresponding author on request.

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References

1. Apfeld, J. & Kenyon, C. Regulation of lifespan by sensory perception in Caenorhabditis elegans. Nature 402, 804–809 (1999).
2. Riera, C. E. & Dillin, A. Emerging role of sensory perception in aging and metabolism. Trends Endocrinol. Metab. 27, 294–303 (2016).
3. Riera, C. E. et al. TRPV1 pain receptors regulate longevity and metabolism by neuromodulation signaling. Cell 157, 1023–1036 (2014).
4. Waterson, M. J., Chan, T. P. & Fletcher, S. D. Adaptive physiological response to perceived scarcity as a mechanism of sensory modulation of life span. J. Gerontol. A 70, 1088–1091 (2015).
5. Fletcher, M. & Kim, D. H. Age-dependent neuroendocrine signaling from sensory neurons modulates the effect of dietary restriction on longevity of Caenorhabditis elegans. PLoS Genet. 13, e1006544 (2017).
6. Linford, N. J., Chan, T. P. & Fletcher, S. D. Reformating sleep architecture in Drosophila through gustatory perception and nutritional quality. Curr. Biol. 8, e1006688 (2012).
7. Snoddi, B., Brynem, M., Kreibich, C. D. & Amdam, G. V. Brood pheromone suppresses physiology of extreme longevity in honeybees (Apis mellifera). J. Exp. Biol. 212, 3795–3801 (2009).
8. Libert, S. et al. Regulation of Drosophila life span by olfaction and food-derived odors. Science 315, 1133–1137 (2007).
9. Gendron, C. M. et al. Drosophila life span and physiology are modulated by sexual perception and reward. Science 343, 544–548 (2014).
10. Harvanek, Z. M. et al. Perceptive costs of reproduction drive ageing and physiology in male Drosophila. Nat. Ecol. Evol. 1, 152 (2017).
11. Ro, J. et al. Serotonin signaling mediates protein interaction during aging. Elife 5, https://doi.org/10.7554/elife.16843 (2016).
12. Shi, C. & Murphy, C. T. Matting induces shrinking and death in Caenorhabditis mothers. Science 343, 536–540 (2014).
13. Maures, T. J. et al. Males shorten the life span of C. elegans hermaphrodites via secreted compounds. Science 343, 541–544 (2014).
14. Choe, D. H., Millar, J. G. & Rust, M. K. Chemical signals associated with life inhibit neurodegeneration in Argentine ants. Proc. Natl. Acad. Sci. USA 106, 8253–8257 (2009).
15. Oliveira, T. A. et al. Death-associated odors induce stress in zebrafish. Horm. Behav. 65, 340–344 (2014).
16. Iglésias, T. L., McElreath, R. & Patricelli, G. L. Western scrub-jay funerals: cacophonous aggregations in response to dead specifics. Anim. Behav. 84, 1103–1111 (2012).
17. Biro, D. et al. Chimpanzee mothers at Bossou, Guinea carry the mumified remains of their dead infants. Curr. Biol. 20, R351–R352 (2010).
18. Anderson, J. R. Comparative thanatology. Curr. Biol. 26, R533–R536 (2016).
19. Keyes, K. M. et al. The burden of loss: unexpected death of a loved one and psychiatric disorders across the life course in a national study. Am. J. Psychiatry 171, 864–871 (2014).
20. Marmar, C. R. et al. Predictors of posttraumatic stress in police and other first responders. Ann. NY Acad. Sci. 1071, 1–18 (2006).
21. Alexander, D. A. & Klein, S. First responders after disasters: a review of stress reactions, at-risk, vulnerability, and resilience factors. Prehosp. Disaster Med. 24, 87–94 (2012).
22. McAfee, A. et al. A death pheromone, oleic acid, triggers hygienic behavior in honey bees (Apis mellifera L.), Sci. Rep. 8, 5719 (2018).
23. Wisman, A. & Selitrennikoff, J. The smell of death: evidence that putrescine elicits threat management mechanisms. Front. Psychol. 6, 1274 (2015).
24. M. Y. et al. The ancient chemistry of avoiding risks of predation and disease. Evol. Biol. 36, 267–281 (2009).
25. Suh, G. S. et al. A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila. Nature 431, 854–859 (2004).
26. Keene, A. C. & Waddell, S. Drosophila olfactory memory: single genes to complex neural circuits. Nat. Rev. Neurosci. 8, 341–354 (2007).
27. Ostojic, I. et al. Positive and negative gustatory inputs affect Drosophila lifespan partly in parallel to dFOXO signaling. Proc. Natl. Acad. Sci. USA 111, 8143–8148 (2014).
28. Waterson, M. J. et al. Water sensor prk28 modulates Drosophila lifespan and physiology through AKH signaling. Proc. Natl. Acad. Sci. USA 111, 8137–8142 (2014).
29. Zhang, G. et al. Hypothalamic programming of systemic ageing involving IKK-beta, NF-kappaB and GmRH. Nature 497, 211–216 (2013).
30. Abun, L. et al. Functional architecture of olfactory ionotropic glutamate receptors. Neuron 69, 46–46 (2010).
31. Ernst, J. et al. Increased pregenual anterior cingulate glucose and lactate concentrations in major depressive disorder. Mol. Psychiatry 22, 113–119 (2017).
32. Miynt, A. M. Kynurenines: from the perspective of major psychiatric disorders. FERS J. 279, 1375–1385 (2012).
33. Liu, M. L. et al. GC-MS based metabolomics identifies of possible novel biomarkers for schizophrenia in peripheral blood mononuclear cells. Mol. Biosyst. 10, 2398–2406 (2014).
34. Yin, J. X. et al. Ketones block amyloid entry and improve cognition in an Alzheimer’s model. Neurobiol. Aging 39, 25–37 (2016).
35. Woo, H. I. et al. Plasma amino acid profiling in major depressive disorder treated with selective serotonin reuptake inhibitors. CNS Neurosci. Ther. 21, 417–424 (2015).
36. Pawlowski, L., Siwanowicz, J., Bigajsk, K. & Przegalinski, E. Central antiserotonergic and antidopaminergic action of pirenperone, a putative 5-HT2 receptor antagonist. Pol. J. Pharm. Pharmac. 37, 179–196 (1985).
37. Hamada, F. N. et al. An internal thermal sensor controlling temperature preference avoidance in Drosophila. Nature 454, 217–220 (2008).
38. Smith, E. D. et al. Age- and calorie-independent life span extension from dietary restriction by bacterial deprivation in Caenorhabditis elegans. BMC Dev. Biol. 8, 49 (2008).
39. Linford, N. J., Ro, J., Chung, B. Y. & Pletcher, S. D. Gustatory and metabolic perception of nutrient stress in Drosophila. Proc. Natl. Acad. Sci. USA 112, 2587–2592 (2015).
40. Alcedo, J. & Kenyon, C. Regulation of C. elegans longevity by specific gustatory and olfactory neurons. Neuron 41, 45–55 (2004).
41. Schneider, J., Murali, N., Taylor, G. W. & Levine, J. D. Can Drosophila melanogaster tell who’s who? PLoS ONE 13, e0205043 (2018).
42. Koziol, B. Z., Lynch, Z. R., Mortimer, N. T. & Schlenke, T. A. Fruit flies mediate offspring after seeing parasites. Science 339, 947–950 (2013).
43. de la Flor, M. et al. Drosophila increase exploration after visually detecting predators. PLoS ONE 12, e0180749 (2017).
44. Hanson, J. L. & Hurley, L. M. Context-dependent fluctuation of serotonin in the auditory midbrain: the influence of sex, reproductive state and experience. J. Exp. Biol. 217, 535–545 (2014).
45. Chao, M. Y., Komatsu, H., Fukuto, H. S., Dionne, H. M. & Hart, A. C. Feeding status and serotonin rapidly and reversibly modulate a Caenorhabditis elegans chemosensory circuit. Proc. Natl. Acad. Sci. USA 101, 15512–15517 (2004).
46. Leiser, S. F. et al. Cell nonautonomous activation of flavin-containing monooxygenase promotes longevity and health span. Science 350, 1375–1378 (2015).
47. Petrascheck, M., Ye, X. & Buck, L. B. A high-throughput screen for chemicals that increase the lifespan of Caenorhabditis elegans. Ann. N Y Acad. Sci. 1170, 698–701 (2009).
48. Petrascheck, M., Ye, X. & Buck, L. B. An antidepressant that extends lifespan in adult Caenorhabditis elegans. Nature 450, 553–556 (2007).
49. Tatum, E. C. et al. Neuronal serotonin release triggers the heat shock response in C. elegans in the absence of temperature increase. Curr. Biol. 25, 163–174 (2015).
50. Curran, K. F. & Chalasani, S. H. Serotonin circuits and anxiety: what can invertebrates teach us? Invert. Neurosci. 12, 81–92 (2012).
51. Mohammad, F. et al. Ancient anxiety pathways influence Drosophila defense behaviors. Curr. Biol. 26, 981–986 (2016).
52. Ries, A. A., Hermanns, T., Poeck, B. & Strauss, R. Serotonin modulates a depression-like state in Drosophila responsive to lithium treatment. Nat. Commun. 8, 15738 (2017).
53. Jiang, M. D., Zheng, Y., Wang, J. L. & Wang, Y. F. Drug induces depression-like phenotypes and alters gene expression profiles in Drosophila. Brain Res. Bull. 122, 222–231 (2017).
54. O’Kane, C. J. Drosophila as a model organism for the study of neuropsychiatric disorders. Curr. Top. Behav. Neurosci. 7, 37–60 (2011).
55. Kato, K., Zweig, R., Schechter, C. B., Barzilai, N. & Atzmon, G. Positive attitude toward life, emotional expression, self-rated health, and depressive symptoms among centenarians and near-centenarians. Aging Ment. Health 20, 930–939 (2016).
56. Delio Buono, M., Urciuoli, O. & De Leo, D. Quality of life and longevity: a study of centenarians. Age Aging 27, 207–216 (1998).
57. Zaninotto, P., Wardle, J. & Steptoe, A. Sustained enjoyment of life and mortality at older ages: analysis of the English Longitudinal Study of Ageing. BMJ 355, i6267 (2016).
58. Anderson, D. J. & Adolphs, R. A framework for studying emotions across species. Cell 157, 187–200 (2014).
59. Boll, W. & Noll, M. The Drosophila Pox neuro gene: control of male courtship behavior and fertility as revealed by a complete dissection of all enhancers. Development 129, 5687–5688 (2002).
60. Pletcher, S. D. & Vosshall, L. B. Atypical Membrane Topology and Heteromeric Function of Drosophila Odorant Receptors In Vivo. PLoS Biol. 4, e20 (2006).
61. Kuo, T. H. et al. Insulin signaling mediates sexual attractiveness in Drosophila. PLoS Genet. 8, e1002684 (2012).
62. Linford, N. J., Ro, J., Harvanek, Z. M. & Pletcher, S. D. FLIC: high-throughput, continuous analysis of feeding behaviors in Drosophila. PLoS ONE 9, e101107 (2014).
63. Linford, N. J., Bülgr, C., Ro, J. & Pletcher, S. D. Measurement of lifespan in Drosophila melanogaster. J Vis Exp, https://doi.org/10.3791/50068 (2013).

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Author contributions
T.S.C., C.M.G., A.S.M., and S.D.P. designed the experiments. T.S.C., C.M.G., A.S.M., M. D., and Z.W.H. performed the experiments. C.M.G., A.S.M., Y.L., and S.D.P. analyzed the data. T.S.C., C.M.G., Y.L., and S.D.P. wrote the manuscript.

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