Taste triggers a homeostatic temperature control in *Drosophila*

Yujiro Umezaki[1], Sergio Hidalgo[2], Erika Nguyen[3], Tiffany Nguyen[3], Jay Suh[3], Sheena S. Uchino[1], Joanna C. Chiu[2], and Fumika N. Hamada[1,4] ‡

[1] Department of Neurobiology, Physiology and Behavior, University of California, Davis, Davis, CA 95616

[2] Department of Entomology and Nematology, University of California, Davis, Davis, CA 95616

[3] Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229, USA

[4] Lead contact

‡Correspondence: fnhamada@ucdavis.edu

Telephone: 530-754-1782

FAX:

Manuscript information: Summary, 150 words; 7 figures; 4 supplemental figures; and 1 supplemental table

Keywords: Gustatory receptors, temperature-sensing neurons, circadian clock, clock genes, starvation, cephalic phase response, body temperature, temperature preference rhythms, hunger signals, and *Drosophila melanogaster*.

SUMMARY

Hungry animals consistently show a desperate desire to obtain food. Even a brief sensory detection of food can trigger bursts of physiological and behavioral changes. However, the underlying mechanisms by which the sensation of food triggers the acute behavioral response remain elusive. We have previously shown in *Drosophila* that hunger drives a preference for low temperature. Because *Drosophila* is a small ectotherm, a preference for low temperature implies a low body temperature and a low metabolic rate. Here, we show that taste sensing triggers a switch from a low to a high temperature preference in hungry flies. We show that taste stimulation by artificial sweeteners or optogenetics triggers an acute warm preference, but is not sufficient to reach the fed state. Instead, nutrient intake is required to reach the fed state. The data suggest that starvation recovery is controlled by two components: taste-evoked and nutrient-induced warm preferences, and that taste and nutrient quality play distinct roles in starvation recovery. Animals are motivated to eat based on time of day or hunger. We found that clock genes and hunger signals profoundly control the taste-evoked warm preferences. Thus, our data suggest that the taste-evoked response is one of the critical layers of regulatory mechanisms representing internal energy homeostasis and metabolism.

INTRODUCTION

Animals are constantly sensing environmental stimuli and changing their behavior or physiology based on their internal state [1-7]. Hungry animals are desperate for food. Immediately after seeing, smelling, or chewing food, even without absorbing nutrients, a burst of physiological changes is suddenly initiated in the body. These responses are known in mammals as the cephalic phase response (CPR). For example, a flood of saliva and gastrointestinal secretions prepares hungry animals to digest food [8-12]. Starvation results in lower body temperatures, and chewing food triggers a rapid increase in heat production, demonstrating CPR in thermogenesis [13-15]. Hungry animals are strongly attracted to food. However, the underlying mechanisms of how the sensation of food without nutrients triggers the behavioral response remain unclear.

To address this question, we used a relatively simple and versatile model organism, *Drosophila melanogaster*. Flies exhibit robust temperature preference behavior [16,17]. Due to the low mass of small ectotherms, their body temperatures are close to the ambient temperature [18,19]. Hunger stress forces flies to change their behavior and physiological response [20,21]. The hungry flies prefer a lower temperature [22], indicating lower body temperatures and lower metabolic rates [22-24]. Notably, this phenomenon of hunger-driven lower body temperature is similar to mammals, as the body temperature of starved animals is lower than that of fed animals [5-7]. Furthermore, flies exhibit robust feeding behavior [25,26], and molecular and neural mechanisms of taste are well documented [2,27-32]. Therefore, we asked whether the taste cue triggers a robust behavioral recovery of temperature preference in starving flies.

Here we show in hungry flies that taste without nutrients induces a switch from a low to a high temperature preference. While taste leads to a warmer temperature preference, nutrient intake causes the flies to prefer an even warmer temperature. This nutrient-induced warm

preference results in a complete recovery from starvation. Thus, taste-evoked warm preference is different from nutrient-induced warm preference and potentially similar physiology as CPR. Therefore, when animals emerge from starvation, they use a two-step approach to recovery: taste-evoked and nutrient-induced warm preference. While a rapid component is elicited by food taste alone, a slower component requires nutrient intake.

Animals are motivated to eat based on their internal state, such as time of day or degree of hunger. The circadian clock drives daily feeding rhythms [33-35] and anticipates meal timing. Daily feeding timing influences energy homeostasis and metabolism [36,37]. Hunger and satiety dramatically affect animal behavior and physiology [1-7] and control feeding. We found that clock genes and hunger signals are strongly required for taste-evoked warm preference. The data suggest that taste-evoked response is an indispensable physiological response that represents internal state. Taken together, our data shed new light on the role of the taste-evoked response and highlight a crucial aspect of our understanding of feeding state and energy homeostasis.

RESULTS

Food detection triggers a warm preference.

Flies exhibit robust temperature preference behavior [16,17]. To examine *Drosophila* temperature preference behavior, flies are released into a chamber with a temperature gradient of 16-34°C [38] and subsequently accumulate at their preferred temperature (Tp) within 30 min. The *white*^{1118} (*w*^{1118}) flies were fed fly food containing carbohydrate, protein and fat sources (Fig. 1A) and preferred 25.2±0.2 ^{O}C (Fig. 1B: fed, white bar). When *w*^{1118} flies were starved overnight with water only, they preferred 21.7±0.3 ^{O}C (Fig. 1A, 1B: overnight starvation (STV), gray bar). Thus, starvation leads to a lower Tp. As we have previously reported, starvation strongly influences temperature preference [22].

To examine how starved flies recover from lower Tp, they were offered fly food (Fig. 1A) and their Tp was tested. After 10 min, 30 min or 1 hr of fly food refeeding, starved flies preferred a temperature similar to that of fed flies (Fig. 1B: statistics shown as green stars, Table S1), suggesting a full recovery from starvation. On the other hand, refeeding after 5 min resulted in a warmer temperature than the starved flies. Nevertheless, Tp did not reach that of the fed flies (Fig. 1B). Therefore, 5 min fly food refeeding caused a partial recovery from the starved state (Fig. 1E, statistics shown as red stars, Table S1). Thus, our data suggest that food intake triggers a warm preference in starved flies.

Sucralose refeeding promotes a warm preference.

Given that only 5 min refeeding caused hungry flies to prefer a warmer temperature (Fig. 1B), we hypothesized that food-sensing cues might be important for the warm preference. Sucralose is an artificial sweetener that activates sweet taste neurons in *Drosophila* [39] and modulates taste behaviors such as the proboscis extension reflex [40-43]. Importantly, sucralose is a non-metabolizable sugar and has no calories. Therefore, to investigate how food-sensing cues are involved in warm preference, we examined how sucralose refeeding changes the temperature preference of starved flies. After starved flies were refed sucralose for 10 min or 1 hr (Fig. 1A), they preferred a warmer temperature; however, Tp was halfway between Tps of fed and starved flies (Fig. 1C: blue, Table S1). Thus, sucralose ingestion induces a warm preference but shows a partial recovery of Tp from the starved state.

While refeeding the flies with food resulted in a full recovery of Tp from the starved state, refeeding them with sucralose resulted in only a partial recovery of Tp. Therefore, starved flies may use both taste cues and nutrients to fully recover Tp from the starved state. To evaluate this possibility, we used glucose, which contains both sweetness (gustatory cues) and nutrients (i.e., metabolizable sugar) (Fig. 1A), and tested glucose refeeding for 5 min, 10 min, and 1 hr. We found that 10 min or 1 hr glucose refeeding resulted in full recovery of Tp from the starved state (Fig. 1D and E: statistics shown in red NS, Table S1) and was significantly different from starved flies (Fig. 1D and E: statistics shown in green stars, Table S1). Thus, our data showed that sucralose refeeding induced partial recovery and glucose refeeding induced full recovery from the starved state.

However, it is still possible that the starved flies consumed glucose more or faster than sucralose during the first 10 min, which could result in a different warming preference. To rule out this possibility, we examined how often starved flies touched glucose, sucralose, or water

during the 30 min using the Fly Liquid-food Interaction Counter (FLIC) system [44]. The FLIC system assays allow us to monitor how much interaction between the fly and liquid food reflects feeding/drinking episodes. We found that starved flies touched glucose and sucralose food at similar frequencies and more frequently than water during the 30 min test period (Fig. 1F, Table S1). The data suggest that flies are likely to consume glucose and sucralose at similar rates and amounts. Therefore, we concluded that the differential effect of sucralose and glucose refeeding on temperature preference was not due to differences in feeding rate or amount. Instead, the gustatory cues likely induce partial Tp recovery, and the gustatory cue plus nutrients induce full Tp recovery.

Activation of sweet taste neurons leads to warm preference.

We first focused on how taste elicits a warm preference. Sweet gustatory receptors (Grs) detect sweet taste. We used sweet Gr mutants and asked whether sweet Grs are involved in taste-evoked warm preference. Two different sweet Gr mutant strains, *Gr5a*$^{-/-}$*; Gr64a*$^{-/-}$ and *Gr5a*$^{-/-}$*;;Gr61a*$^{-/-}$, *Gr64a-f*$^{-/-}$, have reduced sugar sensitivity compared to the control [41,42,45,46]. We found that sweet Gr mutant flies exhibited a normal starvation response in which the Tp of starved flies was lower than that of fed flies (Fig. 2A and B, white and gray bars, Table S1). However, starved sweet Gr mutant flies did not increase Tp after 10 min sucralose refeeding (Fig. 2A and B, blue bars, Table S1). These data suggest that sweet Grs are involved in taste-evoked warm preference.

Sweet Grs are expressed in the sweet Gr-expressing neurons (GRNs) located in the proboscis and forelegs [46,47]. To determine whether sweet GRNs are involved in taste-evoked

warm preference, we silenced all sweet GRNs. We expressed the inward rectifying K^+ channel Kir2.1 (*uas-Kir*) [48] with *Gr64f-Gal4*, which is expressed in all sweet GRNs in the proboscis and forelegs [41,46,47]. Knockdown of all sweet GRNs showed a normal starvation response (Fig. 2C, white and gray bars). However, flies silencing all sweet GRNs failed to show a warm preference after 10 min sucralose refeeding (Fig. 2C, blue bar, Table S1). This phenotype was similar to the data obtained with the sweet Gr mutant strains (Fig. 2A and B). On the other hand, control flies (*Gr64f-Gal4/+* and *uas-Kir/+*) showed a normal starvation response and a taste-evoked warm preference (Fig. 2D and E, gray and blue bars, respectively, Table S1). Thus, our data indicate that sweet GRNs are required for taste-evoked warm preference.

To further investigate whether activation of sweet GRNs induces a warm preference, we used the optogenetic approach, a red light sensitive channelrhodopsin, CsChrimson [49,50]. Starved flies were given water containing 0.8 mM all-trans-retinal (ATR), the chromophore required for CsChrimson activation. These flies were not fed sucralose; instead, gustatory neurons in starved flies were excited by red light pulses (flashing on and off at 10 Hz) for 10 min (Fig. 2F). In this case, although the flies were not refed, the gustatory neurons were artificially excited by CsChrimson activation so that we could evaluate the effect of excitation of sweet GRNs on taste-evoked warm preference.

CsChrimson was expressed in sweet GRNs in the proboscis and legs (all sweet GRNs) using *Gr64f-Gal4*. These flies showed a normal starvation response (Fig. 2G-J, white and gray bars, Table S1). Excitation of all sweet GRNs by red light pulses elicited a warm preference, and Tp was intermediate between fed and starved flies, suggesting partial recovery (Fig. 2G, yellow bar, Table S1). However, neither excitation of Gr5a- (Fig. 2H) nor Gr64a-expressing neurons (Fig. 2I) induced a warm preference (yellow bars, Table S1). While *Gr64a-Gal4* is expressed

only in legs, *Gr5a-Gal4* is expressed in proboscis and legs, but does not cover all sweet GRNs like *Gr64f-Gal4* [46,47]. Notably, control flies (*UAS-CsChrimson/+*) did not show a warm preference to red light pulses with ATR application (Fig. 2J). Taken together, our data suggest that excitation of all sweet GRNs results in a warm preference.

The temperature-sensing neurons are involved in taste-evoked warm preference.

The warm-sensing neurons, anterior cells (ACs) and the cold-sensing *R11F02-Gal4*-expressing neurons control temperature preference behavior [38,51,52]. Small ectotherms such as *Drosophila* set their Tp to avoid noxious temperatures using temperature information from cold- and warm-sensing neurons [16,17,38]. We have previously shown that starved flies choose a lower Tp, the so-called hunger-driven lower Tp [22]. ACs control hunger-driven lower Tp but cold-sensing *R11F02-Gal4*-expressing neurons do not [22]. ACs express transient receptor potential A1 (TrpA1), which responds to a warm temperature >25 ^{O}C [38,53]. The set point of ACs in fed flies, which is ~25 ^{O}C, is lowered in starved flies. Therefore, the lower set point of ACs corresponds to the lower Tp in starved flies.

First, we asked whether ACs are involved in taste-evoked warm preference. Because the ACs are important for the hunger-driven lower Tp [22], the AC-silenced flies did not show a significant difference in Tp between fed and starved conditions for only one overnight starvation [22]. Therefore, we first extended the starvation time to two nights so that the AC-silenced flies showed a significant difference in Tp between fed and starved conditions (Fig. 3A, white and

gray bars, Table S1). Importantly, more prolonged starvation does not affect the ability of w^{1118} flies to recover (Fig. S1).

To examine whether ACs regulate taste-evoked warm preference, we refed sucralose to AC-silenced flies for 10 min. We found that the Tp of the refed flies was still similar to that of the starved flies (Figure 3A, blue bar, Table S1), indicating that sucralose refeeding could not restore Tp and that ACs are involved in taste-evoked warm preference. Significantly, even when the AC-silenced flies were starved for two nights, they were able to recover Tp to the normal food for 10 min and to glucose for 1 hr (Fig. S3D), suggesting that the starved AC-silenced flies were still capable of recovery.

Other temperature-sensing neurons involved in temperature preference behavior are cold-sensing *R11F02-Gal4*-expressing neurons [51,52]. To determine whether *R11F02-Gal4*-expressing neurons are involved in taste-evoked warm preference, we silenced *R11F02-Gal4*-expressing neurons using *uas-Kir*. We found that the flies showed a significant difference in Tp between fed and starved conditions, but the flies did not show a warm preference upon sucralose refeeding (Fig. 3B, gray and blue bars, Table S1). As controls, $TrpA1^{SH}$*-Gal4/+*, *R11F02-Gal4/+* and *uas-Kir/+* flies showed normal starvation response and taste-evoked warm preference (Fig. 3C-E).

To further ensure the results, we used optogenetics to artificially excite warm and cold neurons with $TrpA1^{SH}$*-Gal4* and *R11F02-Gal4*, respectively, by red light pulses for 10 min. We compared the Tp of starved flies with and without ATR under red light. Tp of starved flies with ATR was significantly increased (Fig. 3F and G, yellow bars, Table S1) compared to those without ATR (Fig. 3F and G, gray bars, Table S1). Therefore, these data indicate that ACs and *R11F02-Gal4*-expressing neurons are required for taste-evoked warm preference.

Internal state influences taste-evoked warm preference in hungry flies.

Internal state strongly influences feeding motivation. However, how internal state influences starved animals to exhibit a food response remains unclear. Hunger represents the food-deficient state in the body, which induces the release of hunger signals such as neuropeptide Y (NPY). NPY promotes foraging and feeding behavior in mammals and flies [54]. While intracerebroventricular injection of NPY induces cephalic phase response (CPR) [55], injection of NPY antagonists suppresses CPR in dogs, suggesting that NPY is a regulator of CPR in mammals [56]. Therefore, we first focused on neuropeptide F (NPF) and small neuropeptide F (sNPF), which are the *Drosophila* homologue and orthologue of mammalian NPY, respectively [54], and asked whether they are involved in taste-evoked warm preference. In NPF mutant ($NPF^{-/-}$) or sNPF hypomorph (*sNPF hypo*) mutant, we found that the Tp of fed and starved flies were significantly different, showing a normal starved response (Fig. 4A and B: white vs. gray bars, Table S1). However, they failed to show a taste-evoked warm preference after 10 min sucralose refeeding (Fig. 4A and B: blue bars, Table S1). Thus, NPF and sNPF are required for taste-evoked warm preference after sucralose feeding.

Next, we asked whether NPF and sNPF are involved in the nutrient-induced warm preference during glucose refeeding. We found that starved $NPF^{-/-}$ mutants significantly increased Tp after 1 hr glucose refeeding (Fig. 4A, green and dark green bars, Table S1). We also found that starved *sNPF hypo* mutants significantly increased Tp after 10 min and 1 hr of glucose refeeding (Fig. 4B, green and dark green bars, Table S1). In addition, both $NPF^{-/-}$ and *sNPF hypo* mutants increased Tp after 10 min fly food refeeding (Fig. 4A and B, orange bars,

Table S1). These data suggest that NPF and sNPF are unlikely to be critical for nutrient-induced warm preference.

Factors involving hunger regulate taste-evoked warm preference.

Based on the above results, we hypothesized that hunger signals might be involved in taste-evoked warm preference. To test this hypothesis, we focused on several factors involved in the hunger state. The major hunger signals [20], diuretic hormone 44 (DH44), and adipokinetic hormone (AKH) are the mammalian corticotropin-releasing hormone homologue [57] and the functional glucagon homologue respectively [58,59]. We found that DH44- or AKH-neuron-silenced flies failed to show a taste-evoked warm preference after sucralose refeeding. However, they were able to increase Tp after 10 min glucose or fly food refeeding (Fig. 4D and F, green and orange bars, Table S1), suggesting a normal nutrient-induced warm preference. The control flies (*Dh44-Gal4/+*, *Akh-Gal4/+*, and *UAS-Kir/+*) showed normal warm preference to sucralose (Fig. 4C, E and J, blue bars, Table S1), glucose (Fig. 4C, E and J, green bars, Table S1), and normal fly food refeeding (Fig. 4C, E and J, orange bars, Table S1). The data suggest that DH44 or AKH neurons are required for taste-evoked warm preference, but not for nutrient-induced warm preference.

Insulin-like peptide 6 (Ilp6) is a homologue of mammalian insulin-like growth factor 1 (IGF1), and *ilp6* mRNA expression increases in starved flies [60-62]. Because Ilp6 is important for the hunger-driven lower Tp [22], the *ilp6* mutants did not show a significant difference in Tp between fed and starved conditions for only one overnight starvation [22]. Therefore, we first extended the starvation time to three nights. We found that *ilp6* loss-of-function (*ilp6 LOF*)

mutant failed to show a taste-evoked warm preference after sucralose refeeding (Fig. 4G, blue bars, Table S1), but showed a nutrient-induced warm preference after glucose refeeding (Fig. 4G, green bars, Table S1). These data suggest that Ilp6 is required for taste-evoked warm preference but not for nutrient-induced warm preference after glucose or fly food refeeding.

Unpaired3 (Upd3) is a *Drosophila* cytokine that is upregulated under nutritional stress [63]. We found that *upd3* mutants failed to show a taste-evoked warm preference after sucralose refeeding (Fig. 4H, blue bars, Table S1), but showed a nutrient-induced warm preference after glucose refeeding (Fig. 4G and H, green bars, Table S1). These data suggest that Upd3 is required for taste-evoked warm preference, but not for nutrient-induced warm preference after glucose refeeding. Notably, *upd3* mutants failed to show nutrient-induced warm preference upon normal food feeding (Fig. 4H, orange bars, Table S1).

We also examined the role of the satiety factor Unpaired2 (Upd2), a functional leptin homologue in flies, in taste-evoked warm preference [64]. *upd2* mutants failed to show a warm preference after refeeding of sucralose, glucose or fly food (Fig. 4I, blue, green and orange bars, Table S1). Thus, our data suggest that factors involved in the hunger state are required for taste-evoked warm preference.

Flies show a taste-evoked warm preference at all times of the day.

Animals anticipate feeding schedules at a time of day that is tightly controlled by the circadian clock [37]. Flies show a rhythmic feeding pattern: one peak in the morning [44] or two peaks in the morning and evening [65]. Because food cues induce a warm preference, we wondered whether

feeding rhythm and taste-evoked warm preference are coordinated. If so, they should show a parallel phenotype.

Since flies exhibit one of the circadian outputs, the temperature preference rhythm (TPR) [66], Tp gradually increases during the day and peaks in the evening. First, we tested starvation responses at Zeitgeber time (ZT) 1-3, 4-6, 7-9, and 10-12 in light and dark (LD) conditions. We found that both w^{1118} and *yellow*^{1} *white*^{1} ($y^{1}w^{1}$) flies exhibited higher Tp in the fed state and lower Tp in the starved state at all times during the day (Fig. 5A and B: black vs. gray line; Table S1). Next, we refed sucralose to starved flies at all time points tested and examined taste-evoked warm preference. While starved $y^{1}w^{1}$ flies showed a taste-evoked warm preference at all time points (Fig. 5B1, gray vs. blue line, Table S1), starved w^{1118} flies showed a significant taste-evoked warm preference at ZT 4-6 and 10-12 (Fig. 5A1, gray vs. blue line, Table S1).

Because starved w^{1118} flies showed an advanced phase shift of TPR with a peak at ZT 7-9 (Fig. 5A, gray), it is likely that the highest Tp simply masks the taste-evoked warm preference at ZT 7-9. We also focused on nutrient- (carbohydrate-) induced warm preference. Starved w^{1118} and $y^{1}w^{1}$ flies successfully increased Tp after 10 min glucose refeeding (Fig. 5A2 and B2, green line, Table S1). Glucose refeeding for 1 hr resulted in Tp similar to that of fed w^{1118} flies (Fig. 5A3, green line). Because the feeding rhythm peaks in the morning or morning/evening [44,65], our data suggest that the feeding rhythm and taste-evoked warm preference do not occur in parallel.

Circadian clock genes are required for taste-evoked warm preference, but not nutrient-induced warm preference.

We asked whether the circadian clock is involved in taste-evoked warm preference. We used clock gene null mutants, *period*^{01} (*per*^{01}) and *timeless*^{01} (*tim*^{01}). Although they showed significant starvation responses (Fig. 5C and D, black and gray lines, Table S1), neither starved *per*^{01} nor *tim*^{01} mutants could exhibit taste-evoked warm preference upon sucralose refeeding (Fig. 5C1 and D1, blue lines, Table S1). Nevertheless, they fully recovered upon glucose refeeding in LD at any time of day (Fig. 5C2 and D2, green lines, Table S1). Therefore, our data suggest that clock genes are required for taste-evoked warm preference, but not for nutrient-induced warm preference.

However, starved *per*^{01} and *tim*^{01} mutants may eat sucralose less frequently than glucose, which could result in a failure to show a taste-evoked warm preference. Therefore, we examined how often starved *per*^{01} and *tim*^{01} mutants touched glucose, sucralose, or water during the 30 min using FLIC assays [44] (Fig. S2A-C). Interestingly, starved *per*^{01} and *tim*^{01} mutants touched water significantly more often than glucose or sucralose (Fig. S2B and C, Table S1). Although starved *tim*^{01} flies touched glucose slightly more than sucralose for only 10 min, this phenotype is not consistent with *per*^{01} and *w*^{1118} flies. Thus, we conclude that in *per*^{01} and *w*^{1118} flies, the differential response between taste-evoked and nutrient-induced warm preferences is not due to feeding rate or amount.

The sweet GRNs and temperature-sensing neurons contribute both taste-evoked and nutrient (carbohydrate)-induced warm preference.

Our data suggest that taste-evoked and nutrient- (carbohydrate-) induced warm preferences are controlled separately. We examined how these differences are coordinated. We show that genes involved in internal state tend to control taste-evoked warm preferences. Since gustatory or temperature-sensitive neurons are required for taste-evoked warm preference (Figs. 2 and 3), we asked whether they contribute to nutrient-induced warm preference.

To this end, we used all-sweet-GRNs-silenced flies (*Gr64f-Gal4>uas-Kir*), two Grs mutant strains (*Gr5a*$^{-/-}$*; Gr64a*$^{-/-}$ and *Gr5a*$^{-/-}$*;;Gr61a*$^{-/-}$*, Gr64a-f*$^{-/-}$), and the warm- or cold-neuron-silenced flies (*TrpA1*^{SH}*-Gal4* or *R11F02-Gal4>uas-Kir*). We found that all these starved flies did not increase Tp after 10 min glucose intake (Fig. S3A-E, green bars, Table S1), but increased Tp after 10 min refeeding with fly food containing carbohydrate, fat, and protein (Fig. S3A-E, orange bars, Table S1). As an exception, AC-silenced flies increased Tp after 1 hr glucose intake (Fig. S3D, green bars, Table S1). All control flies showed normal responses to both 10 min glucose refeeding and fly food intake (Fig. S3F-I, green bars, Table S1). The data suggest that gustatory neurons and temperature-sensing neurons are required for warm preference in carbohydrate refeeding, but not in other foods such as fat or protein (see Discussion). Because flies have sensory neurons that detect fatty acids [67-69] or amino acids [70-73], these neurons may drive the response to fly food intake. This is likely why the sweet- or temperature-insensitive flies can still recover after eating fly food (Fig S3A-E, orange bars).

DISCUSSION

When animals are hungry, sensory detection of food (sight, smell, or chewing) initiates digestion even before the food enters the stomach. The food-evoked responses are also observed in thermogenesis, heart rate, and respiratory rate in mammals [13,14,74]. These responses are referred to as the cephalic phase response (CPR) and contribute to the physiological regulation of digestion, nutrient homeostasis, and daily energy homeostasis [10]. While starved flies show a cold preference, we show here that the food cue, such as the excitation of gustatory neurons, triggers a warm preference, and the nutritional value triggers an even higher warm preference. Thus, when flies exit the starvation state, they use a two-step approach to recovery, taste-evoked and nutrient-induced warm preferences. The taste-evoked warm preference in *Drosophila* may be a physiological response potentially equivalent to CPR in mammals. Furthermore, we found that internal needs, controlled by hunger signals and circadian clock genes, influence taste-evoked warm preference. Thus, we propose that the taste-evoked response plays an important role in recovery and represents another layer of regulation of energy homeostasis.

Tp is determined by the taste cue.

Starved flies increase Tp in response to a nutrient-free taste cue (Fig. 1C and E), resulting in a taste-evoked warm preference. We showed that silencing of ACs or cold neurons caused a loss of

taste-evoked warm preference (Figs. 3 and S3), and that excitation of ACs or cold neurons induced a taste-evoked warm preference (Fig. 3). The data suggest that both warm and cold neurons are important for taste-evoked warm preference: while ACs are required for the hunger-driven lower Tp [22], both ACs and cold neurons are likely to be important for this taste-evoked warm preference.

The hunger-driven lower Tp is a slower response because starvation gradually lowers their Tp, [22]. In contrast, the taste-evoked warm preference is a rapid response. Once ACs and cold neurons are directly or indirectly activated, starved flies quickly move to a warmer area. This is interesting because even when ACs and cold neurons are activated by warm and cold, respectively, the activation of these neurons causes a warm preference. Given that the sensory detection of food (sight, smell, or chewing food) triggers CPR, the activation of these sensory neurons may induce CPR. Tp in sucralose-fed hungry flies is between that of fed and starved flies (Figs. 2, 3, and S3), making it difficult to detect the smaller temperature differences using the calcium imaging experiments. Therefore, we speculate that both ACs and cold neurons may facilitate rapid recovery from starvation so that flies can quickly return to their preferred temperature - body temperature - to a normal state.

Internal state influences taste-evoked warm preference.

We show that mutants of genes involved in hunger and the circadian clock fail to show taste-evoked warm preference, suggesting that hunger and clock genes are important for taste-evoked warm preference. At a certain time of day, animals are hungry for food [44,65]. Therefore, the

"hungry" state acts as a gatekeeper, opening the gate of the circuits when hungry flies detect the food information that leads to taste-evoked warm preference (Fig. S4, blue arrow). While most hunger signals are important only for taste-evoked warm preference, some hunger signals are also required for both taste-evoked and nutrient-induced warm preferences (Fig. 4). The data suggest that internal state is more likely to be associated with taste-evoked warm preference than nutrient-induced warm preference. It is noteworthy that the sensory signals contribute to both taste-evoked warm preference and nutrient-induced warm preferences (Figs. S1, S3 and S4, blue and green arrows). Therefore, taste-evoked warm preference and nutrient-induced warm preference are differentiated at the level of the internal state, but not at the sensory level. This idea is analogous to appetitive memory formation. Sweet taste and nutrients regulate the different layers of the memory formation process. Recent evidence suggests that the rewarding process can be subdivided; the sweet taste is for short-term memory and the nutrient is for long-term memory [75-77]. The data suggest that the taste-evoked response functions differently from the nutrient-induced response. Therefore, taste sensation is not just the precursor to nutrient sensing/absorption, but plays an essential role in the rapid initiation of a taste-evoked behavior that would help the animal survive.

How do hunger signals or clock genes contribute to taste-evoked warm preference?

The hunger signaling hormones/peptides studied in this project are important for taste modulation. For example, mammalian NPY and its *Drosophila* homolog NPF modulate the output of taste signals [43,78,79]. The AKH receptor is expressed in a subset of gustatory neurons that may modulate taste information for carbohydrate metabolism [80]. Therefore, the hunger signals

are likely to modulate the downstream of the sensory neurons, which may result in a taste-evoked warm preference.

Circadian clock genes control and coordinate the expression of many clock-controlled genes in the body [81-84]. Therefore, we expect that the absence of clock genes will disrupt the molecular and neural networks of cellular homeostasis, including metabolism, that are essential for animal life. For example, taste neurons express clock genes, and impaired clock function in taste neurons disrupts daily rhythms in feeding behavior [85]. Temperature-sensing neurons transmit hot or cold temperature information to central clock neurons [86-90]. Therefore, the disrupted central clock in clock mutants may respond imprecisely to temperature signals. There are many possible reasons why the lack of clock gene expression in the brain is likely to cause abnormal taste-evoked warm preference.

In addition, hunger signals may contribute to the regulation of circadian output. DH44 is located in the dorsomedial region of the fly brain, the pars intercerebralis (PI), and DH44-expressing neurons play a role in the output pathway of the central clock [91,92]. Insulin-producing cells (IPCs) are also located in addition to DH44-expressing neurons [93-96]. IPCs receive a variety of information, including circadian [92,97] and metabolic signals [60-62,64]. and then transduce the signals downstream to release Ilps. Both Upd2 and Ilp6, which are expressed in the fat body respond to metabolic states and remotely regulate Ilp expression [60-62,64]. Insect fat body is analogous to the fat tissues and liver in the vertebrates [98,99]. Therefore, each hunger signal may have its specific function for taste-evoked warm preference. Further studies are needed to describe the entire process.

Taste-evoked warm preference may be CPR in flies.

The introduction of food into the body disrupts the internal milieu, so CPR is a necessary process that helps animals prepare for digestion. Specifically, in mammals, taste leads to an immediate increase in body temperature and metabolic rate. Starvation results in lower body temperatures, and chewing food, even before it enters the stomach, triggers a rapid increase in heat production, demonstrating CPR in thermogenesis [13,14].

Starved flies have a lower Tp [22]. Because *Drosophila* is a small ectotherm, the lower Tp indicates a lower body temperature [19,20]. Even when the flies do not receive food, the sweet taste and the excitation of sweet neurons induce starved flies to show a warm preference, which eventually leads to a warmer body temperature. Thus, the taste-evoked warm preference in *Drosophila* may be a physiological response equivalent to one of the CPRs observed in mammals. Thus, in both flies and mammals, starvation leads to lower body temperatures, and food cues quickly initiate a rise in body temperature before food enters their body.

CPR is essential because both starved mammals and starved flies must rapidly regulate their body temperature to survive.

As soon as starving flies taste food, the sensory signals trigger CPR. They can move to a warmer place to prepare to raise their body temperature (Fig. S4, blue arrows). CPR may allow flies to choose a more hospitable place to restore their physiological state and allow for higher metabolism, and eventually they move to the next step, such as foraging and actively searching for a mate before competitors arrive. As such, CPR may be a strategy for the fly's survival. Similarly, starvation or malnutrition in mammals causes lower body temperatures [6,7,100], and

biting food triggers heat production, which is CPR [13-15]. Thus, while starvation causes lower body temperatures in both flies and mammals, food cues initiate CPR in body temperature elevation and nutrient intake, resulting in full recovery from the starved state. Our data suggest that *Drosophila* CPR may be a physiological response equivalent to CPR observed in other animals. Thus, *Drosophila* may shed new light on the regulation of CPR and provide a deeper understanding of the relationship between CPR and metabolism.

Acknowledgements

We are grateful to Drs. Anupama Dahanukar, Hubert Amrein, Greg Suh, and Paul A. Garrity and the Bloomington Drosophila Fly Stock Center for the fly lines. We thank members of the Hamada laboratory for critical comments and advice on the manuscript, Dr. Richard A. Lang and members of his laboratory for comments and kindly sharing reagents, Dr. Satoshi Namekawa for kindly sharing reagents, and Matthew Batie and Nathan T. Petts for design and construction of the behavioral assays and red-light illumination apparatus. This research was supported by a Trustee Grant and RIP funding from Cincinnati Children's Hospital, JST (Japan Science and Technology)/Precursory Research for Embryonic Science and Technology (PRESTO), the March of Dimes, and NIH R01 grant GM107582 and NIH R21 grant NS112890 to F.N.H. S.H.S. is a Latin American Fellow in the Biomedical Sciences supported by the Pew Charitable Trusts. Research in the laboratory of J.C.C. is supported by NIH R01 grant DK124068.

Contributions

F.N.H and Y.U designed the experiments. Y.U, E.N., T.N. and J.S. performed the temperature preference behavioral assays and data analysis. Y.U., S.H.S., S.S.U. and J.C. performed the feeding experiments and the data analysis. F.N.H and Y.U wrote the manuscript.

Declaration of Interest

The authors declare no competing interests

REFERENCES

1. Root, C.M., Ko, K.I., Jafari, A., and Wang, J.W. (2011). Presynaptic facilitation by neuropeptide signaling mediates odor-driven food search. Cell *145*, 133-144. 10.1016/j.cell.2011.02.008.

2. Kain, P., and Dahanukar, A. (2015). Secondary taste neurons that convey sweet taste and starvation in the Drosophila brain. Neuron *85*, 819-832. 10.1016/j.neuron.2015.01.005.

3. Soria-Gomez, E., Bellocchio, L., Reguero, L., Lepousez, G., Martin, C., Bendahmane, M., Ruehle, S., Remmers, F., Desprez, T., Matias, I., et al. (2014). The endocannabinoid system controls food intake via olfactory processes. Nat Neurosci *17*, 407-415. 10.1038/nn.3647.

4. Hanci, D., and Altun, H. (2016). Hunger state affects both olfactory abilities and gustatory sensitivity. Eur Arch Otorhinolaryngol *273*, 1637-1641. 10.1007/s00405-015-3589-6.

5. Keys, A., Brožek, J., Henschel, A., Mickelsen, O., and Taylor, H.L. (1950). The Biology of Human Starvation. University of Minnesota Press.

6. Piccione, G., Caola, G., and Refinetti, R. (2002). Circadian modulation of starvation-induced hypothermia in sheep and goats. Chronobiology international *19*, 531-541.

7. Sakurada, S., Shido, O., Sugimoto, N., Hiratsuka, Y., Yoda, T., and Kanosue, K. (2000). Autonomic and behavioural thermoregulation in starved rats. The Journal of physiology *526 Pt 2*, 417-424.

8. Chen, Y., and Knight, Z.A. (2016). Making sense of the sensory regulation of hunger neurons. Bioessays *38*, 316-324. 10.1002/bies.201500167.

9. Power, M.L., and Schulkin, J. (2008). Anticipatory physiological regulation in feeding biology: cephalic phase responses. Appetite *50*, 194-206. 10.1016/j.appet.2007.10.006.

10. Zafra, M.A., Molina, F., and Puerto, A. (2006). The neural/cephalic phase reflexes in the physiology of nutrition. Neuroscience and biobehavioral reviews *30*, 1032-1044. 10.1016/j.neubiorev.2006.03.005.

11. Pavlov, I.P. (1902). The Work of the Digestive Glands. London : Charles Griffin.

12. Garrison, J.L., and Knight, Z.A. (2017). Linking smell to metabolism and aging. Science *358*, 718-719. 10.1126/science.aao5474.

13. LeBlanc, J. (2000). Nutritional implications of cephalic phase thermogenic responses. Appetite *34*, 214-216. 10.1006/appe.1999.0283.

14. LeBlanc, J., and Cabanac, M. (1989). Cephalic postprandial thermogenesis in human subjects. Physiol Behav *46*, 479-482.

15. LeBlanc, J., Cabanac, M., and Samson, P. (1984). Reduced postprandial heat production with gavage as compared with meal feeding in human subjects. The American journal of physiology *246*, E95-101. 10.1152/ajpendo.1984.246.1.E95.

16. Sayeed, O., and Benzer, S. (1996). Behavioral genetics of thermosensation and hygrosensation in Drosophila. Proceedings of the National Academy of Sciences of the United States of America *93*, 6079-6084.

17. Dillon, M.E., Wang, G., Garrity, P.A., and Huey, R.B. (2009). Review: Thermal preference in Drosophila. J Therm Biol *34*, 109-119. 10.1016/j.jtherbio.2008.11.007.

18. Stevenson, R.D. (1985). Body size and limits to the daily range of body temperature in terrestrial ectotherms. Am. Nat. , 102-117.

19. Stevenson, R.D. (1985). The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. The American Naturalist *126*.

20. Lin, S., Senapati, B., and Tsao, C.H. (2019). Neural basis of hunger-driven behaviour in Drosophila. Open Biol *9*, 180259. 10.1098/rsob.180259.

21. Zhang, D.W., Xiao, Z.J., Zeng, B.P., Li, K., and Tang, Y.L. (2019). Insect Behavior and Physiological Adaptation Mechanisms Under Starvation Stress. Frontiers in physiology *10*, 163. 10.3389/fphys.2019.00163.

22. Umezaki, Y., Hayley, S.E., Chu, M.L., Seo, H.W., Shah, P., and Hamada, F.N. (2018). Feeding-State-Dependent Modulation of Temperature Preference Requires Insulin Signaling in Drosophila Warm-Sensing Neurons. Current biology : CB. 10.1016/j.cub.2018.01.060.

23. Berrigan, D., and Partridge, L. (1997). Influence of temperature and activity on the metabolic rate of adult Drosophila melanogaster. Comp Biochem Physiol A Physiol *118*, 1301-1307.

24. Schilman, P.E., Waters, J.S., Harrison, J.F., and Lighton, J.R. (2011). Effects of temperature on responses to anoxia and oxygen reperfusion in Drosophila melanogaster. The Journal of experimental biology *214*, 1271-1275. 10.1242/jeb.052357.

25. Ja, W.W., Carvalho, G.B., Mak, E.M., de la Rosa, N.N., Fang, A.Y., Liong, J.C., Brummel, T., and Benzer, S. (2007). Prandiology of Drosophila and the CAFE assay. Proceedings of the National Academy of Sciences of the United States of America *104*, 8253-8256. 10.1073/pnas.0702726104.

26. Itskov, P.M., Moreira, J.M., Vinnik, E., Lopes, G., Safarik, S., Dickinson, M.H., and Ribeiro, C. (2014). Automated monitoring and quantitative analysis of feeding behaviour in Drosophila. Nature communications *5*, 4560. 10.1038/ncomms5560.

27. Wang, G.H., and Wang, L.M. (2019). Recent advances in the neural regulation of feeding behavior in adult Drosophila. J Zhejiang Univ Sci B *20*, 541-549. 10.1631/jzus.B1900080.

28. Pool, A.H., and Scott, K. (2014). Feeding regulation in Drosophila. Curr Opin Neurobiol *29*, 57-63. 10.1016/j.conb.2014.05.008.

29. Snell, N.J., Fisher, J.D., Hartmann, G.G., Zolyomi, B., Talay, M., and Barnea, G. (2022). Complex representation of taste quality by second-order gustatory neurons in Drosophila. Current biology : CB *32*, 3758-3772 e3754. 10.1016/j.cub.2022.07.048.

30. Kim, H., Kirkhart, C., and Scott, K. (2017). Long-range projection neurons in the taste circuit of Drosophila. Elife *6*. 10.7554/eLife.23386.

31. Liu, W.W., Mazor, O., and Wilson, R.I. (2015). Thermosensory processing in the Drosophila brain. Nature *519*, 353-357. 10.1038/nature14170.

32. Frank, D.D., Jouandet, G.C., Kearney, P.J., Macpherson, L.J., and Gallio, M. (2015). Temperature representation in the Drosophila brain. Nature *519*, 358-361. 10.1038/nature14284.

33. Cedernaes, J., Waldeck, N., and Bass, J. (2019). Neurogenetic basis for circadian regulation of metabolism by the hypothalamus. Genes Dev *33*, 1136-1158. 10.1101/gad.328633.119.

34. Longo, V.D., and Panda, S. (2016). Fasting, Circadian Rhythms, and Time-Restricted Feeding in Healthy Lifespan. Cell Metab *23*, 1048-1059. 10.1016/j.cmet.2016.06.001.

35. Hirayama, M., Mure, L., S, and Panda, S. (2018). Circadian regulation of energy intake in mammals. Current Opinion in Physiology *5*, 141-148.

36. Greenhill, C. (2018). Benefits of time-restricted feeding. Nat Rev Endocrinol *14*, 626. 10.1038/s41574-018-0093-2.

37. Chaix, A., Manoogian, E.N.C., Melkani, G.C., and Panda, S. (2019). Time-Restricted Eating to Prevent and Manage Chronic Metabolic Diseases. Annu Rev Nutr *39*, 291-315. 10.1146/annurev-nutr-082018-124320.

38. Hamada, F.N., Rosenzweig, M., Kang, K., Pulver, S.R., Ghezzi, A., Jegla, T.J., and Garrity, P.A. (2008). An internal thermal sensor controlling temperature preference in Drosophila. Nature *454*, 217-220. nature07001 [pii] 10.1038/nature07001.

39. Biolchini, M., Murru, E., Anfora, G., Loy, F., Banni, S., Crnjar, R., and Sollai, G. (2017). Fat storage in Drosophila suzukii is influenced by different dietary sugars in relation to their palatability. PloS one *12*, e0183173. 10.1371/journal.pone.0183173.

40. Park, J.H., Carvalho, G.B., Murphy, K.R., Ehrlich, M.R., and Ja, W.W. (2017). Sucralose Suppresses Food Intake. Cell Metab *25*, 484-485. 10.1016/j.cmet.2017.02.011.

41. Dahanukar, A., Lei, Y.T., Kwon, J.Y., and Carlson, J.R. (2007). Two Gr genes underlie sugar reception in Drosophila. Neuron *56*, 503-516. 10.1016/j.neuron.2007.10.024.

42. Dus, M., Min, S., Keene, A.C., Lee, G.Y., and Suh, G.S. (2011). Taste-independent detection of the caloric content of sugar in Drosophila. Proceedings of the National Academy of Sciences of the United States of America *108*, 11644-11649. 10.1073/pnas.1017096108.

43. Wang, Q.P., Lin, Y.Q., Zhang, L., Wilson, Y.A., Oyston, L.J., Cotterell, J., Qi, Y., Khuong, T.M., Bakhshi, N., Planchenault, Y., et al. (2016). Sucralose Promotes Food Intake through NPY and a Neuronal Fasting Response. Cell Metab *24*, 75-90. 10.1016/j.cmet.2016.06.010.

44. Ro, J., Harvanek, Z.M., and Pletcher, S.D. (2014). FLIC: high-throughput, continuous analysis of feeding behaviors in Drosophila. PloS one *9*, e101107. 10.1371/journal.pone.0101107.

45. Yavuz, A., Jagge, C., Slone, J., and Amrein, H. (2014). A genetic tool kit for cellular and behavioral analyses of insect sugar receptors. Fly *8*, 189-196. 10.1080/19336934.2015.1050569.

46. Fujii, S., Yavuz, A., Slone, J., Jagge, C., Song, X., and Amrein, H. (2015). Drosophila sugar receptors in sweet taste perception, olfaction, and internal nutrient sensing. Current biology : CB *25*, 621-627. 10.1016/j.cub.2014.12.058.

47. Thoma, V., Knapek, S., Arai, S., Hartl, M., Kohsaka, H., Sirigrivatanawong, P., Abe, A., Hashimoto, K., and Tanimoto, H. (2016). Functional dissociation in sweet taste receptor neurons between and within taste organs of Drosophila. Nature communications *7*, 10678. 10.1038/ncomms10678.

48. Baines, R.A., Uhler, J.P., Thompson, A., Sweeney, S.T., and Bate, M. (2001). Altered electrical properties in Drosophila neurons developing without synaptic transmission. The Journal of neuroscience : the official journal of the Society for Neuroscience *21*, 1523-1531.

49. Klapoetke, N.C., Murata, Y., Kim, S.S., Pulver, S.R., Birdsey-Benson, A., Cho, Y.K., Morimoto, T.K., Chuong, A.S., Carpenter, E.J., Tian, Z., et al. (2014). Independent optical excitation of distinct neural populations. Nat Methods *11*, 338-346. 10.1038/nmeth.2836.

50. Simpson, J.H., and Looger, L.L. (2018). Functional Imaging and Optogenetics in Drosophila. Genetics *208*, 1291-1309. 10.1534/genetics.117.300228.

51. Ni, L., Klein, M., Svec, K.V., Budelli, G., Chang, E.C., Ferrer, A.J., Benton, R., Samuel, A.D., and Garrity, P.A. (2016). The Ionotropic Receptors IR21a and IR25a mediate cool sensing in Drosophila. Elife *5*. 10.7554/eLife.13254.

52. Umezaki, Y., Hayley, S.E., Chu, M.L., Seo, H.W., Shah, P., and Hamada, F.N. (2018). Feeding-State-Dependent Modulation of Temperature Preference Requires Insulin Signaling in Drosophila Warm-Sensing Neurons. Current biology : CB *28*, 779-787.e773. 10.1016/j.cub.2018.01.060.

53. Tang, X., Platt, M.D., Lagnese, C.M., Leslie, J.R., and Hamada, F.N. (2013). Temperature integration at the AC thermosensory neurons in Drosophila. The Journal of neuroscience : the official journal of the Society for Neuroscience *33*, 894-901. 10.1523/JNEUROSCI.1894-12.2013.

54. Nassel, D.R., and Wegener, C. (2011). A comparative review of short and long neuropeptide F signaling in invertebrates: Any similarities to vertebrate neuropeptide Y signaling? Peptides *32*, 1335-1355. 10.1016/j.peptides.2011.03.013.

55. Geoghegan, J.G., Lawson, D.C., Cheng, C.A., Opara, E., Taylor, I.L., and Pappas, T.N. (1993). Intracerebroventricular neuropeptide Y increases gastric and pancreatic secretion in the dog. Gastroenterology *105*, 1069-1077. 10.1016/0016-5085(93)90951-8.

56. Lee, M.C., Lawson, D.C., and Pappas, T.N. (1994). Neuropeptide Y functions as a physiologic regulator of cephalic phase acid secretion. Regul Pept *52*, 227-234. 10.1016/0167-0115(94)90057-4.

57. Dus, M., Lai, J.S., Gunapala, K.M., Min, S., Tayler, T.D., Hergarden, A.C., Geraud, E., Joseph, C.M., and Suh, G.S. (2015). Nutrient Sensor in the Brain Directs the Action of the Brain-Gut Axis in Drosophila. Neuron *87*, 139-151. 10.1016/j.neuron.2015.05.032.

58. Lee, G., and Park, J.H. (2004). Hemolymph sugar homeostasis and starvation-induced hyperactivity affected by genetic manipulations of the adipokinetic hormone-encoding gene in Drosophila melanogaster. Genetics *167*, 311-323.

59. Kim, S.K., and Rulifson, E.J. (2004). Conserved mechanisms of glucose sensing and regulation by Drosophila corpora cardiaca cells. Nature *431*, 316-320. 10.1038/nature02897.

60. Okamoto, N., Yamanaka, N., Yagi, Y., Nishida, Y., Kataoka, H., O'Connor, M.B., and Mizoguchi, A. (2009). A fat body-derived IGF-like peptide regulates postfeeding growth in Drosophila. Dev Cell *17*, 885-891. 10.1016/j.devcel.2009.10.008.

61. Slaidina, M., Delanoue, R., Gronke, S., Partridge, L., and Leopold, P. (2009). A Drosophila insulin-like peptide promotes growth during nonfeeding states. Dev Cell *17*, 874-884. 10.1016/j.devcel.2009.10.009.

62. Bai, H., Kang, P., and Tatar, M. (2012). Drosophila insulin-like peptide-6 (dilp6) expression from fat body extends lifespan and represses secretion of Drosophila insulin-like peptide-2 from the brain. Aging Cell *11*, 978-985. 10.1111/acel.12000.

63. Woodcock, K.J., Kierdorf, K., Pouchelon, C.A., Vivancos, V., Dionne, M.S., and Geissmann, F. (2015). Macrophage-derived upd3 cytokine causes impaired glucose homeostasis and reduced lifespan in Drosophila fed a lipid-rich diet. Immunity *42*, 133-144. 10.1016/j.immuni.2014.12.023.

64. Rajan, A., and Perrimon, N. (2012). Drosophila cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion. Cell *151*, 123-137. 10.1016/j.cell.2012.08.019.

65. Xu, K., Zheng, X., and Sehgal, A. (2008). Regulation of feeding and metabolism by neuronal and peripheral clocks in Drosophila. Cell Metab *8*, 289-300. 10.1016/j.cmet.2008.09.006.

66. Kaneko, H., Head, L.M., Ling, J., Tang, X., Liu, Y., Hardin, P.E., Emery, P., and Hamada, F.N. (2012). Circadian Rhythm of Temperature Preference and Its Neural Control in Drosophila. Current biology : CB *22*, 1851-1857. S0960-9822(12)00934-7 [pii] 10.1016/j.cub.2012.08.006.

67. Masek, P., and Keene, A.C. (2013). Drosophila fatty acid taste signals through the PLC pathway in sugar-sensing neurons. PLoS genetics *9*, e1003710. 10.1371/journal.pgen.1003710.

68. Ahn, J.E., Chen, Y., and Amrein, H. (2017). Molecular basis of fatty acid taste in Drosophila. Elife *6*. 10.7554/eLife.30115.

69. Brown, E.B., Shah, K.D., Palermo, J., Dey, M., Dahanukar, A., and Keene, A.C. (2021). Ir56d-dependent fatty acid responses in Drosophila uncover taste discrimination between different classes of fatty acids. Elife *10*. 10.7554/eLife.67878.

70. Croset, V., Schleyer, M., Arguello, J.R., Gerber, B., and Benton, R. (2016). A molecular and neuronal basis for amino acid sensing in the Drosophila larva. Sci Rep *6*, 34871. 10.1038/srep34871.

71. Ganguly, A., Pang, L., Duong, V.K., Lee, A., Schoniger, H., Varady, E., and Dahanukar, A. (2017). A Molecular and Cellular Context-Dependent Role for Ir76b in Detection of Amino Acid Taste. Cell Rep *18*, 737-750. 10.1016/j.celrep.2016.12.071.

72. Chen, Y.D., and Dahanukar, A. (2017). Molecular and Cellular Organization of Taste Neurons in Adult Drosophila Pharynx. Cell Rep *21*, 2978-2991. 10.1016/j.celrep.2017.11.041.

73. Steck, K., Walker, S.J., Itskov, P.M., Baltazar, C., Moreira, J.M., and Ribeiro, C. (2018). Internal amino acid state modulates yeast taste neurons to support protein homeostasis in Drosophila. Elife *7*. 10.7554/eLife.31625.

74. Nederkoorn, C., Smulders, F.T., and Jansen, A. (2000). Cephalic phase responses, craving and food intake in normal subjects. Appetite *35*, 45-55. 10.1006/appe.2000.0328.

75. Musso, P.Y., Lampin-Saint-Amaux, A., Tchenio, P., and Preat, T. (2017). Ingestion of artificial sweeteners leads to caloric frustration memory in Drosophila. Nature communications *8*, 1803. 10.1038/s41467-017-01989-0.

76. Gruber, F., Knapek, S., Fujita, M., Matsuo, K., Bracker, L., Shinzato, N., Siwanowicz, I., Tanimura, T., and Tanimoto, H. (2013). Suppression of conditioned odor approach by feeding is independent of taste and nutritional value in Drosophila. Current biology : CB *23*, 507-514. 10.1016/j.cub.2013.02.010.

77. Huetteroth, W., Perisse, E., Lin, S., Klappenbach, M., Burke, C., and Waddell, S. (2015). Sweet taste and nutrient value subdivide rewarding dopaminergic neurons in Drosophila. Current biology : CB *25*, 751-758. 10.1016/j.cub.2015.01.036.

78. Inagaki, H.K., Jung, Y., Hoopfer, E.D., Wong, A.M., Mishra, N., Lin, J.Y., Tsien, R.Y., and Anderson, D.J. (2014). Optogenetic control of Drosophila using a red-shifted channelrhodopsin reveals experience-dependent influences on courtship. Nat Methods *11*, 325-332. 10.1038/nmeth.2765.

79. Herness, S., and Zhao, F.L. (2009). The neuropeptides CCK and NPY and the changing view of cell-to-cell communication in the taste bud. Physiol Behav *97*, 581-591. 10.1016/j.physbeh.2009.02.043.

80. Bharucha, K.N., Tarr, P., and Zipursky, S.L. (2008). A glucagon-like endocrine pathway in Drosophila modulates both lipid and carbohydrate homeostasis. The Journal of experimental biology *211*, 3103-3110. 10.1242/jeb.016451.

81. Giebultowicz, J.M. (2018). Circadian regulation of metabolism and healthspan in Drosophila. Free Radic Biol Med *119*, 62-68. 10.1016/j.freeradbiomed.2017.12.025.

82. Mazzoccoli, G., Pazienza, V., and Vinciguerra, M. (2012). Clock genes and clock-controlled genes in the regulation of metabolic rhythms. Chronobiology international *29*, 227-251. 10.3109/07420528.2012.658127.

83. Lin, Y., Han, M., Shimada, B., Wang, L., Gibler, T.M., Amarakone, A., Awad, T.A., Stormo, G.D., Van Gelder, R.N., and Taghert, P.H. (2002). Influence of the period-dependent circadian clock on diurnal, circadian, and aperiodic gene expression in Drosophila melanogaster. Proceedings of the National Academy of Sciences of the United States of America *99*, 9562-9567. 10.1073/pnas.132269699.

84. Bozek, K., Relogio, A., Kielbasa, S.M., Heine, M., Dame, C., Kramer, A., and Herzel, H. (2009). Regulation of clock-controlled genes in mammals. PloS one *4*, e4882. 10.1371/journal.pone.0004882.

85. Chatterjee, A., Tanoue, S., Houl, J.H., and Hardin, P.E. (2010). Regulation of gustatory physiology and appetitive behavior by the Drosophila circadian clock. Current biology : CB *20*, 300-309. 10.1016/j.cub.2009.12.055.

86. Tang, X., Roessingh, S., Hayley, S.E., Chu, M.L., Tanaka, N.K., Wolfgang, W., Song, S., Stanewsky, R., and Hamada, F.N. (2017). The role of PDF neurons in setting preferred temperature before dawn in Drosophila. Elife *6*. 10.7554/eLife.23206.

87. Alpert, M.H., Frank, D.D., Kaspi, E., Flourakis, M., Zaharieva, E.E., Allada, R., Para, A., and Gallio, M. (2020). A Circuit Encoding Absolute Cold Temperature in Drosophila. Current biology : CB *30*, 2275-2288.e2275. 10.1016/j.cub.2020.04.038.

88. Marin, E.C., Büld, L., Theiss, M., Sarkissian, T., Roberts, R.J.V., Turnbull, R., Tamimi, I.F.M., Pleijzier, M.W., Laursen, W.J., Drummond, N., et al. (2020). Connectomics Analysis Reveals First-, Second-, and Third-Order Thermosensory and Hygrosensory Neurons in the Adult Drosophila Brain. Current biology : CB *30*, 3167-3182.e3164. 10.1016/j.cub.2020.06.028.

89. Yadlapalli, S., Jiang, C., Bahle, A., Reddy, P., Meyhofer, E., and Shafer, O.T. (2018). Circadian clock neurons constantly monitor environmental temperature to set sleep timing. Nature. 10.1038/nature25740.

90. Jin, X., Tian, Y., Zhang, Z.C., Gu, P., Liu, C., and Han, J. (2021). A subset of DN1p neurons integrates thermosensory inputs to promote wakefulness via CNMa signaling. Current biology : CB *31*, 2075-2087 e2076. 10.1016/j.cub.2021.02.048.

91. King, A.N., Barber, A.F., Smith, A.E., Dreyer, A.P., Sitaraman, D., Nitabach, M.N., Cavanaugh, D.J., and Sehgal, A. (2017). A Peptidergic Circuit Links the Circadian Clock to Locomotor Activity. Current biology : CB *27*, 1915-1927 e1915. 10.1016/j.cub.2017.05.089.

92. Barber, A.F., Fong, S.Y., Kolesnik, A., Fetchko, M., and Sehgal, A. (2021). Drosophila clock cells use multiple mechanisms to transmit time-of-day signals in the brain. Proceedings of the National Academy of Sciences of the United States of America *118*. 10.1073/pnas.2019826118.

93. Cao, C., and Brown, M.R. (2001). Localization of an insulin-like peptide in brains of two flies. Cell and tissue research *304*, 317-321. 10.1007/s004410100367.

94. Brogiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R., and Hafen, E. (2001). An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control. Current biology : CB *11*, 213-221.

95. Ikeya, T., Galic, M., Belawat, P., Nairz, K., and Hafen, E. (2002). Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in Drosophila. Current biology : CB *12*, 1293-1300.

96. Rulifson, E.J., Kim, S.K., and Nusse, R. (2002). Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science *296*, 1118-1120. 10.1126/science.1070058.

97. Cavanaugh, D.J., Geratowski, J.D., Wooltorton, J.R., Spaethling, J.M., Hector, C.E., Zheng, X., Johnson, E.C., Eberwine, J.H., and Sehgal, A. (2014). Identification of a circadian output circuit for rest:activity rhythms in Drosophila. Cell *157*, 689-701. 10.1016/j.cell.2014.02.024.

98. Li, S., Yu, X., and Feng, Q. (2019). Fat Body Biology in the Last Decade. Annu Rev Entomol *64*, 315-333. 10.1146/annurev-ento-011118-112007.

99. Arrese, E.L., and Soulages, J.L. (2010). Insect fat body: energy, metabolism, and regulation. Annu Rev Entomol *55*, 207-225. 10.1146/annurev-ento-112408-085356.

100. Cintron-Colon, R., Sanchez-Alavez, M., Nguyen, W., Mori, S., Gonzalez-Rivera, R., Lien, T., Bartfai, T., Aid, S., Francois, J.C., Holzenberger, M., and Conti, B. (2017). Insulin-like growth factor 1 receptor regulates hypothermia during calorie restriction. Proceedings of the National Academy of Sciences of the United States of America *114*, 9731-9736. 10.1073/pnas.1617876114.

101. Goda, T., Leslie, J.R., and Hamada, F.N. (2014). Design and analysis of temperature preference behavior and its circadian rhythm in Drosophila. Journal of visualized experiments : JoVE, e51097. 10.3791/51097.

FIGURE

Figure 1. Hungry flies switch from cold to warm preference upon food detection.

(A) A schematic diagram of the experimental conditions. Flies were reared on fly food. Emerging flies were kept on fly food for one or two days (orange) and then starved with water-absorbing paper for one or two nights (gray). Starved flies were refed with either fly food (orange), 2 mL sucralose (blue), or glucose solution (green) absorbed paper and then examined for temperature preference (Tp) behavioral assays. Details are provided in the experimental procedures.

(B-D) Comparison of Tp of *white*^{1118} (w^{1118}) control flies between fed (white bar), starved (STV; gray bar), and refed (orange, blue, or green bar) states. Starved flies were refed with fly food (orange bar) for 5, 10, 30, or 60 min (1 hr) (B), 2.8 mM sucralose solution (blue bar) for 10 min or 1 hr (C), or 2.8 mM (equivalent to 5%) glucose solution (green bar) for 5 min, 10 min, or 1 hr (D). Behavioral experiments were performed at the specific time points, Zeitgeber time (ZT) 4-7. ZT0 and ZT12 are light on and light off, respectively. Dots on each bar indicate individual Tp in assays. Numbers in italics indicate the number of trials. The Shapiro-Wilk test was performed to test for normality. One-way ANOVA was performed to compare Tp between each refeeding condition. Tukey's post hoc test was used to compare differences between experimental and starved conditions (green stars). Data are presented as mean Tp with SEM. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$. ****$p < 0.0001$. NS indicates no significance.

(E) Comparison of Tp among the following conditions in w^{1118} (control) flies: fed (white bar), starved overnight (O/N) (water or STV; gray bar), refed with fly food (fly food; orange bar),

sucralose (blue bars), or glucose (green bars). The same data are used in Fig. 1B-D. The Shapiro-Wilk test was performed to test for normality. One-way ANOVA was performed to compare Tp between each refeeding condition. Red (or green) stars indicate Tukey's post hoc test comparing differences between experimental and fed (or starved) conditions, respectively. *p < 0.05. **p < 0.01. ***p < 0.001. ****p < 0.0001. NS indicates not significant.

(F) Feeding assay: The number of touches to water (gray), 2.8 mM (equivalent to 5%) glucose (green), or 2.8 mM sucralose in each solution (blue) was examined using w^{1118} flies starved for 1 day. A cumulative number of touches to water or sugar solution for 0-30 min was plotted. Two-way ANOVA was used for multiple comparisons. Blue and green stars show Fisher's LSD post hoc test comparing sucralose (blue stars) or glucose (green stars) solution feeding to water drinking. All data shown are means with SEM. *p < 0.05. **p < 0.01. ***p < 0.001. ****p < 0.0001.

Figure 2. Gustatory neurons are essential for taste-evoked warm preference.

(A-E) Comparison of Tp of flies between fed (white bar), starved (STV; gray bar), and refed (blue bar) states. Starved flies were refed with sucralose (blue bars) for 10 min.

(F) Schematic of optogenetic activation assay.

(G-J) Comparison of Tp of flies between fed (white bar), starved (STV; gray bar), and starved with all-trans-retinal (ATR; yellow bars), which is the chromophore required for CsChrimson activation. Gustatory neurons in starved flies were excited by red light pulses (flashing on and off at 10 Hz) for 10 min. Behavioral experiments were conducted at ZT4-7. Dots on each bar indicate individual Tp in assays. Numbers in italics indicate the number of trials. Shapiro-Wilk

test was performed for normality test. One-way ANOVA was used for statistical analysis. Green stars indicate Tukey's post hoc test compared between each experiment and the starved condition. Pooled data are shown in Fig. S3. All data shown are means with SEM. *p < 0.05. **p < 0.01. ***p < 0.001. ****p < 0.0001. NS indicates not significant.

Figure 3. Both warm- and cold-temperature-sensing neurons are involved with taste-evoked warm preference.

(A-E) Comparison of Tp of flies between fed (white bar), starved (STV; gray bar), and refed (blue bar) conditions. Starved flies were refed with sucralose (blue bars) for 10 min. The Shapiro-Wilk test was performed to test for normality. One-way ANOVA or Kruskal-Wallis test was used for statistical analysis. Green stars indicate Tukey's post hoc test or Dunn's test compared between each experiment and the starved condition. Pooled data are shown in Fig. S3. **(F and G)** Comparison of Tp between starved (STV; gray bar) and all-trans-retinal (ATR; yellow bars) starved flies. Warm neurons (F) or cold neurons (G) in starved flies expressed CsChrimson, which was excited by red light pulses for 10 min. The Shapiro-Wilk test was performed to test for normality. Student's t-test or Kolmogorov-Smirnov test was used for statistical analysis. (G) *tubGal80ts; R11F02-Gal4>uas-CsChrimson* flies were reared at 18°C, and emerged adults were collected and stored at 29°C. See Materials and Methods for details. These behavioral experiments were conducted on ZT4-7. All data shown are means with SEM. *p < 0.05. **p < 0.01. ***p < 0.001. ****p < 0.0001. NS indicates no significance. The same data from *UAS-Kir/+* in Fig. 2E are used in Fig. 3E.

Figure 4. Hunger signals are involved in taste-evoked warm preference.

Comparison of Tp of flies between fed (white bar), starved (STV; gray bar), and refed states (orange, blue, green, or dark green). Starved flies were refed with fly food (orange bar), sucralose (blue bar), or glucose (green bar) for 10 min. These behavioral experiments were conducted on ZT4-7. Dots on each bar indicate individual Tp in assays. Numbers in italics indicate the number of trials. Shapiro-Wilk test was performed for normality test. One-way ANOVA was used for statistical analysis. Green stars indicate Tukey's post hoc test compared between each experiment and the starved condition. Pooled data are shown in Fig. S3. All data shown are means with SEM. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$. ****$p < 0.0001$. NS indicates not significant.

Figure 5. Clock genes are involved in taste-evoked warm preference.

(A-D) Comparison of Tp of flies between fed (white circles), starved (STV; gray triangles), and refed (blue, green, or dark green squares) states. Flies were starved for 24 hrs. Starved flies were refed with sucralose (blue squares, A1-D1) or glucose for 10 min (green squares, A2-D2) or 1 hr (dark green squares, A3). After the 10 min or 1 hr refeeding, the temperature preference behavior assays were performed immediately at ZT1-3, 4-6, 7-9, and 10-12 in LD. The Shapiro-Wilk test was performed to test for normality. One-way ANOVA or Kruskal-Wallis test was used for statistical analysis. Stars indicate Tukey's post hoc test or Dunn's test compared between each experiment and starved condition at the same time point. All data shown are means with SEM. *$p<0.05$. **$p<0.01$. ***$p<0.001$. ****$p < 0.0001$.

(E) Comparison of Tp during daytime in each feeding state. One-way ANOVA or Kruskal-Wallis test was used for statistical analysis between ZT1-3 and ZT7-9 or ZT10-12. R and AR indicate rhythmic and arrhythmic, respectively, during the daytime.

Figure S1. The starvation period is unlikely to affect the ability to recover (Related to Fig. 3)

Comparison of Tp of w^{1118} control flies between fed (white bar), starved (STV; gray bar), and refed (STV; gray bar) and refed (blue or green bar). Flies were starved for overnight (STV1ON; gray bar) or 1.5 ON (STV1.5ON; dark gray bar), i.e. starved for 18-21 hrs. starved for 18-21 hrs or 26-29 hrs. Starved flies were refed with sucralose for 10 min (blue bar) or glucose for 10 min (green bar). These behavioral experiments were performed on ZT4-7. Dots on each bar indicate individual Tp in the assays. Numbers in italics indicate the number of trials. Per Shapiro-Wilk test was performed to test for normality. One-way ANOVA or Kruskal-Wallis test was used for statistical analysis. Green stars indicate Tukey's post hoc test or Dunn's test was used to compare between each experiment and starved condition. and starved condition. All data shown are means with sem. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$. ****$p < 0.0001$. NS indicates no significance. The same data from STV1ON, STV1ON+refed sucralose for 10 min and STV1ON+refed sucralose for 10 min in Fig. 1C-E are used in Fig. S1.

Figure S2. Number of touches to water, sucralose and glucose using *yw*, *per*^{01} and *tim*^{01} flies. (Related to Figures 1, 5, and 6)

Feeding assay: The number of touches to water (gray), 2.8 mM (equivalent to 5%) glucose (green), or 2.8 mM sucralose solution (blue) was examined using *yw*, *per*^{01}, or *tim*^{01} flies starved for 1 day. Cumulative number of touches for 0-30 min was plotted. Two-way ANOVA was used for multiple comparisons. Blue and green stars show Fisher's LSD post hoc test comparing sucralose (blue stars) or glucose (green stars) solution feeding to water drinking. Black stars show the statistical difference between sucralose and glucose refeeding. All data shown are means with SEM. *p < 0.05. *F*p < 0.01. ***p < 0.001. ****p < 0.0001.

Figure S3. Taste- or temperature-sensing neurons are not important for nutrient-(carbohydrate-) induced warm preference (Related to Figures 2 and 3).

Comparison of Tp of flies between fed (white bar), starved (STV; gray bar), and refed (orange, blue, green, or dark green bar) conditions. Starved flies were refed with fly food for 10 min (fly food; orange bar), sucralose for 10 min (blue bar), or glucose for 10 min (green bar) or 1 hr (dark green bar). These behavioral experiments were conducted on ZT4-7. The dots on each bar indicate individual Tp in the assays. Numbers in italics indicate the number of experiments. The Shapiro-Wilk test was performed to test for normality. One-way ANOVA or Kruskal-Wallis test was used for statistical analysis. Green stars indicate Tukey's post hoc test or Dunn's test compared between each experiment and the starved condition. All data shown are means with sem. *p < 0.05. **p < 0.01. ***p < 0.001. ****p < 0.0001. NS indicates no significance. The same data from *UAS-Kir/+* in Fig. 2E are used in Fig. S3I.

Figure S4. MODEL

A schematic diagram of Tp recovery by taste-evoked warm preference (blue arrows) and nutrient-induced warm preference (green arrows).

Materials and Methods

All flies were reared under 12 hr-light/12 hr-dark (LD) cycles at 25°C and 60-70% humidity in an incubator (DRoS33SD, Powers Scientific Inc.) with an electric timer (light on: 8am; light off: 8pm). The light intensity is 1000-1400 lux. All flies were reared on custom fly food recipe, with the following composition per 1 L of food: 6.0 g sucrose, 7.3 g agar, 44.6 g cornmeal, 22.3 g yeast and 16.3 mL molasses as described previously [52]. *white*^{1118} (*w*^{1118}) and *yellow*^{1} *white*^{1} (*y*^{1}*w*^{1}) flies were used as control flies. *EP5Δ; Gr64a*^{1} (*Gr5a*$^{-/-}$*; Gr64a*$^{-/-}$) was kindly provided by Dr. Anupama Dahanukar [41]. *R1; Gr5a-LexA; +; ΔGr61a, ΔGr64a-f* (*Gr5a*$^{-/-}$*; Gr61a*$^{-/-}$*, Gr64a-f*$^{-/-}$) were kindly provided by Dr. Hubert Amrein [45,46]. *Dh44-Gal4* was kindly provided by Dr. Greg Suh [42]. *TrpA1*^{SH}*-gal4* was kindly provided by Dr. Paul A. Garrity [38]. Other fly lines were provided by Bloomington Drosophila stock center and Vienna Drosophila Stock Center.

Temperature preference behavioral assay

The temperature preference behavior assays were examined using a gradient of temperatures, ranging from 16-34°C and were performed for 30 min in an environmental room maintained at 25°C /60%–70% humidity as described previously [22]. We prepared a total of 40-50 flies (male and female flies were mixed) for fed condition experiments and 90-100 flies for overnight(s) starved and refed condition experiments for one trial. Flies were never reused in subsequent trials. In starved and refed experiments, we prepared double the amount of flies required for one trial, as almost half of them died by starvation stress. Others climbed on the wall and ceiling (starved flies are usually hyperactive (Yang et al. 2015 PNAS)), even though we applied slippery coating

chemicals (PTFE; Cat# 665800, Sigma or byFormica PTFE Plus, https://byformica.com/products/fluon-plus-ptfe-escape-prevention-coating) to the plexiglass covers.

The behavioral assays were performed for 30 min at Zeitgeber time (ZT) 4-7 (Light on and light off are defined as ZT0 and ZT12, respectively), and starvation was initiated at ZT9-10 (starved for 1, 2 or 3 O/N are 18-21 hrs, 42-45 hrs or 66-69 hrs, respectively). As for STV1.5 for Fig. S1, starvation was initiated at ZT1-2 (26-29 hrs). For the starvation assays, the collected flies were maintained with our fly food for at least one day for their maturation and subsequently moved into plastic vials with 3 mL of distilled water which is absorbed by a Kimwipe paper. For the refeeding experiments, starved flies were transferred into plastic vials with 2 mL of 2.8 mM sugar solution (Sucralose water/ Glucose water) for a certain time period. Sugar solution is absorbed by the half size of a Kimwipe.

After the 30-min behavioral assay, the number of flies whose bodies were completely located on the apparatus were counted. Flies whose bodies were partially or completely located on the walls of the apparatus cover were not included in the data analysis. The percentage of flies within each one-degree temperature interval on the apparatus was calculated by dividing the number of flies within each one-degree interval by the total number of flies on the apparatus. The location of each one-degree interval was determined by measuring the temperature at 6 different points on the bottom of the apparatus. Data points were plotted as the percentage of flies within a one-degree temperature interval. The weighted mean of Tp was calculated by totaling the product of the percentage of flies within a one-degree temperature interval and the corresponding temperature (e.g., fractional number of flies x 15.5°C + fractional number of flies x 16.5°C

+…… fractional number of flies x 34.5°C). If the SEM of the averaged Tp was not < 0.3 after the five trials, we performed additional trials around 10 times until the SEM reached < 0.3.

Temperature preference rhythm (TPR) assay

For the TPR assays, we performed temperature preference behavior assays in different time windows during the day (zeitgeber time (ZT) or circadian time (CT) 1-3, 4-6, 7-9, and 10-12) as described previously [66,101]. Because starvation duration directly affects flies' Tp [22], starvation was initiated at each time window to adjust the starvation duration at each time point, which means flies were starved for 24 hrs or 48 hrs but not 1 or 2 O/N. Each behavioral assay was not examined during these time periods (ZT or CT0-1 and 11.5-13) because of large phenotype variation around light on and light off.

Optogenetic activation

For the optogenetic activation of the target neurons for behavioral assays, red-light-sensitive channelrodopsin, *UAS-CsChrimson*, was crossed with each Gal4 drivers. Flies were reared on fly food at 25°C and 60-70% humidity under LD cycles in an incubator (DRoS33SD, Powers Scientific Inc.) with an electric timer. After flies emerged, we collected adult flies and kept them with fly food for 1-2 days. Next day, flies were transferred into water with or without 0.8 mM all-trans retinal (ATR; #R2500, Sigma) diluted in dimethyl sulfoxide (DMSO; #472301, Sigma) for 2 O/N. To activate flies expressing UAS-CsChrimson crossed with Gal4 drivers, we employed 627 nm red-colored light-emitted diode (LED) equipped with pulse photoillumination

system (10 Hz, 0.08 $mWmm^{-2}$). Flies were irradiated with pulsed red light for 10 min, which is equivalent to refeeding time period. This photoillumination system was used in an incubator (Sanyo Scientific, MIR-154), and followed by temperature preference behavioral assays.

Optogenetic assay for *tubGal80^{ts}; R11F02-Gal4>uas-CsChrimson*

Because *R11F02-Gal4>uas-CsChrimson* flies died during the pupal stage, Gal4/Gal80^{ts} system was used to restrict *uas-CsChrimson* expression to the adult stage for optogenetc assays against the cold-sensing neurons (*R11F02-Gal4*-expressing neurons). Gal80^{ts} is a temperature sensitive allele of Gal80, which binds Gal4 and inhibits *CsChrimson* gene expression at 18°C. However, Gal80 is degraded at 29°C; in turn, the Gal4 activates *CsChrimson* gene expression (Southall et al. 2008 CSH protocol). *tubGal80^{ts}; R11F02-Gal4>uas-CsChrimson* flies were reared with fly food at 18°C. Emerged adult flies were collected and kept with fly food at 29°C. Next day, flies were transferred to water condition (starved condition) with or without 0.8 mM ATR for 2 O/N. Starved flies with or without ATR application were exposed to pulsed red light for 10 min (equivalent to refeeding period) and then immediately loaded in the behavior apparatus for behavioral assays to measure their Tp.

Feeding assay

To measure single fly feeding, we used *Drosophila* Fly Liquid Interaction Counter (FLIC) system (Ro et al. 2013 Plos one; Sable systems International https://www.sablesys.com). Groups of 1-2-day-old male and female flies were starved for 24 hrs, which was started from between

ZT4-5 (12-1pm). Individual flies were then loaded into FLIC monitors. Flies were acclimatized to the monitors for 30 min, with access to water provided in the food wells. To start the feeding assay, water was replaced with 2.8 mM Sucralose or 2.8 mM Glucose solution (equivalent to 5% Glucose conc.) and the number of licks (touches) were recorded for 30 min. Assays were performed at ZT4-7. To account for potential confounding effect of startle response when changing the food, wells where water was replaced with new water were used as a control. FLIC raw data was analyzed using the FLIC R code master (Pletcher Lab, available at https://github.com/PletcherLab/FLIC_R_Code). The numbers of licks were obtained and summed into 5-min windows bin, while the cumulative licks were obtained by the consecutive sum of the licks in these bins.

Fig. 1

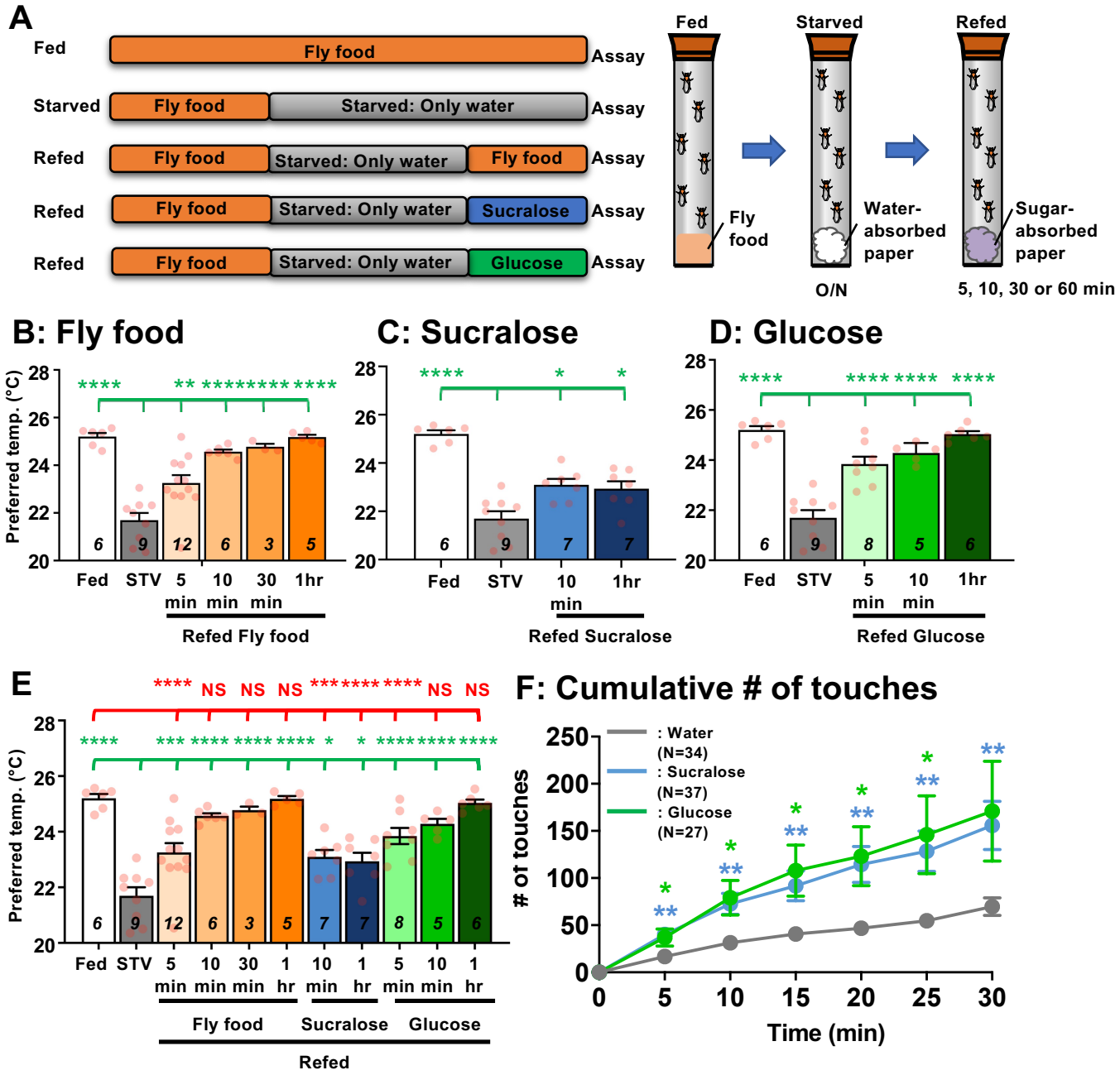

A
Fed
Fly food
Assay
Starved
Fly food
Starved: Only water
Assay
Refed
Fly food
Starved: Only water
Fly food
Assay
Refed
Fly food
Starved: Only water
Sucralose
Assay
Refed
Fly food
Starved: Only water
Glucose
Assay
Fed
Starved
Refed
Fly food
Water-absorbed paper
Sugar-absorbed paper
O/N
5, 10, 30 or 60 min
B: Fly food
C: Sucralose
D: Glucose
Preferred temp. (°C)
Fed
STV
5 min
10 min
30 min
1hr
Refed Fly food
Refed Sucralose
Refed Glucose
E
NS
Fly food
Sucralose
Glucose
Refed
F: Cumulative # of touches
: Water (N=34)
: Sucralose (N=37)
: Glucose (N=27)
# of touches
Time (min)

Fig. 2

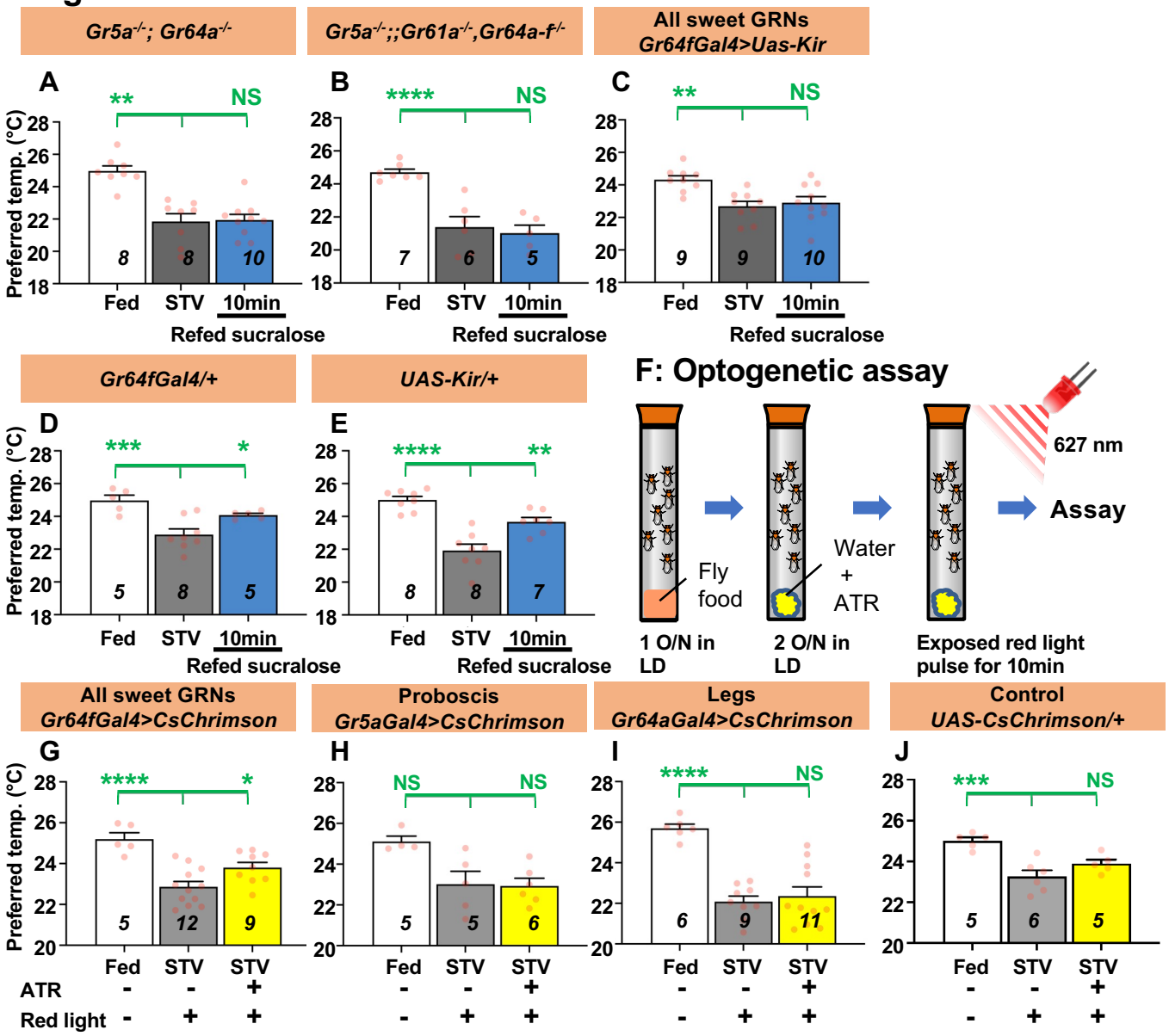

Gr5a-/-; Gr64a-/-
Gr5a-/-;;Gr61a-/-,Gr64a-f-/-
All sweet GRNs
Gr64fGal4>Uas-Kir
A
B
C
**
NS
****
Preferred temp. (°C)
Fed
STV
10min
Refed sucralose
Gr64fGal4/+
UAS-Kir/+
D
E
***
*
F: Optogenetic assay
Fly food
Water + ATR
627 nm
Assay
1 O/N in LD
2 O/N in LD
Exposed red light pulse for 10min
All sweet GRNs
Gr64fGal4>CsChrimson
Proboscis
Gr5aGal4>CsChrimson
Legs
Gr64aGal4>CsChrimson
Control
UAS-CsChrimson/+
G
H
I
J
NS
ATR
Red light

Fig. 3

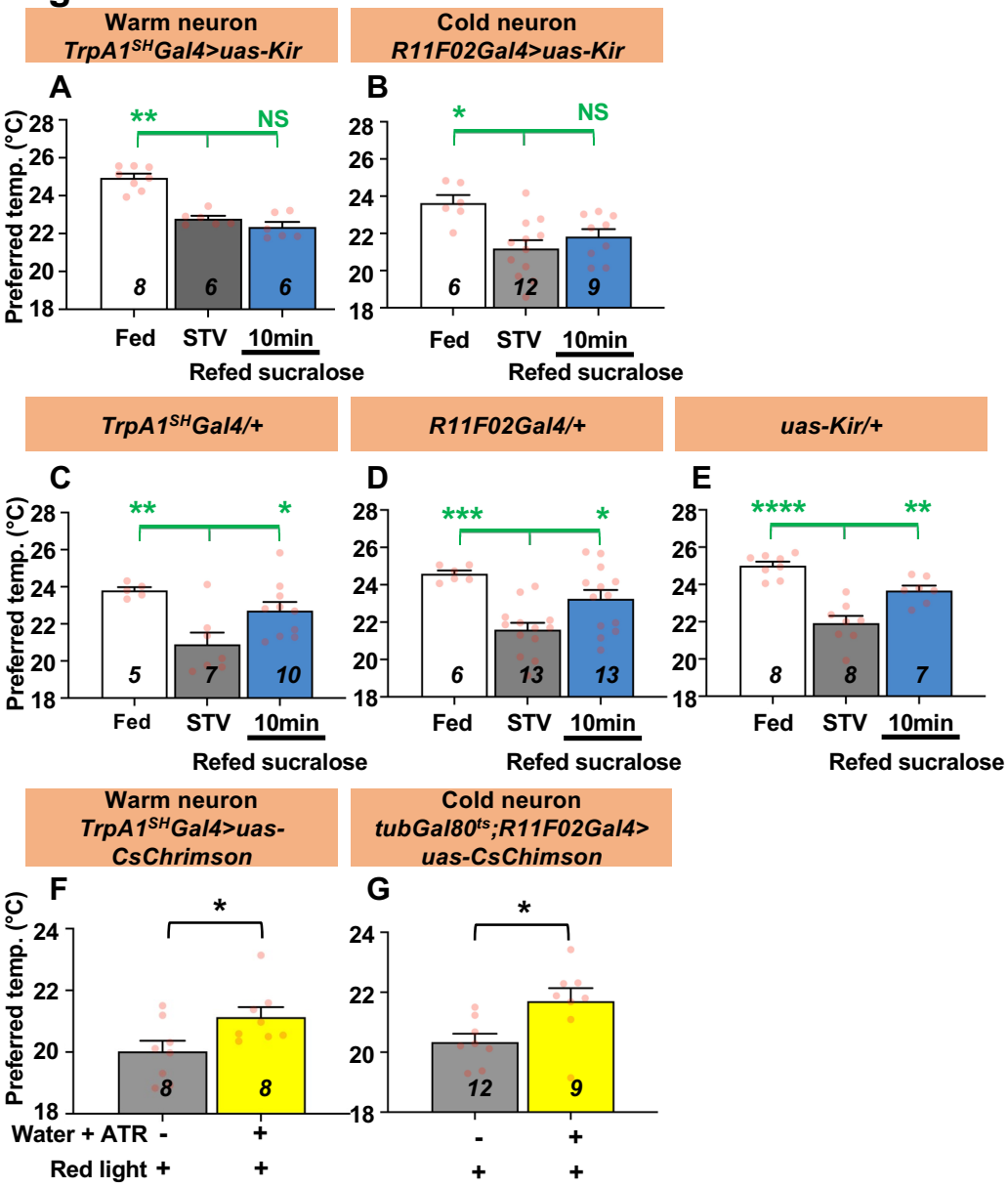

Warm neuron
TrpA1SHGal4>uas-Kir
Cold neuron
R11F02Gal4>uas-Kir
A
B
Preferred temp. (°C)
**
NS
*
NS
8
6
6
6
12
9
Fed
STV
10min
Refed sucralose
TrpA1SHGal4/+
R11F02Gal4/+
uas-Kir/+
C
D
E
**
*
***
*
****
**
5
7
10
6
13
13
8
8
7
Warm neuron
TrpA1SHGal4>uas-CsChrimson
Cold neuron
tubGal80ts;R11F02Gal4>uas-CsChimson
F
G
*
*
8
8
12
9
Water + ATR
Red light

Fig. 4

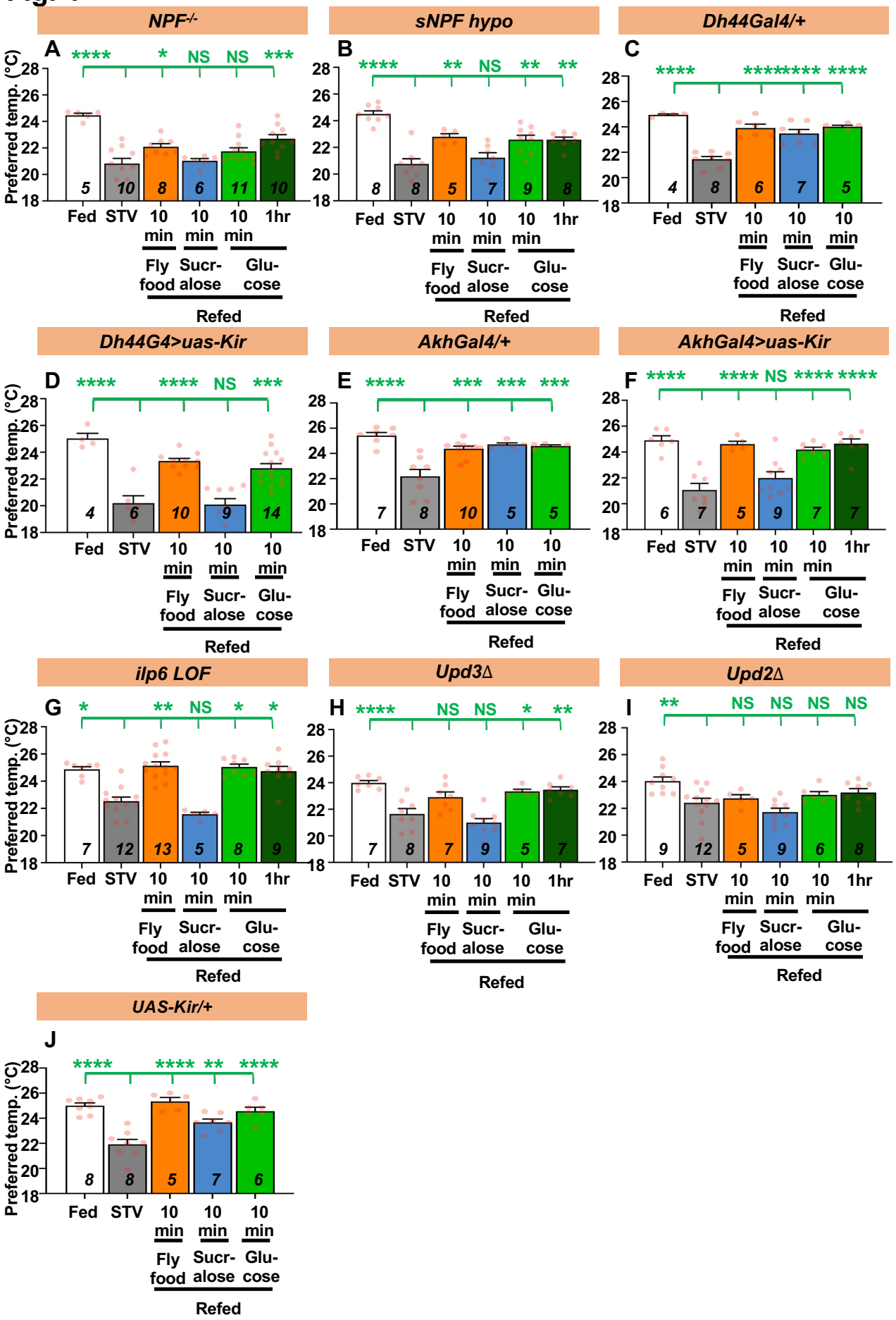
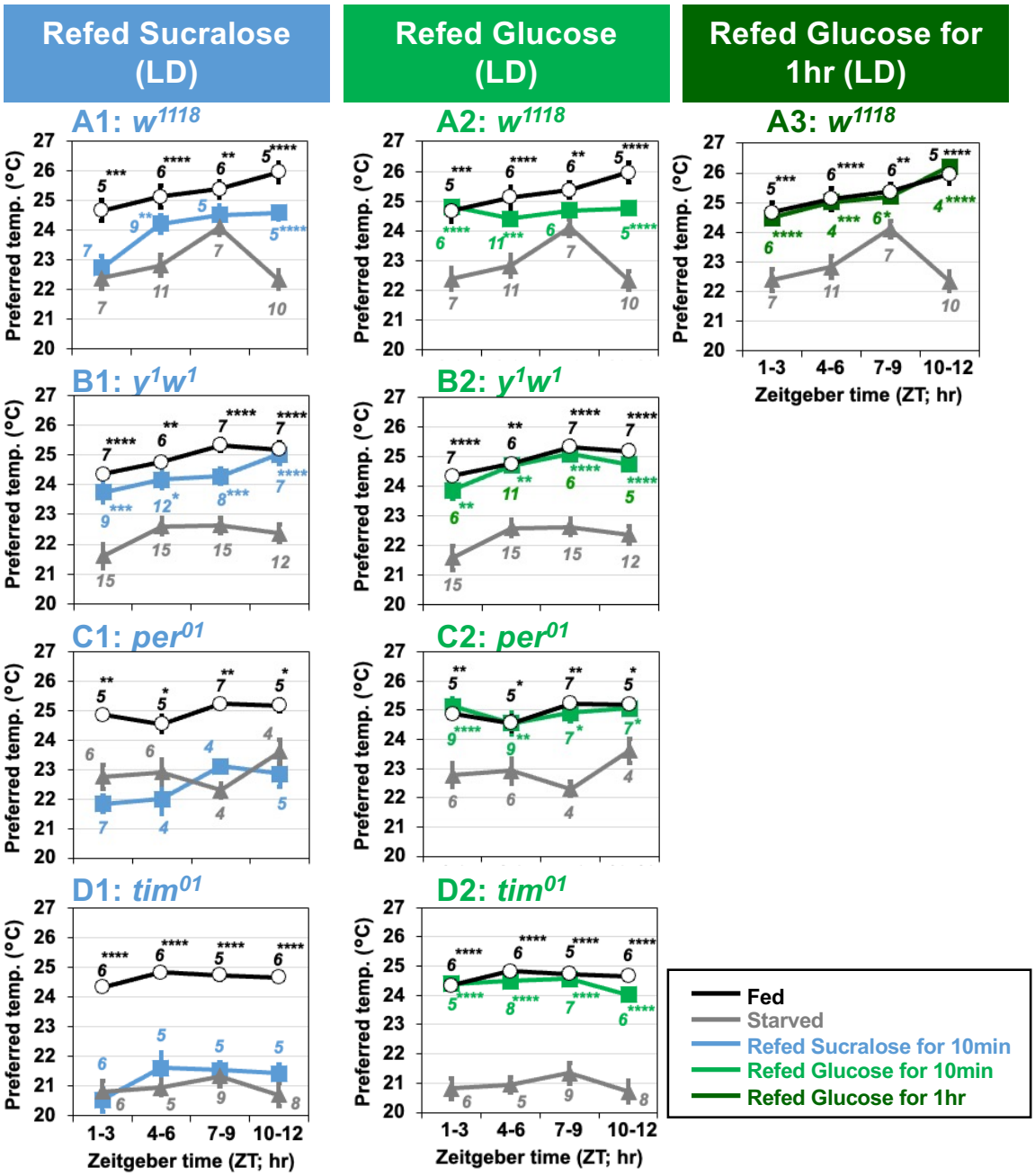

NPF-/-
A
**** * NS NS ***
5 10 8 6 11 10
sNPF hypo
B
**** ** NS ** **
8 8 5 7 9 8
Dh44Gal4/+
C
**** ******** ****
4 8 6 7 5
Dh44G4>uas-Kir
D
**** **** NS ***
4 6 10 9 14
AkhGal4/+
E
**** *** *** ***
7 8 10 5 5
AkhGal4>uas-Kir
F
**** **** NS **** ****
6 7 5 9 7 7
ilp6 LOF
G
* ** NS * *
7 12 13 5 8 9
Upd3Δ
H
**** NS NS * **
7 8 7 9 5 7
Upd2Δ
I
** NS NS NS NS
9 12 5 9 6 8
UAS-Kir/+
J
**** **** ** ****
8 8 5 7 6
Preferred temp. (°C)
28 26 24 22 20 18
Fed
STV
10 min Fly food
10 min Sucr-alose
10 min Glu-cose
1hr
Refed

Fig. 5



E

LD	Fed	Starvation	Refed Sucralose	Refed Glucose	Refed Glucose for 1hr
A: w1118	R	R	R	AR	R
B: y1w1	R	AR	AR	R	
C: per01	AR	AR	AR	AR	
D: tim01	AR	AR	AR	AR	

Fig. S1

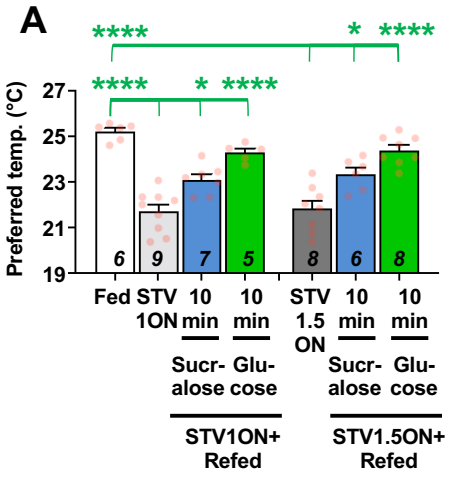

A
****
*
****
****
*
****
Preferred temp. (°C)
27
25
23
21
19
6
9
7
5
8
6
8
Fed
STV 1ON
10 min
10 min
STV 1.5 ON
10 min
10 min
Sucr-alose
Glu-cose
Sucr-alose
Glu-cose
STV1ON+ Refed
STV1.5ON+ Refed

Fig. S2

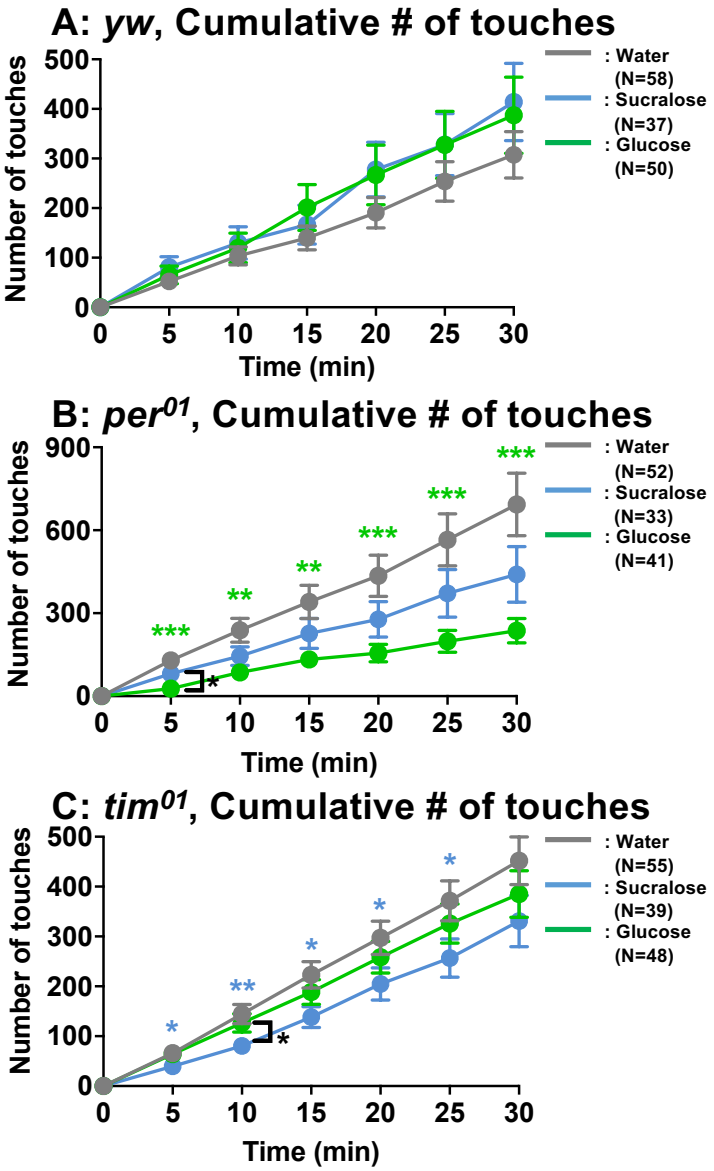

A: yw, Cumulative # of touches
Number of touches
Time (min)
: Water (N=58)
: Sucralose (N=37)
: Glucose (N=50)
B: per01, Cumulative # of touches
Number of touches
Time (min)
: Water (N=52)
: Sucralose (N=33)
: Glucose (N=41)
C: tim01, Cumulative # of touches
Number of touches
Time (min)
: Water (N=55)
: Sucralose (N=39)
: Glucose (N=48)

Fig. S3

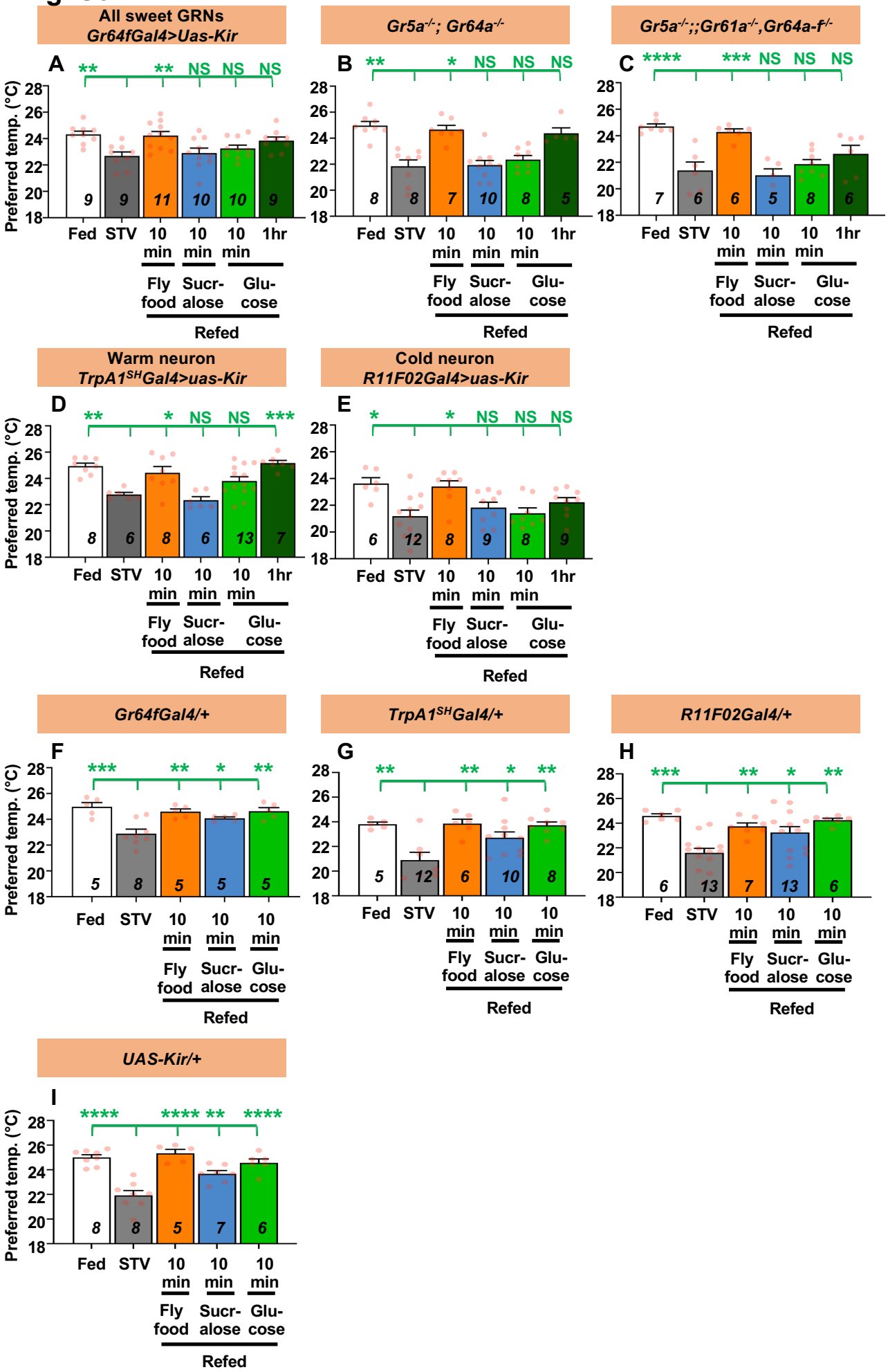
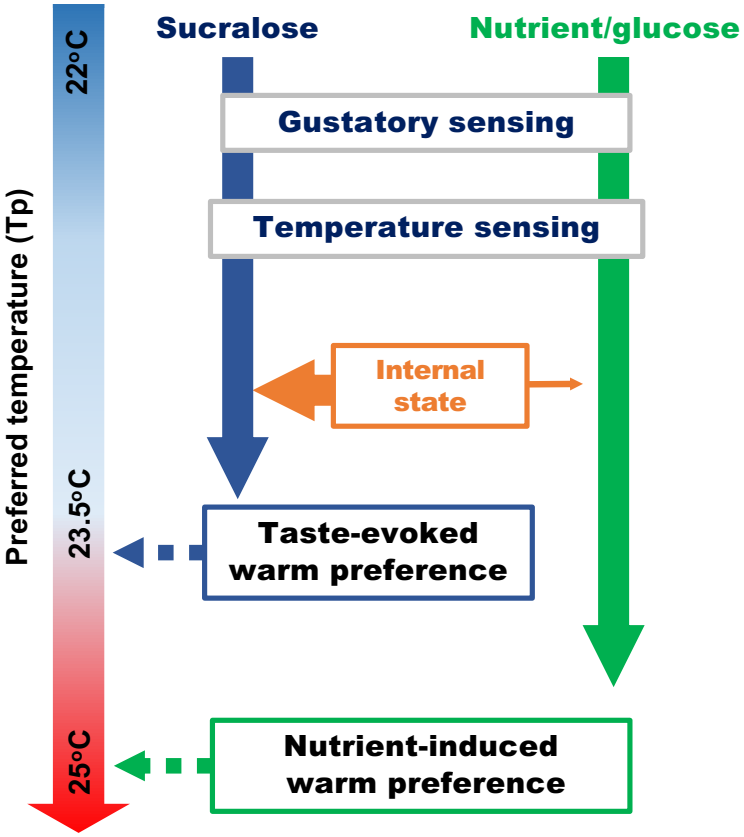

All sweet GRNs
Gr64fGal4>Uas-Kir
A
Gr5a-/-; Gr64a-/-
B
Gr5a-/-;;Gr61a-/-,Gr64a-f-/-
C
Warm neuron
TrpA1SHGal4>uas-Kir
D
Cold neuron
R11F02Gal4>uas-Kir
E
Gr64fGal4/+
F
TrpA1SHGal4/+
G
R11F02Gal4/+
H
UAS-Kir/+
I
Preferred temp. (°C)
Fed
STV
10 min
1hr
Fly food
Sucr-alose
Glu-cose
Refed

Fig. S4


Sucralose
Nutrient/glucose
Gustatory sensing
Temperature sensing
Internal state
Taste-evoked warm preference
Nutrient-induced warm preference
Preferred temperature (Tp)
22°C
23.5°C
25°C