Gustatory and metabolic perception of nutrient stress in *Drosophila*

Nancy J. Linford[a], Jennifer Ro[b], Brian Y. Chung[a], and Scott D. Pletcher[a,c,1]

[a]Department of Molecular and Integrative Physiology, [b]Cellular and Molecular Biology Program, and [c]Geriatrics Center, University of Michigan, Ann Arbor, MI 48109

Edited by Michael Rosbash, Howard Hughes Medical Institute, Brandeis University, Waltham, MA, and approved January 21, 2015 (received for review February 14, 2014)

Sleep loss is an adaptive response to nutrient deprivation that alters behavior to maximize the chances of feeding before imminent death. Organisms must maintain systems for detecting the quality of the food source to resume healthy levels of sleep when the stress is alleviated. We determined that gustatory perception of sweetness is both necessary and sufficient to suppress starvation-induced sleep loss when animals encounter nutrient-poor food sources. We further find that blocking specific dopaminergic neurons phenocopies the absence of gustatory stimulation, suggesting a specific role for these neurons in transducing taste information to sleep centers in the brain. Finally, we show that gustatory perception is required for survival, specifically in a low nutrient environment. Overall, these results demonstrate an important role for gustatory perception when environmental food availability approaches zero and illustrate the interplay between sensory and metabolic perception of nutrient availability in regulating behavioral state.

sleep | *Drosophila* | activity | sensory perception | feeding

Starvation is a condition of extreme nutrient stress that leads to rapid death. On detecting the absence of environmental nutrient sources, organisms use multiple strategies to adjust resource allocation to maximize the chances of finding a food source, including inducing longer foraging searches (1) and limiting sleep behavior (2, 3). Sleep loss in *Drosophila melanogaster* is a characteristic response to nutrient deprivation that appears ∼12 h after the removal of a food source; in males, it is followed by death in another 12 h (2). Sleep loss is thought to represent a cost to the organism (4–6), and mechanisms for evaluating the environment and terminating this behavioral response when food is available would likely confer an adaptive benefit. A deeper understanding of how organisms perceive and respond to environmental stress could offer substantial benefit to humans attempting to maintain maximal health in the face of food shortages and unstable environmental conditions. The strategies used by organisms to evaluate the sufficiency of a food source and to initiate or suppress sleep loss under very low nutrient conditions remain largely unknown and represent one path toward understanding global stress response.

Results

We have observed, as previously reported, that adult *Drosophila* spontaneously adjust their behavioral patterns to reduce sleep when starved (Fig. 1*A*) (2, 7). To more completely understand how organisms modulate sleep in response to food availability, we measured the extent of nutrient deprivation required to induce sleep loss. These assays consist of monitoring the activity of male Canton-S flies on a complete medium (10% sugar:Brewer's yeast medium; *Materials and Methods*) for 1 d (day 1) to estimate baseline sleep in the fully fed condition, followed by data collection from 1 or more days on a 1% agar-only starvation medium (day 2+), which provides water and humidity but not nutrients. We modified this procedure by augmenting the agar medium with either 50 mM D-glucose or 550 mM D-glucose. Interestingly, 50 mM D-glucose was sufficient to promote normal sleep patterns (Fig. 1*B*), despite evidence that the animals remained in a state of severe malnourishment (Fig. 1*C*). Indeed, median survival on 50 mM D-glucose medium was only 5.1 d, which was only slightly longer than complete starvation (1.1 d) and substantially less than 550 mM D-glucose (21.2 d; Fig. 1*C*). The discordance between the amount of nutrients apparently required to support life and the amount sufficient to eliminate starvation-induced sleep loss suggested that the animals may be capable of regulating sleep through evaluations of the food source that are independent of its energetic value.

Previous work has established that sensory perception of the food source influences sleep architecture but not total sleep in fully fed *Drosophila* (8), and we wondered whether it also regulates sleep loss in response to nutrient stress. We tested modulatory roles for gustatory perception using a mutant line containing a deletion of the *Gr64a-f* sweet-sensing receptor gene cluster (*ΔGr64*) (9), which has been previously reported to suppress proboscis extension in response to glucose, as well as to several other sweet tastants (trehalose, arabinose, maltose, sucrose, and glycerol) but not to fructose. Concentrations of D-glucose that promoted normal sleep behavior in control (*ΔGr64*/+) heterozygous flies were ineffective in flies homozygous for the *ΔGr64* mutation (Fig. 2*A*), with the defect particularly apparent at low nutrient concentrations. This defect was fully rescued by *Gal4-UAS*-based rescue of the *ΔGr64* deletion (Fig. 2a) or by transgenic expression of an ectopic copy of the *Gr64a-f* genomic region (Figure S1a). We note that high concentrations of D-glucose still promoted sleep in *ΔGr64* mutant flies, albeit not to the extent observed in control animals, suggesting that taste perception may be particularly important at low nutrient concentrations. There

Significance

Starvation induces a suite of costly behavioral and metabolic responses to maximize the possibility of finding a new nutrient source. It is therefore advantageous for an organism to switch out of the starvation state when food is encountered. How does an animal know when a food source is found and ultimate starvation is unlikely? This new work addresses this fundamental question, which is essential for broadening our understanding of how organisms interpret information from their environment to enact changes in complex behaviors and physiology. We describe an intriguing mechanism that combines information from both sensory and metabolic perception depending on the nutrient density in the food source.

Author contributions: N.J.L. and S.D.P. designed research; N.J.L., J.R., and B.Y.C. performed research; N.J.L., J.R., B.Y.C., and S.D.P. analyzed data; and N.J.L. and S.D.P. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. Email: spletch@umich.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1401501112/-/DCSupplemental.

NEUROSCIENCE

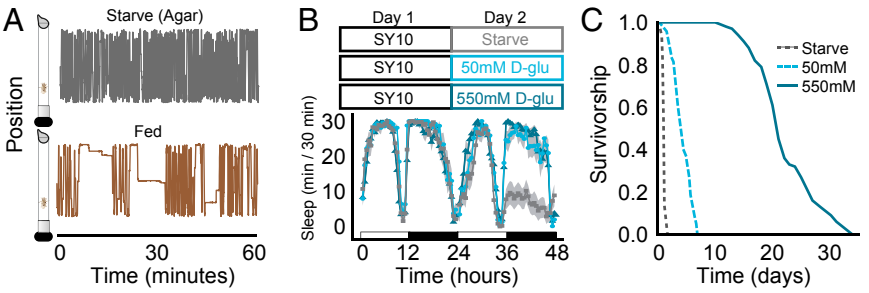

Fig. 1. Sleep behavior is regulated by nutrient availability. (*A*) Sample video trace of the fly (Canton S male) position over time on a complete food (10% sugar:yeast, SY10) or after 20-h starvation. The *y* axis represents the fly position in a tube resting horizontally with a video camera above. (*B*) Sleep behavior (30-min bins) during day 1 on SY10 food followed by day 2 on the indicated test medium. (*C*) Survival on starvation medium or the indicated amount of D-glucose. All points with error bars represent the mean ± SEM from 30 to 100 flies.

are multiple reports of a gustatory-independent nutrient sensor that regulates behavior and food preference under starvation conditions (8, 10–13), and it is likely that this sensor compensates for lack of gustatory perception to promote normal sleep behavior when nutrients are replete.

To ensure that the sleep loss phenotype observed in *ΔGr64* mutant flies is due to their failure to taste glucose rather than a deficiency in their metabolic response to nutrients in general, we repeated the experiments, replacing D-glucose with fructose, which has roughly the same caloric value but can be perceived by *ΔGr64* mutant flies (8, 9, 14, 15). Consistent with taste as a causal factor, *ΔGr64* mutants exhibited a normal pattern of sleep behavior that was statistically indistinguishable from the control and genetic rescue lines (Fig. 2*A* and Fig. S1*B*). Together, these data suggest that behavioral decisions affecting sleep regulation rely heavily on gustatory perception when nutrients are scarce.

One alternative interpretation of the data presented thus far is that sleep loss is not regulated per se but instead occurs as flies approach death—at any given point in time, a greater proportion of flies in low nutrient environments will be near death than their better-fed siblings. Data from flies carrying the *ΔGr64* mutation effectively refute this hypothesis. On their first day of 50 mM D-glucose feeding, *ΔGr64* flies exhibit nearly identical sleep loss to levels observed using starved flies (either *ΔGr64* or control; Fig. 2*B*). However, the fraction of each population that is predicted to be near death is very different; 100% of the starved

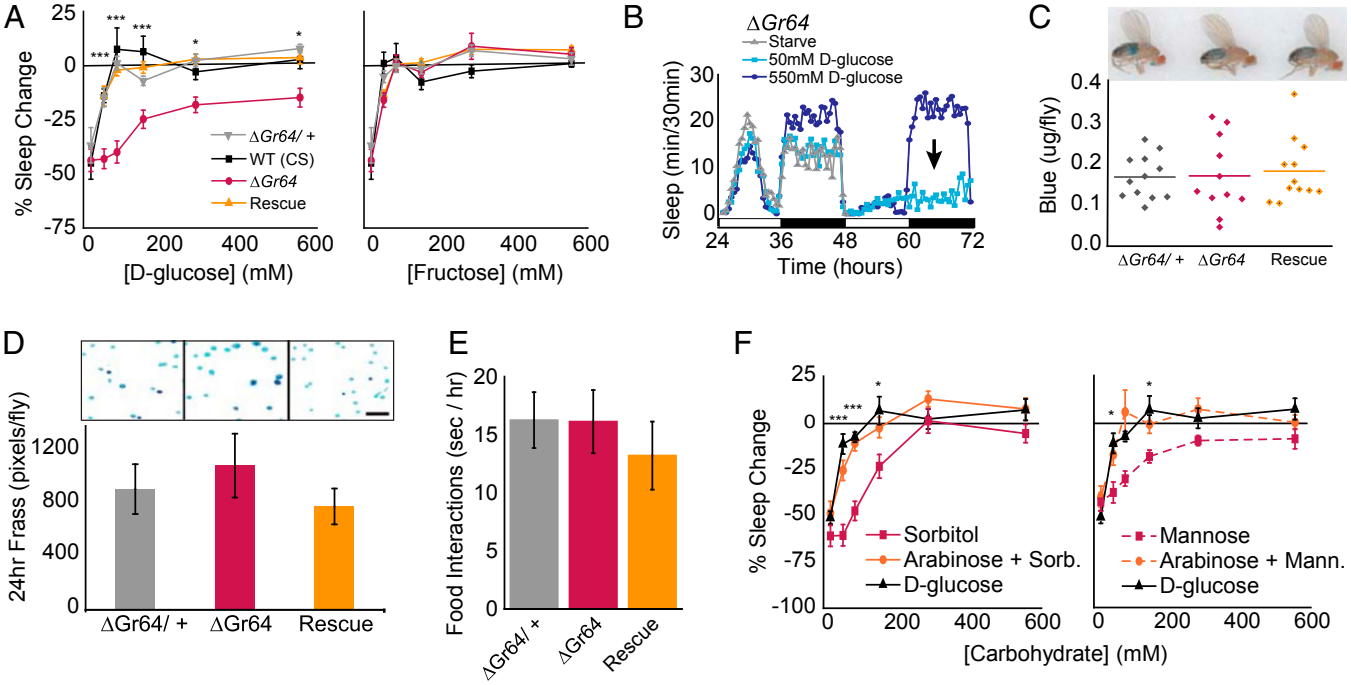

Fig. 2. Appetitive gustatory perception is required for low environmental nutrient density to promote sleep. (*A*) Deletion of the *Gr64a-f* gene cluster significantly impaired normal sleep behavior on a D-glucose but not fructose medium. Where noted, the *ΔGr64* mutant was significantly different from all other groups with no differences between the other groups. For *A* and *F*, all points with error bars represent the mean ± SEM from 30 to 100 flies. Statistical significance was determined by one-way ANOVA with a Tukey posttest. (*B*) Sleep behavior (30-min bins) for *ΔGr64* on the test day (24–48 h) and the following day. The arrow indicates the persistence of sleep loss on 50 mM D-glucose. (*C*) Abdominal food content after 2 h on 50 mM D-glucose containing 0.5% FD&C Blue #1. $P = 0.89$, one-way ANOVA. (*D*) Frass production during the test day on 50 mM D-glucose containing 0.5% FD&C Blue #1. $n = 3$ vials of 15 flies. (Scale bar, 1 mm.) $P = 0.56$, one-way ANOVA. (*E*) Food interactions during day 2 for flies exposed to 50 mM D-glucose in liquid. $n = 4$–6 flies per group. $P = 0.43$, one-way ANOVA. (*F*) Sleep change when flies (Canton-S) were fed the indicated concentrations of carbohydrates that were nutritious but nonsweet (sorbitol or mannose) relative to D-glucose and a dietary rescue with the addition of arabinose (Table S1). Where noted, sorbitol or mannose was significantly different from the other groups with no differences between groups. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. See also Fig. S1.

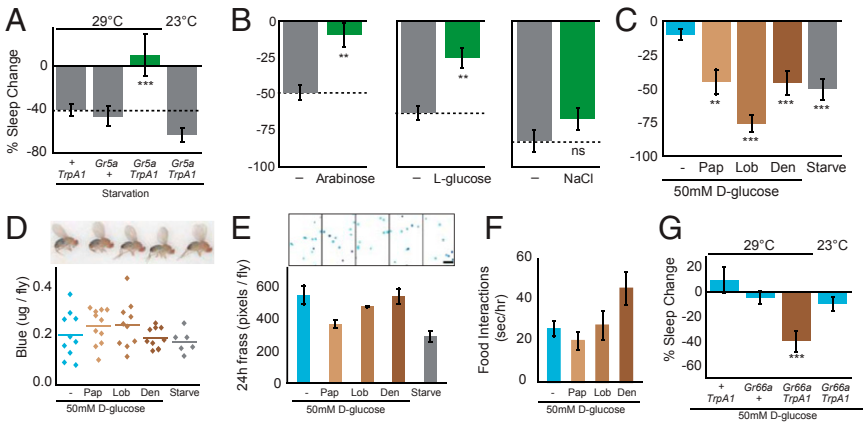

Fig. 3. Appetitive gustatory perception is sufficient to prevent sleep loss. (*A*) Activation of *Gr5a*-containing sweet-sensing neurons with *Gr5a-Gal4/UAS-TrpA1* (29 °C) on 1% agar starvation medium was sufficient to promote an increase in sleep relative to each of the control groups. One-way ANOVA with Tukey's posttest, $n = 30–100$. (*B*) Feeding with sweet nonnutritious compounds (250 mM arabinose or L-glucose) but not a salty compound (100 mM NaCl) was sufficient to promote an increase in sleep relative to starvation. Student *t* test. Each compound is compared with its contemporaneous control, $n = 30–100$. (*C*) Addition of 1 mM of the bitter tastants papaverine (Pap), lobeline (Lob), and denatonium (Den) to 50 mM D-glucose caused sleep loss relative to glucose alone. One-way ANOVA with Tukey's posttest, $n = 30–100$. (*D*) Abdominal food content following 2 h on the indicated test food containing 0.5% FD&C Blue #1. $P = 0.27$, one-way ANOVA. (*E*) Frass production on the test day for the indicated foods containing 0.5% FD&C Blue #1 relative to glucose alone. $n = 3$ vials of 15 flies. (Scale bar, 1 mm.) One-way ANOVA with Tukey's posttest. (*F*) Total time of interactions between individual flies and the indicated foods. Adding a bitter tastant to 50 mM D-glucose did not change flies' tendency to interact with a food. One-way ANOVA with Tukey's posttest, $n = 9–16$ flies per group. Flies' interaction with a food containing Pap was significantly lower than a food containing Den ($P = 0.034$). (*G*) Activation of *Gr66a*-containing bitter-sensing neurons with *Gr66a-Gal4/UAS-TrpA1* (29 °C) on 50 mM D-glucose was sufficient to promote a decrease in sleep relative to all of the control groups. One-way ANOVA with Tukey's posttest, $n = 30–100$. Summary data are expressed as the mean ± SEM. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. See also Fig. S2.

population will die in the subsequent 24 h, whereas less than 5% of 50 mM-fed mutants will do so (see also Fig. 5). Furthermore, if sleep loss was strongly coupled to death, we would expect to observe its onset much later in the *ΔGr64* mutant animals on 50 mM D-glucose compared with starved controls, which does not happen (Fig. 2*B*). The sustained sleep loss in the *ΔGr64* mutant flies, therefore, is consistent with a model where the animals regulate sleep based on the perception of food.

A second alternative explanation is that *ΔGr64* mutant flies respond normally to a given amount of nutrient intake but are effectively "self-starving" through an undocumented effect of the mutation on feeding behavior. To test this model, we estimated food intake in three ways. First, we replaced the test medium with a blue dye-labeled medium for 2 h near the end of the assay, which corresponds to the period of the circadian day when food intake is thought to be maximal (16). There was no difference in the amounts of blue dye between mutant and control flies (Fig. 2*C*). Second, we measured 24-h fecal deposition by preloading the flies on blue dye during the pretreatment period on complete medium and measuring blue frass (excrement) deposits on the side of the vial during a 24-h exposure to the 50 mM D-glucose test medium, on which the mutant phenotype was most prominent (17). There was no difference in the total deposition per fly (Fig. 2*D*). Third, we used a novel assay [the fly liquid food interaction counter (FLIC)] that quantifies food interactions by allowing the fly to complete a low-voltage electrical circuit by touching or consuming a liquid food while standing on a metal base (18). We found that there was no difference between the *ΔGr64* and control flies in the amount of time spent interacting with the food ($P = 0.56$; Fig. 2*E*).

The abnormal sleep response profile observed in *ΔGr64* mutants can be recapitulated in WT (Canton S) animals using alternative nutrient sources that offer nutrition without stimulating gustatory sensilla (sorbitol and mannose) (11–13, 19). We found that providing carbohydrate nutrition without sweetness in the feeding medium led to a response profile that largely phenocopied the concentration-dependent sleep response of the gustatory mutant, with "starvation-like" sleep loss at low environmental nutrient concentrations relative to control groups exposed to D-glucose (Fig. 2*F* and Table S1). This defect was fully rescued by the addition of a nonnutritive sweetener (arabinose or L-glucose) (11–13) to the feeding medium in combination with either mannose or sorbitol (Fig. 2*F*, Fig. S1*C*, and Table S1). As before, these results were not driven by differences in food uptake; we confirmed the presence of blue dye in the abdomens of flies exposed to all concentrations of mannose and sorbitol (Fig. S1*D*). These results further support the notion that appetitive gustatory perception is required to promote normal sleep behavior, particularly when environmental nutrient availability is low.

Having established that gustatory perception is required to promote normal sleep behavior in the presence of nutrients, we next asked whether appetitive signals alone are sufficient to prevent starvation-induced sleep loss. To simulate sweet taste in the absence of nutrients, we expressed the temperature-sensitive activating ion channel TRPA1 (20) under control of the *Gr5a-GAL4* driver, which is broadly expressed in sweet-sensing neurons (21). We found that this manipulation eliminated starvation-induced sleep loss (Fig. 3*A*) when the neurons are activated (29 °C) but not in control, nonactivating conditions (23 °C). Neuronal activation only during the daytime period when flies are most actively feeding recapitulated the reversal of sleep loss observed when those same neurons were activated continuously during the 48 h of starvation (Fig. S2). We also tested two sweet but nonnutritional sugars, arabinose and L-glucose, and both significantly suppressed sleep loss (81% and 60%, respectively; Fig. 3*B*, Fig. S3 *A–D*, and Table S1). On the other hand, salt (NaCl, 100 mM) had no significant effect (19%; Fig. 3*B* and Fig. S3 *A* and *B*). We conclude that gustatory perception of sweetness is sufficient to promote normal sleep in the absence of available nutrients, even when death is imminent (Fig. S3*D*).

If gustatory perception of sweetness regulates sleep behavior, we would anticipate that the gustatory perception of bitterness (22, 23), which has been shown to counteract sweet-responsive phenotypes, would block the ability of sweet taste inputs to promote sleep. We found that addition of 1 mM denatonium,

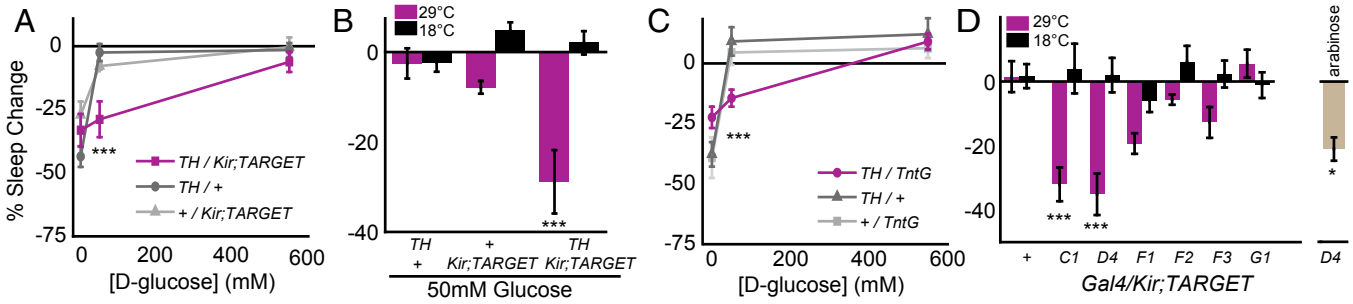

Fig. 4. Blockade of dopaminergic neurotransmission phenocopies loss of appetitive gustatory perception. (*A* and *B*) Blockade of TH-expressing neurons with *TH-Gal4/UAS-Kir$^{2.1}$;tub-Gal80^{ts}* (TARGET) at 29 °C or (*C*) constitutive blockade using *TH-GAL4/UAS-TntG* at 25 °C suppressed sleep at 50 mM D-glucose relative to genetic controls at that glucose concentration. (*D*) Sleep loss on 50 mM D-glucose or 200 mM arabinose (where indicated) in the presence of dopaminergic subset Gal4 drivers with *UAS-Kir$^{2.1}$; TARGET* relative to the *UAS-Kir$^{2.1}$; TARGET* alone control. **P* < 0.05; ***P* < 0.01; ****P* < 0.001, one-way ANOVA with Tukey's posttest. All data are expressed as the mean ± SEM from 30 to 100 flies. See also Fig. S3.

lobeline, or papaverine (24) to 50 mM D-glucose substantially blocked the capacity for this sugar to suppress starvation-induced sleep loss (Fig. 3*C*). As before, we ruled out feeding differences as the cause for these observations. Abdominal blue dye levels from the 2-h dye feeding assay (Fig. 3*D*), frass deposition (Fig. 3*E* and Fig. S3*E*), and total food interactions (Fig. 3*F* and Fig. S3*F*) were not decreased by the addition of bitter substances, which is consistent with idea that flies rapidly habituate to noxious but nontoxic tastants when there is no better choice available (24). Furthermore, expression of TRPA1 under control of the *Gr66a-GAL4* driver, which is broadly expressed in bitter-sensing neurons (21), led to sleep loss in the presence of 50 mM D-glucose (Fig. 3*G*) when the neurons were activated (29 °C) but not at a lower temperature, where the neurons fired normally (23 °C). Together, these results indicate that multiple sensory modalities coordinate information about the potential quality of the food source to regulate sleep behavior, as predicted by our model.

We note that our findings, which indicate a strong relationship between gustatory perception and starvation-induced sleep loss, differ from a prior report demonstrating that a nonnutritive sweetener, sucralose, failed to suppress sleep loss (2). We confirmed this prior observation. However, although we observed that sucralose was appetitive to control flies, it was also aversive to *ΔGr64* mutant animals (Fig. S3*G*). The receptor-mediated signaling pathway for sucralose is not fully described in *Drosophila,* and these data suggest that sucralose may activate both sweet- and bitter-sensing neurons, making it a more complicated stimulus than is currently appreciated. Based on our model, a compound with both bitter and sweet properties would be unable to attenuate starvation-induced sleep loss.

Dopamine-containing neurons have been implicated as downstream mediators of sweet sensory input for other behavioral outputs including proboscis extension response (25, 26). We therefore tested whether dopaminergic neurons play a role in transducing sensory information to sleep regulatory centers. We selectively suppressed the activity of dopaminergic [tyrosine hydroxylase (TH)-containing] neurons by expressing the inward-rectifying potassium channel KIR$^{2.1}$ (27) in combination with *tub-Gal80^{ts}* (*TARGET*) (28) under control of the *TH-Gal4* driver. The *TH-Gal4* construct has been shown to express in all of the major subsets of dopaminergic neurons (29). This approach allowed us to bypass developmental effects and only adjust neuronal activity during the experimental period. If gustatory stimulation leads to activation of dopaminergic neurotransmission, we would predict that blockade of TH-containing neurons (*TH-Gal4/UAS-Kir$^{2.1}$;TARGET*) would phenocopy the *ΔGr64* defect and lead to sleep loss at low nutrient concentrations. We found exactly this (Fig. 4 *A* and *B*). When the flies were exposed to a high temperature (29 °C, neurons blocked) only during the experiment, we saw a specific impairment of the response to low glucose (Fig. 4*A*) that was not present at the low temperature (18 °C, neurons firing normally; Fig. 4*B*). We observed a similar result with constitutive expression of the tetanus toxin light chain (*UAS-tntG*), which blocks neurotransmission through an independent mechanism and does not require temperature manipulations (Fig. 4*C*).

To begin narrowing down the specific neurons involved, we tested other *Gal4* drivers (labeled with the letters C–G) that were created in the laboratory of Mark Wu from fragments of the *TH* enhancer region. These drivers have been shown in a prior report to express in more confined, dopamine-producing neuron subsets based on TH antibody colabeling (29). We found that expression of *UAS-Kir$^{2.1}$;TARGET* in both the *D4* and *C1* subsets eliminated the ability of 50 mM D-glucose to suppress starvation-induced sleep loss (Fig. 4*D* and Fig. S4*A*). Expression by *G1* and *F2* had no effect, whereas *F1* and *F3* partially suppressed sleep loss but the effect was not statistically significant. The *D1* driver combined with *UAS-Kir$^{2.1}$;TARGET* caused death within 24 h when placed at 29 °C. Based on the expression profile of the *D4* driver, we can effectively say that the PAM, PAL, PPM1, and PPL2 dopaminergic clusters are unlikely to be involved. Notably, the PPM1 neuron subset contains *TH-Gal4*-positive nondopaminergic cells that we can effectively rule out with this analysis. To confirm that D4 dopaminergic neurons are in the gustatory sensing pathway, we also tested flies expressing *UAS-Kir$^{2.1}$;TARGET* under control of the *D4* driver using the sweet but nonnutritive sugar arabinose and also observed sleep suppression, consistent with a role for these dopaminergic neurons in the regulation of sleep by gustatory cues (Fig. 4*D*). Further work will be required to determine the specific neuron(s) that relay gustatory signals to the sleep centers of the brain and whether dopamine alone, or a combination of neurotransmitters and neuropeptides, are involved.

Finally, we asked whether the lack of appetitive gustatory perception and its associated sleep dysregulation have broader consequences on organismal health. *ΔGr64* mutants are not impaired in their survival under starvation (Fig. 5*A* and Table S2), and their lifespan is not adversely affected by sleep deprivation using the guest-host paradigm, which is a model for sleep stress where the pairing of a male and female in the same activity tube significantly reduces sleep in both animals (30) and eventually leads to death (Fig. 5*B* and Table S2). Thus, we conclude that the *ΔGr64* animals are not sick or broadly stress sensitive.

Our model predicts that the negative consequences of losing sweet taste sensitivity would be most substantial under conditions of low nutrient availability. Consistent with this, we see that the *ΔGr64* mutants are short-lived on the diet to which they are taste blind (D-glucose) relative to fructose when the nutrient concentration is 50 mM (69% difference in mean lifespan; Fig. 5*C* and

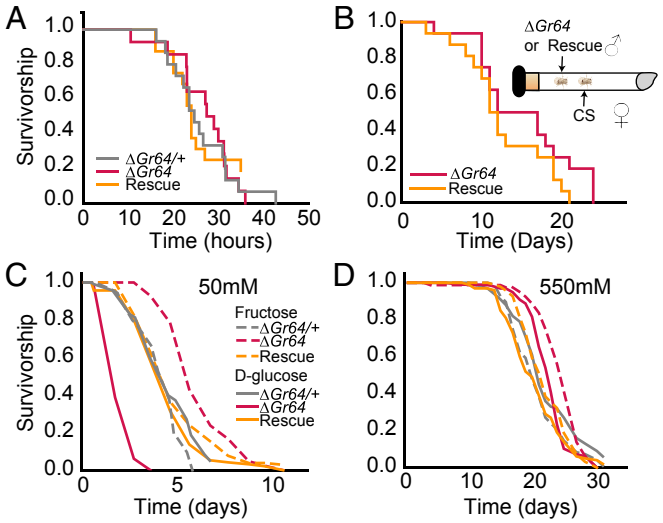

Fig. 5. Loss of appetitive gustatory perception impairs survival in low nutrient environments. Survival in response to starvation (*A*) or sleep deprivation stress from the presence of a female "guest" in an activity monitor tube on a complete SY10 food (*B*) is not impaired by the *ΔGr64* deletion. Survival on 50 mM (*C*) but not 550 mM (*D*) D-glucose is impaired by the *ΔGr64* deletion relative to the fructose control. Number of flies, median survival, and *P* values are in Table S2.

Table S2). There is no significant difference between fructose and D-glucose for either the *ΔGr64*/+ control (1% lifespan difference; Fig. 5*C* and Table S2) or the genomic rescue lines (<1% lifespan difference; Fig. 5*C* and Table S2). We observed a similar reduced survival on low nutrients when the *ΔGr64* deletion mutant was crossed to a different *Gr64* mutant that contains a deletion only in *Gr64a-c* (19), indicating that the a–c region of the gene cluster may be important for survival under nutrient stress (Table S2). However, under conditions of high nutrient density, the effects of the *ΔGr64* mutation on survival are largely absent (Fig. 5*D* and Table S2). Thus, the negative effects of losing sweet-sensory perception are dependent on the environmental nutrient concentration. Interestingly, *ΔGr64* mutant survival on both low and high fructose (which the animals can taste) is significantly enhanced relative to the two control backgrounds (Fig. 5 *C* and *D* and Table S2). Together our results suggest an essential role for gustatory perception in defining normal behavioral and physiological responses to nutrient variability, particularly under conditions of nutrient stress.

Discussion

We showed that gustatory perception of sweetness is both necessary and sufficient to suppress starvation-induced sleep loss when animals encounter nutrient-poor food sources. Notably, gustatory perception is also required for a normal lifespan, specifically when animals are maintained in a low nutrient environment, establishing that gustatory perception is a critical mechanism for transmitting information about environmental quality to central regulatory centers that control sleep behavior and survival. Although there are apparently multiple types of sensors that relay the complexity of dietary information, the impact of gustatory perception on both sleep and survival is most profound when environmental nutrient density is low. Overall, these results demonstrate an important role for gustatory perception when environmental food availability approaches zero, and they illustrate the interplay between sensory and metabolic perception of nutrient availability in regulating physiology and other complex behaviors.

We find that dopaminergic neuron activity is required for appropriate regulation of sleep behavior specifically under conditions of very low nutrient availability. These data support a model where gustatory perception signals through dopaminergic neurons to transmit information about food availability to sleep centers. It is also possible that dopaminergic neurons act to relay the gustatory information back to sensory neurons, as has been reported for starvation (25). We believe this second model is less likely due to the fact that known dopamine-gustatory feedback is dependent on the DOPECR receptor and that we find no effect of *DopEcr* mutation or RNAi on gustatory-dependent sleep behavior (Fig. S4*B*). The precise neurons involved in this interaction remain unknown, but neurons in the PPM2 cluster are strong candidates due to overlapping expression in the C1 and D4 dopamine subset Gal4 lines (29). These data and others are beginning to reveal the importance of specific subsets of neurons for regulating distinct behavioral phenotypes.

Why might animals rely on gustatory perception rather than an assessment of nutritional content per se to drive key food-related behaviors? One possibility is that in the wild gustatory and nutritional cues are nearly always paired, and therefore taste may be a very reliable predictor of nutrient quality. Metabolic assessment of quality may have lower sensitivity, and it is almost surely slower. As we understand more about the nature of metabolic nutrient sensor(s) in flies and other organisms, the nature of the relationship between the gustatory and metabolic information will clarify. For now, we propose that the gustatory and metabolic processing pathways are mostly distinct. However, the ΔGr64 mutant is partially defective in suppressing starvation-induced sleep loss, even at very high dietary glucose concentrations (Fig. 2*A* and Fig. S1*A*), which leaves open the possibility of either a compensatory change in metabolic processing in response to sensory deficit or more direct cross-talk between gustatory and metabolic signaling.

We speculate that the ability for gustatory stimulation to attenuate starvation-induced sleep loss may be evolutionarily conserved and that this may provide an interesting therapeutic angle for limiting the negative effects of fasting or periods of anticipated food deprivation. Under conditions where sensory perception is impaired, including aging and neurodegenerative disease, nutritional intervention may provide an important approach to limit the disorders of sleep behavior that can potentially accelerate disease progression and reduce independence. Together, our work supports a key role for sensory perception in the regulation of complex behaviors that serve to support the normal functions of the organism, and it serves as an essential step in understanding how organisms process information from their environment.

Materials and Methods

Husbandry. All fly stocks were maintained on a standard cornmeal-based larval growth medium and maintained in a temperature and humidity-controlled environment (25 °C, 60% humidity) with a 12:12-h light:dark cycle. Before experimentation, the F0 generation was mated in egg collection chambers, and eggs were collected on grape agar medium and distributed at 10 μL/vial to provide consistent larval density. Following eclosion, flies were mated for 2 d, and then males were separated into SY10 medium at a constant density of 25 flies per vial for 2–4 d (31) before experimentation.

Fly Stocks. The genotype of the ΔGr64 line was *R1;R2/+;ΔGr64/ΔGr64* and the rescue was *R1;R2/Gr5a-GAL4; ΔGr64,UAS-Gr64abcdGFPf/ΔGr64.* Heterozygous controls used for comparison with the ΔGr64 mutant were *R1;R2/+; ΔGr64/+*. These stocks were a kind gift from the Amrein Laboratory, College Station, TX (9). The *Gr64* genomic rescue flies were made by inserting the CH322-176A20 genomic fragment (32) into the 59D3 chromosome location. Transgenesis was performed by BestGene. *Gr64a*^{2}, *Gr5a-Gal4*, *Gr66a-Gal4*, and *UAS-Kir*$^{2.1}$ flies were kind gifts from A. Dahanukar, University of California, Riverside, CA (19); K. Scott, University of California, Berkeley, CA (21); J. Carlson, Yale University, New Haven, CT (22); and R. Baines, The University of Manchester, Manchester, UK (33), respectively. The *TH-Gal4* subset lines (C1-G1) were a kind gift from M. Wu (29). *tubGAL80*^{ts} (7017), *UAS-tntG*

(28838), *and TH-Gal4* (8848) were from the Bloomington Stock Center, and the *UAS* and *Gal4* lines were backcrossed six generations into the w^{1118} background. All other experiments used the Canton-S control strain.

Sleep Behavior. Flies were transferred to 5-mm polycarbonate tubes fitted with a food cup (trimmed pipet tip with glued end) containing 50 μL of SY10 medium and allowed to equilibrate within a Trikinetics activity monitoring apparatus (Trikinetics) for at least 1 d (day 0). For all temperature manipulations, the flies were placed at the test temperature at the start of day 0. Baseline sleep behavior was subsequently monitored for 24 h (day 1 ZT0-24). At ZT0 (lights-on) of day 2, the food cup was replaced with the specified test medium (1% Bacto-agar with or without additions) with minimal disturbance to the fly. Flies were not starved before placement on the test medium. Sleep behavior was monitored for at least 24 h on the test medium. Sleep was calculated as the total time spent in periods of 5 min or more of continuous inactivity (34) and grouped into 30-min bins. The percent sleep change was calculated for each fly during hours 12–24 of each day using the following equation: (day 1 – day 2)/day 1 × 100. If a fly died within the test day (no further activity counts), the sleep calculation was adjusted to include only the window of time over which the fly survived on both day 1 and day 2. Within each experimental cohort, the values were normalized to a group of flies experiencing SY10 on both day 1 and day 2 to correct for time-dependent effects (typically less than 10%). All analysis was conducted using custom scripts in the R statistical software package that are available from the authors on request. The observed total sleep levels on day 1 for the genotypes used here are listed in Table S3.

Feeding Behavior.

Abdominal blue food. Flies were transferred from SY10 medium to vials containing the test medium for 24 h and then transferred to test medium containing 0.5% FD&C blue #1 for 2 h (35). Individual flies were frozen and homogenized in 40 μL of PBS + 0.01% Triton X-100 using a Qiagen TissueLyser. The lysate was centrifuged at 2,250 × *g* for 20 min. Twenty microliters of the resulting supernatant was analyzed at 630 nm using a half-diameter 96-well plate using a standard curve with the blue dye. A control group fed without blue dye was run simultaneously to determine the nonspecific 630-nm absorbance, and this value was subtracted from all measurements.

Blue frass. Groups of 15 flies were placed on SY10 medium containing 0.5% FD&C blue #1 for 24 h (day 1, baseline day) and then transferred to test medium containing 0.5% FD&C blue #1 in 28.5 × 95-mm (standard wide) vials fitted with a layer of transparency film on the inner surface of the vial. After 24 h, the transparency film was removed and imaged to determine the total number of spots and the area of each spot.

Food interactions. Individual interactions with the food were counted using the FLIC, a novel apparatus that continuously (roughly 500 times/s) monitors feeding behavior by recording an electrical signal for every interaction a fly makes with a liquid food source (for details, see ref. 18). Flies were placed individually into FLIC measurement arenas for 6 h with the indicated food type, and the total number of seconds spent interacting with the food was recorded.

Video Analysis. We observed and recorded the position of flies in 5-mm activity monitoring tubes as described previously (8). Briefly, we recorded movies at 1 frame/s and used an in-house software system (DTrack) to calculate the centroid position for each fly and plotted the position along the axis of the tube over time. This software is available from the authors on request.

Survival. Flies were prepared for survival experiments as previously described (31), with a slight modification. Male flies were transferred to the test medium (1% agar with or without the indicated carbohydrate) between days 3 and 10 after eclosion, and the time of transfer is indicated as time 0. Flies were transferred to new food three times per week, and survival was recorded every 1–2 d.

ACKNOWLEDGMENTS. We thank Zach Harvanek for his role in development of the FLIC apparatus and Tammy Chan for transgenesis. This work was supported by funding from the Ellison Medical Foundation (S.D.P.), National Institutes of Health (NIH) Grants K01AG031917 (to N.J.L.) and R01AG030593 (to S.D.P.), National Institute of General Medical Sciences Grant T32 GM007315 (to J.R.), National Institute on Aging (NIA) Grant 5T32AG000114-29 (to J.R.), and a pilot award from the University of Michigan Geriatrics Center and Nathan Shock Center of Excellence in the Basic Biology of Aging (to N.J.L.). This work used the resources of the Drosophila Aging Core of the Nathan Shock Center of Excellence in the Biology of Aging funded by NIA Grant P30-AG-013283.

1. Wiersma P, Salomons HM, Verhulst S (2005) Metabolic adjustments to increasing foraging costs of starlings in a closed economy. *J Exp Biol* 208(Pt 21):4099–4108.
2. Keene AC, et al. (2010) Clock and cycle limit starvation-induced sleep loss in Drosophila. *Curr Biol* 20(13):1209–1215.
3. MacFadyen UM, Oswald I, Lewis SA (1973) Starvation and human slow-wave sleep. *J Appl Physiol* 35(3):391–394.
4. Rechtschaffen A, Gilliland MA, Bergmann BM, Winter JB (1983) Physiological correlates of prolonged sleep deprivation in rats. *Science* 221(4606):182–184.
5. Shaw PJ, Tononi G, Greenspan RJ, Robinson DF (2002) Stress response genes protect against lethal effects of sleep deprivation in Drosophila. *Nature* 417(6886):287–291.
6. Bergmann BM, et al. (1996) Are physiological effects of sleep deprivation in the rat mediated by bacterial invasion? *Sleep* 19(7):554–562.
7. Erion R, DiAngelo JR, Crocker A, Sehgal A (2012) Interaction between sleep and metabolism in Drosophila with altered octopamine signaling. *J Biol Chem* 287(39): 32406–32414.
8. Linford NJ, Chan TP, Pletcher SD (2012) Re-patterning sleep architecture in Drosophila through gustatory perception and nutritional quality. *PLoS Genet* 8(5):e1002668.
9. Slone J, Daniels J, Amrein H (2007) Sugar receptors in Drosophila. *Curr Biol* 17(20): 1809–1816.
10. Dus M, Min S, Keene AC, Lee GY, Suh GS (2011) Taste-independent detection of the caloric content of sugar in Drosophila. *Proc Natl Acad Sci USA* 108(28):11644–11649.
11. Burke CJ, Waddell S (2011) Remembering nutrient quality of sugar in Drosophila. *Curr Biol* 21(9):746–750.
12. Fujita M, Tanimura T (2011) Drosophila evaluates and learns the nutritional value of sugars. *Curr Biol* 21(9):751–755.
13. Stafford JW, Lynd KM, Jung AY, Gordon MD (2012) Integration of taste and calorie sensing in Drosophila. *J Neurosci* 32(42):14767–14774.
14. Jiao Y, Moon SJ, Montell C (2007) A Drosophila gustatory receptor required for the responses to sucrose, glucose, and maltose identified by mRNA tagging. *Proc Natl Acad Sci USA* 104(35):14110–14115.
15. Jiao Y, Moon SJ, Wang X, Ren Q, Montell C (2008) Gr64f is required in combination with other gustatory receptors for sugar detection in Drosophila. *Curr Biol* 18(22): 1797–1801.
16. Xu K, Zheng X, Sehgal A (2008) Regulation of feeding and metabolism by neuronal and peripheral clocks in Drosophila. *Cell Metab* 8(4):289–300.
17. Cognigni P, Bailey AP, Miguel-Aliaga I (2011) Enteric neurons and systemic signals couple nutritional and reproductive status with intestinal homeostasis. *Cell Metab* 13(1):92–104.
18. Ro J, Harvanek ZM, Pletcher SD (2014) FLIC: High-throughput, continuous analysis of feeding behaviors in Drosophila. *PLoS ONE* 9(6):e101107.
19. Dahanukar A, Lei YT, Kwon JY, Carlson JR (2007) Two Gr genes underlie sugar reception in Drosophila. *Neuron* 56(3):503–516.
20. Kang K, et al. (2010) Analysis of Drosophila TRPA1 reveals an ancient origin for human chemical nociception. *Nature* 464(7288):597–600.
21. Marella S, et al. (2006) Imaging taste responses in the fly brain reveals a functional map of taste category and behavior. *Neuron* 49(2):285–295.
22. Weiss LA, Dahanukar A, Kwon JY, Banerjee D, Carlson JR (2011) The molecular and cellular basis of bitter taste in Drosophila. *Neuron* 69(2):258–272.
23. Masek P, Scott K (2010) Limited taste discrimination in Drosophila. *Proc Natl Acad Sci USA* 107(33):14833–14838.
24. Zhang YV, Raghuwanshi RP, Shen WL, Montell C (2013) Food experience-induced taste desensitization modulated by the Drosophila TRPL channel. *Nat Neurosci* 16(10): 1468–1476.
25. Inagaki HK, et al. (2012) Visualizing neuromodulation in vivo: TANGO-mapping of dopamine signaling reveals appetite control of sugar sensing. *Cell* 148(3):583–595.
26. Marella S, Mann K, Scott K (2012) Dopaminergic modulation of sucrose acceptance behavior in Drosophila. *Neuron* 73(5):941–950.
27. Nitabach MN, Blau J, Holmes TC (2002) Electrical silencing of Drosophila pacemaker neurons stops the free-running circadian clock. *Cell* 109(4):485–495.
28. McGuire SE, Mao Z, Davis RL (2004) Spatiotemporal gene expression targeting with the TARGET and gene-switch systems in Drosophila. *Sci STKE* 2004(220):pl6.
29. Liu Q, Liu S, Kodama L, Driscoll MR, Wu MN (2012) Two dopaminergic neurons signal to the dorsal fan-shaped body to promote wakefulness in Drosophila. *Curr Biol* 22(22):2114–2123.
30. Gilestro GF, Tononi G, Cirelli C (2009) Widespread changes in synaptic markers as a function of sleep and wakefulness in Drosophila. *Science* 324(5923):109–112.
31. Linford NJ, Bilgir C, Ro J, Pletcher SD (2013) Measurement of lifespan in Drosophila melanogaster. *J Vis Exp* (71):pii50068.
32. Venken KJ, et al. (2009) Versatile P[acman] BAC libraries for transgenesis studies in Drosophila melanogaster. *Nat Methods* 6(6):431–434.
33. Baines RA, Uhler JP, Thompson A, Sweeney ST, Bate M (2001) Altered electrical properties in Drosophila neurons developing without synaptic transmission. *J Neurosci* 21(5):1523–1531.
34. Huber R, et al. (2004) Sleep homeostasis in Drosophila melanogaster. *Sleep* 27(4): 628–639.
35. Tanimura T, Isono K, Takamura T, Shimada I (1982) Genetic dimorphism in the taste sensitivity to trehalose inDrosophila melanogaster. *J Comp Physiol* 147(4):433–437.