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Independent, reciprocal neuromodulatory control of sweet and bitter taste sensitivity during starvation in *Drosophila*

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SUMMARY

An organism's behavioral decisions often depend upon the relative strength of appetitive and aversive sensory stimuli, the relative sensitivity to which can be modified by internal states like hunger. However, whether sensitivity to such opposing influences is modulated in a unidirectional or bidirectional manner is not clear. Starved flies exhibit increased sugar and decreased bitter sensitivity. It is widely believed that only sugar sensitivity changes, and that this masks bitter sensitivity. Here we use gene- and circuit-level manipulations to show that sweet- and bitter-sensitivity are independently and reciprocally regulated by starvation in *Drosophila*. We identify orthogonal neuromodulatory cascades that oppositely control peripheral taste sensitivity for each modality. Moreover, these pathways are recruited at increasing hunger levels, such that low-risk changes (higher sugar sensitivity) precede high-risk changes (lower sensitivity to potentially toxic resources). In this way, state intensity-dependent, reciprocal regulation of appetitive and aversive peripheral gustatory sensitivity permits flexible, adaptive feeding-decisions.

Keywords

Neuromodulation; Drosophila; gustatory system; decision making; dopamine; neuropeptide

INTRODUCTION

Changes in internal states, such as emotion, arousal, starvation and sleep, affect behavioral choices in animals (Blanchard and Blanchard, 1989; Sternson et al., 2013; Taghert and Nitabach, 2012). Typically, these state-dependent influences are multidimensional, scalable and time-variant: one state can modulate multiple physiological and behavioral parameters;

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SUPPLEMENTAL INFOROMATION

Supplemental Information includes Extended Experimental Procedures, figures can be found with this article online at xxx

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and the quantitative or qualitative changes caused by such modulation can vary with the intensity or duration of the state (Anderson and Adolphs, 2014). These prominent features of state-control enable animals to adjust their behavioral responses properly according to context or internal demands. However, understanding how these features are instantiated in the nervous system is challenging because it requires a comprehensive analysis of state-control pathways, including the identification of interoceptive mechanisms, neuromodulatory influences, targets of neuromodulation, and consequent behavioral changes (Bargmann, 2012).

The control of feeding in starved *Drosophila melanogaster* provides an attractive model for state-dependent control of behavior, because of the organism's relatively simple nervous system, easily quantified behavioral responses, and our growing understanding of the gustatory, interoceptive, and neuromodulatory systems in this species (reviewed in (Itskov and Ribeiro, 2013; Pool and Scott, 2014)). *Drosophila* detects gustatory cues in foods with their taste bristles on the labellum and other parts of the body (Montell, 2009; Thorne et al., 2004). Sugar, low concentrations of salt, fatty acids and other attractive tastants are detected by gustatory receptor 5a and 64f (Gr5a and Gr64f)-expressing gustatory receptor neurons (GRNs), while toxic compounds, such as bitter substances and high concentrations of salt, are detected by Gr66-expressing GRNs (Dahanukar et al., 2007; Marella et al., 2006; Masek and Keene, 2013; Scott et al., 2001; Wang et al., 2004; Weiss et al., 2011; Zhang et al., 2013). Multiple candidate interoceptive receptors and cells have been also identified in *Drosophila* (Dus et al., 2013; Kim and Rulifson, 2004; Kreneisz et al., 2010; Miyamoto et al., 2012). As in mammals (Andrews et al., 2008; Luquet et al., 2005; Sternson et al., 2013), some of these interoceptive neurons express neuropeptides/neurohormones, such as adipokinetic hormone (AKH) and *Drosophila* insulin-like peptides (DILPs) (Kim and Rulifson, 2004; Kreneisz et al., 2010). In addition, various other neuromodulators have been shown to regulate feeding responses in starved adult *Drosophila* (Itskov and Ribeiro, 2013; Nassel and Wegener, 2011; Pool and Scott, 2014; Taghert and Nitabach, 2012). In particular, dNPF and sNPF, neuropeptides related to mammalian NPY, modulate multiple feeding related behaviors, including the formation and expression of food-associated memory, enhancement of food-related olfactory sensitivity, and control of food intake during starvation (Beshel and Zhong, 2013; Hergarden et al., 2012; Krashes et al., 2009; Lee et al., 2004; Root et al., 2011).

Many animal species become less selective in their food choices during periods of energy deficit. They do so by enhancing their sensitivity to nutritious resources, such as sugar (Dethier, 1976; Gillette et al., 2000; Inagaki et al., 2012; Kawai et al., 2000; Page et al., 1998; Sengupta, 2013). In *Drosophila*, starvation enhances behavioral sensitivity to sugar, at least in part, via increased dopamine (DA) release onto Gr5a-expressing sugar-sensing GRNs, which increases calcium responses to GR activation (Inagaki et al., 2012; Marella et al., 2012). Starvation also decreases sensitivity to unpalatable and potentially toxic compounds, such as bitter tastants. The prevailing view is that this decrease in bitter sensitivity is not independently controlled, but rather is an indirect consequence of the "masking effect" of enhanced sugar sensitivity (Figure $1A_1$) (Moss and Dethier, 1983).

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Here we identify a pathway in *Drosophila* controlling the reduction of bitter taste sensitivity during starvation, which is mechanistically independent of the increase in sweet tastant sensitivity. This pathway combines with the masking effect of enhanced sugar sensitivity, to increase acceptance of resources containing unpalatable, potentially toxic contaminants, during periods of energy deficit (Figure $1A_3$). Thus the multi-dimensional features of the "hunger" state reflect bidirectional, independent and reciprocal neuromodulatory mechanisms, rather than a unidirectional control process.

RESULTS

Bitter sensitivity decreases during starvation independently of increased sugar sensitivity

To quantify food acceptance behavior, we presented a drop of solution containing sugar and/or bitter tastants to the labellum, where GRNs are located. When sugar is presented, *Drosophila* extend their proboscis, a reaction known as the proboscis extension reflex (PER) (Dethier, 1976). We selected this method over others because it provides quantification of gustatory sensitivity independently of food intake. As previously reported (Inagaki et al., 2012; Meunier et al., 2007), when flies are wet starved (WS; deprived of food but not water), sugar sensitivity is increased, as indicated by a leftward shift in the PER dose-response curve (Figure $1B_1$). In addition, the mean acceptance threshold to sugar, S_{50} (the sucrose concentration at which 50% of the flies show a PER) (Inagaki et al., 2012) is decreased (Figure $1B_2$; note that the y-axis is inverted: as sensitivity increases, S_{50} decreases). Importantly, the magnitude of both effects increased significantly with longer starvation times (1 day vs. 2 days), suggesting a scalable, time-variant underlying state change (Fig. $1B_{1–2}$).

Next, we tested behavioral sensitivity to unpalatable tastants by presenting a sugar solution mixed with various concentrations of bitter substances. Consistent with a previous report (Meunier et al., 2003), the admixture of a bitter tastant (lobeline) suppressed the PER to sugar in fed flies, in a dose dependent manner (Figure $1C_1$,"Fed"). We quantified this effect by measuring the fraction of flies not showing a PER; thus a higher value of this metric reflects a stronger suppression of the PER by bitter compounds in the presence of a fixed amount of sucrose. Genetic silencing experiments indicated that Gr66a GRNs are required for the effect of bitter substances to suppress the PER (Figure S1A), consistent with earlier studies (Gordon and Scott, 2009; Wang et al., 2004). Interestingly, during starvation there was a progressive reduction in bitter sensitivity, as indicated by a rightward shift in the dose-response curve for PER inhibition as a function of lobelline concentration (Figure $1C_1$, "WS"). Consistent with this, themean threshold response to bitter, B_{50} (the bitter concentration required to inhibit the PER in 50% of the flies that responded to a given, fixed concentration of sugar; Figure S1B), significantly increased with starvation duration (Figure $1C_2$; note that the y axis is inverted).

Because bitter sensitivity during starvation is quantified as the suppression of a behavioral response to sucrose, it was possible that when flies are starved, their absolute bitter sensitivity does not change, but is relatively reduced as an indirect consequence of "masking" by increased sugar sensitivity. Studies in the blowfly, *Phormia regina*, support this idea (Moss and Dethier, 1983), and we confirmed the masking effect for *Drosphila*

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(Figure S1C). In order to determine whether there were any independent changes in bitter sensitivity during starvation, it was necessary to offset the effect of increased sugar sensitivity in our PER assays. To do this, we reduced the fixed concentrations of sucrose used in our bitter titration experiments, at different starvation times. Thus in fed, 1 day WS and 2 day WS flies, we used 800mM, 300mM and 200mM sucrose, respectively, concentrations that yielded equivalent sub-saturating PER responses (50–60 %; see red boxes in Figure $1B_1$ and $1D_1$). Using such a "sugar-normalized PER assay", we still observed a statistically significant decrease in lobeline sensitivity following food deprivation (Figure $1D_{1,2}$). Absolute sensitivity to other bitter tastants (caffeine and coumamine) also decreased during starvation (data not shown). Therefore during starvation, sensitivity to bitter tastants is reduced, in part, independently of the increase in sugar sensitivity (c.f. Fig. $1B_2$ vs. $1D_2$).

We next compared the kinetics of these reciprocal changes in gustatory sensitivity. Sugar sensitivity increased most strongly during the first 6 hours of starvation and continued more gradually from 6 to 48 hours (Figure $1E_1$). In contrast bitter sensitivity, as measured using sugar-normalized PER assays, did not decrease until after 24 hours of starvation (Figure $1E_2$). Thus, bitter sensitivity decreased more slowly than the increase in sweet sensitivity during starvation, suggestive of independent and inverse regulation (Fig. $1A_{2,3}$). In order to confirm the independence of these bi-directional changes in gustatory sensitivity, we investigated their underlying cellular and molecular mechanisms.

dNPF acts upstream of DA to control sugar but not bitter sensitivity

We first asked whether DA, a neuromodulator that increases sugar sensitivity during starvation (Inagaki et al., 2012; Marella et al., 2012), also decreased bitter sensitivity. To do this, we fed non-starved flies with L-dopa, a precursor of DA, which is known to increase DA levels in the fly brain (Bainton et al., 2000). As previously reported, L-dopa feeding increased sugar sensitivity in non-starved wild-type flies, mimicking the effect of starvation (Figure $2A_{1–2}$) (Inagaki et al., 2012). In contrast, L-dopa feeding did not cause a decrease in bitter sensitivity (Figure $2B_{1–2}$).

To further investigate this issue, we genetically silenced DA neurons by expressing the inwardly rectifying potassium channel KIR2.1 under the control of *th*-GAL4, a GAL4 line driven by the *tyrosine hydroxylase* (*th*) promoter. Expression of Kir2.1 was restricted to the adult phase using tub-$Gal80^{ts}$, to avoid developmental lethality (Riemensperger et al., 2011). Consistent with a previous report (Marella et al., 2012), inactivation of DA neurons attenuated the increase in sugar sensitivity during starvation (Figure $2C_{1–2}$ and $S2A_{1–2}$). In contrast, silencing of DA neurons did not affect bitter sensitivity (Figure 2D). The effect of DA to increase sugar sensitivity (Inagaki et al., 2012; Marella et al., 2012) is mediated by the receptor DopEcR (Srivastava et al., 2005), expressed on Gr5a GRNs (Inagaki et al., 2012). A hypomorphic mutation in *DopEcR* also did not affect bitter sensitivity (Figure 2E). Together, these data indicate that DA modulates sugar but not bitter sensitivity during starvation.

We next investigated neuropeptides that might control bitter sensitivity during starvation. dNPF, an orthologue of mammalian neuropeptide Y, has been shown to promote ingestion

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of unpalatable foods in both larval and adult *Drosophila* (Hergarden et al., 2012; Wu et al., 2003; Wu et al., 2005). To determine whether dNPF might directly suppress bitter sensitivity in adult flies, we artificially stimulated dNPF-expressing (dNPF$^+$) neurons using dTrpA1 (Hamada et al., 2008) and performed PER assays at 31°C. Activation of dNPF$^+$ neurons enhanced the sugar sensitivity of fed flies, as if they were starved, in comparison to flies of the same genotype tested at 21°C (Figure 2F$_{1–2}$). In contrast, activation of dNPF$^+$ neurons did not affect behavioral sensitivity to bitter tastants in sugar-normalized PER assays (Figure 2G$_{1–2}$ and S2C$_{1–4}$). None of the genetic control flies exhibited different sugar sensitivities at the permissive and non-permissive temperatures (Figure S2B$_{1–4}$: note that genetic background has a significant effect on baseline gustatory sensitivity. For genetic manipulations using the GAL4-UAS system, +/UAS-effector, GAL4/+, and genetic background-matched +/+ controls were always tested in parallel to show that the behavioral effects were specific to the GAL4/UAS-effector genotype). Conversely, Kir2.1-mediated silencing of dNPF$^+$ neurons inhibited the starvation-dependent increase in sugar sensitivity (Supplementary Figure S2D$_{1–5}$), but did not interfere with the starvation-dependent decrease in bitter sensitivity (Supplementary Figure S2D$_{6–8}$ and S2E). Thus, as in the case of DA neurons, the activity of dNPF$^+$ neurons enhances sugar sensitivity, but does not independently influence bitter sensitivity.

Since both dNPF$^+$ neurons and DA enhance sugar sensitivity during starvation, we sought to determine whether these neuromodulators function in the same or in parallel neuronal pathway(s). Immunostaining experiments indicated that dNPF and DA neurons are distinct (Figure S2F$_{1–3}$). We therefore combined thermogenetic activation of dNPF$^+$ neurons (d*npf-GAL4/UAS-dTrpA1*) with a hypomorphic mutation in *DopEcR*, which is expressed in sugar-sensing GRNs and mediates the influence of DA on these cells (Inagaki et al., 2012). A homozygous *DopEcR* mutation completely blocked the increase in sugar sensitivity caused by activation of dNPF$^+$ neurons in fed flies (Figure 2H$_{1–2}$). Conversely, genetic inhibition of dNPF$^+$ neurons did not block the effect of L-dopa feeding to increase sugar sensitivity (Figure 2I$_{1–2}$). These data suggest that dNPF$^+$ neurons act genetically upstream of DA neurons to increase sugar sensitivity (Figure 2J). Importantly, perturbations of this dNPF-DA pathway had no effect on sugar-independent changes in bitter sensitivity during starvation.

sNPF modulates bitter sensitivity during starvation without affecting sugar sensitivity

Next, we sought to identify neuromodulatory systems that mediate the decrease in bitter sensitivity during starvation. sNPF, an NPY-related protein in *Drosophila*, has been implicated in many hunger related behaviors (Nassel and Wegener, 2011), including the control of food intake in larvae (Lee et al., 2008; Lee et al., 2004) and food-related olfactory sensitivity in adults (Root et al., 2011). To ascertain whether sNPF is also involved in starvation-mediated control of gustatory sensitivity, we tested the behavioral sensitivity of *sNPF* mutant flies to bitter and sugar using the PER assay. We used two independent hypomorphic piggyBac transposon insertion (Thibault et al., 2004) alleles of *sNPF*, *sNPF^{c00448}* (Lee et al., 2008), and *sNPF^{f07577}* (Figure S3A: These *sNPF* mutant flies were introgressed into a wild type CS gentic background for at least six generations).

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Food-deprived, homozygous *sNPF*^{c00448} and *sNPF*^{f07577} mutant flies were more bitter sensitive than starved genetic controls, but showed normal changes in sugar sensitivity (Figure 3A$_{2–3}$ and 3B, red curves/bars; Figure S3A, S3B and S3C). Interestingly, *sNPF/+* heterozygotes also showed a similar phenotype (Figure 3A$_{2–3}$ and 3B$_2$, green curves/bars), indicating haploinsufficiency of this neuropeptide gene. Importantly under fed conditions, *sNPF* mutant flies did not show any change in bitter sensitivity (Figure 3A$_1$), indicating that the mutation affected starvation-dependent changes rather than baseline responsiveness. Consistent with this, sugar- normalized PER assays indicated that 1-day WS vs. unstarved homozygous *sNPF* mutant flies showed no difference in bitter sensitivity, in contrast to genetic background-matched wild-type controls (Figure 3C$_{1–2}$). Flies trans-heterozygous for *sNPF*^{c00448} and *sNPF*^{f07577} also showed a similar phenotype (Figure 3C$_3$).

In larvae, sNPF regulates food intake and growth (Lee et al., 2008; Lee et al., 2004). To show that the bitter sensitivity phenotype in adult flies is not due to developmental effects, we rescued the expression of sNPF specifically in the adult nervous system. We expressed sNPF protein in neurons of *sNPF* hypomophic mutant flies using *UAS-sNPF* under the control of *elav-GeneSwitch* (*elav-GS*), a pan-neuronally expressed, hormone (RU486) inducible form of GAL4 (Osterwalder et al., 2001). Rescue of sNPF expression by RU486 feeding in adult flies resulted in a recovery of the starvation-induced decrease in bitter sensitivity (Figure 3D$_2$ and 3E$_2$), without affecting sugar sensitivity (Figure 3E$_1$ and 3SD$_2$). RU486 feeding did not affect bitter sensitivity in control flies lacking *elav-GS*, showing this is not an artifact caused by the inducer (Figure 3D$_1$, 3E$_{1–2}$, and S3D$_1$). Altogether these results indicate that, 1) sNPF expression is necessary for the decrease in bitter sensitivity during starvation, 2) this effect is not due to a developmental function, and 3) neuronal sNPF regulates bitter sensitivity. Importantly, none of the genetic manipulations of *sNPF* described above affected sugar sensitivity (Figure 3B$_1$, 3E$_1$, S3B, S3C and S3D), suggesting that sNPF independently modulates bitter sensitivity during starvation.

Subsets of sNPF-expressing neurons regulate bitter sensitivity

There is a large number of sNPF positive neurons, including ~4000 Kenyon cells and ~280 other neurons in the brain (Nassel et al., 2008; Nassel and Wegener, 2011) (Figure S4). To identify the subset of sNPF-expressing neurons (sNPF$^+$ neurons) that controls bitter sensitivity, we genetically silenced different subsets of sNPF$^+$ neurons by driving KIR2.1 expression using a panel of 11 GAL4 lines each containing different DNA fragments from the *sNPF* gene (Lee et al., 2009; Pfeiffer et al., 2008). Expression of KIR2.1 was restricted to adulthood using Gal80^{ts}, and bitter sensitivity was analyzed after 1 day of wet starvation. Silencing neurons in 3 of these lines, *GMR20D06-GAL4*, *GMR21B10-GAL4* and *GMR20F11-GAL4*, attenuated the starvation-induced decrease in bitter sensitivity (Figure 4A$_2$ and 4A$_4$), without affecting sugar sensitivity (Figure 4A$_1$ and 4A$_3$).

We next sought to identify the cells within these GAL4 lines responsible for the phenotype. None of the lines labeled GRNs projecting to the SEZ (Figure 4B$_{1–3}$ and S4A). Instead, these lines labeled small numbers of neurons in the central brain, some of which were stained by an anti-sNPF antibody (Figure 4B$_{1–3}$). Notably all 3 lines exhibited co-expression of anti-sNPF immunoreactivity and GAL4-driven GFP in a cluster of 11–12 so-called lateral

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neurosecretory cells (LNCs) (Nassel et al., 2008) (Cells surrounded by yellow dashed line in Figure 4B$_{1–3}$ and red dashed rectangle in Figure 4C). Importantly, anti-sNPF immunocreactivity in these neurons was reduced in *sNPF* mutants (Figure S4B$_{1–2}$). Consistent with previous observations using another GAL4 line (Kapan et al., 2012), axonal projections from LNCs to the SEZ were observed in all 3 lines (blue dashed rectangle in Figure 4B$_{1–3}$ and 4C). In contrast, sNPF promoter lines whose silencing did not effect bitter sensitivity did not exhibit expression in LNCs (with the exception of *GMR21B01-GAL4* (Figure 4D and Figure S4C), which is expressed in a subset of LNCs and only weakly decreased bitter sensitivity (Figure 4A)). Although it is formally possible that different subsets of neurons in each of the 3 GAL4 lines (*GMR20D06-*, *GMR21B10-* and *GMR20F11-* GAL4 lines) are responsible for the common phenotype, the simplest interpretation is that the effect is due in all 3 cases to silencing of the LNC sNPF$^+$ neurons.

To investigate whether the *sNPF* gene itself regulates bitter sensitivity in these LNCs, we knocked down *sNPF* using an sNPF RNAi under the control of the *GMR20D06-*, *GMR21B10-*, and *GMR20F11-*GAL4 lines. This manipulation decreased bitter sensitivity during starvation, again without affecting sugar sensitivity (Figure 4E$_{1–4}$). Line *GMR20F11-GAL4* showed a non-significant trend overall, but showed a significant decrease in the B_{50} value at a concentration of 0.16mM lobeline (Figure 4E$_2$, brown line$_{,,}$ asterisk). Furthermore, selective rescue of sNPF expression under the control of *GMR20D06-* or *GMR21B10-GAL4* in the *sNPF* hypomorphic mutant background restored the starvation-dependent decrease in bitter sensitivity (Figure 4F$_{2–3}$; compare to the phenotype of *sNPF* hypomorphic mutants in Figure 3C$_{2–3}$). By contrast, driving *UAS-sNPF* expression in a different subset of sNPF$^+$ neurons labeled by a line called *sNPF-GAL4* (Lee et al., 2009) did not rescue the mutant phenotype (Figure 4F$_1$ and S4D). Therefore, sNPF expression in a specific subset of sNPF$^+$ neurons (LNCs) is necessary and sufficient for the effect of this neuropeptide to regulate bitter sensitivity.

Importantly, none of LNCs labeled by line 21B10-GAL4 co-expressed dNPF or TH (Figure S4E and S4F). These data, together with the lack of any effect of *sNPF* mutations or neuronal silencing on sugar sensitivity, suggest that the sNPF system and the dNPF-DA pathway independently regulate bitter- and sugar-sensitivity, respectively, at the neural circuit level (Figure 4G).

sNPFR is necessary and sufficient for bitter sensitivity control

If sNPF controls the starvation-dependent decrease in bitter sensitivity, one might predict that its receptor should have a similar function. sNPF receptor (sNPFR) is the only identified G-protein coupled receptor for sNPF in *Drosophila* (Feng et al., 2003; Mertens et al., 2002; Reale et al., 2004). Over-expression of sNPFR using *UAS-sNPFR* under the control of the pan-neuronal *nsyb-GAL4* driver (Fei et al., 2010) enhanced the starvation-dependent decrease in bitter sensitivity (Figure 5A$_{1–2}$, B$_2$, and S5B). Conversely, pan-neuronal knock-down of sNPFR using an *sNPFR* RNAi attenuated the starvation-dependent decrease in bitter sensitivity (Figure 5C$_{1–3}$, and 5D$_2$: these transgenic flies also contained *UAS-Dicer2* to enhance the effects of RNAi). Importantly, these manipulations did not affect bitter sensitivity in fed flies (Figure 5A$_1$ and 5C$_1$), or sugar sensitivity (Figure 5B$_1$, 5D$_1$, S5A$_{1–3}$

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and S5C$_{1–3}$). These data support the conclusion that sNPF plays an important role in modulating starvation-dependent changes in bitter sensitivity.

Downstream cellular targets of sNPF modulation

We next investigated potential cellular targets of modulation by sNFP/sNPFR in the control of bitter sensitivity. As LNCs, sNPF and sNPFR have been implicated in the regulation of insulin -producing cells (IPCs) (Kapan et al., 2012; Lee et al., 2008), we first tested whether IPCs may control bitter sensitivity in response to sNPF. However, neither IPC-specific knock down of sNPFR expression, using an *Ins3P-GAL4* driver (Buch et al., 2008), nor ablation of IPCs using *UAS-hid* (Grether et al., 1995), affected bitter sensitivity in starved flies (Figure S5D$_{1–2}$ S5E$_{1–4}$; cell ablation was histologically confirmed; see Figure S5F$_{1–2}$). Therefore IPCs are unlikely to mediate the modulatory influence of sNPF/sNPFR on bitter sensitivity during starvation.

Since LNCs project to the SEZ (Figure 4B$_{1–3}$), we next asked whether the sNPF-sNPFR pathway might modulate the sensitivity of primary bitter-sensing GRNs. To test this hypothesis, we performed functional calcium imaging of bitter-sensing GRNs in wild type and *sNPF* hypomorphic mutant flies. To monitor calcium transients in bitter-sensing GRNs, we expressed a genetically encoded calcium indicator, GCaMP3.0 (Tian et al., 2009), under the control of *Gr66-GAL4* (Scott et al., 2001). Consistent with previous reports (Marella et al., 2006), the axonal terminals of bitter-sensing GRNs in the subesophageal zone (SEZ) exhibited increased GCaMP3.0 fluorescence in response to increasing concentrations of lobeline applied to the labellum (Figure 5E and 5F$_1$, blue line). Strikingly, 2 day wet starved wild-type flies showed a statistically significant reduction in GCaMP3.0 fluorescence evoked by application of 0.07mM lobeline, and a non-significant decrease at 0.31 mM lobeline (Figure 5F$_1$, red line and 5G). Importantly, this starvation-dependent reduction in bitter responsiveness was virtually abolished in heterozygous *sNPF*^{c00448} mutant flies (Figure 5F$_2$ and 5G), paralleling the effect of this mutation on behavioral sensitivity to bitter tastants (Fig. 3A and 3B).

To determine whether sNPF acts directly on Gr66 GRNs, we manipulated levels of sNPFR in these cells. Neither over-expression nor knock-down of *sNPFR* in bitter-sensing GRNs, using Gr66 and Gr33-GAL4 drivers (Moon et al., 2009) and *UAS-Dicer2*, affected bitter sensitivity in starved flies (Figure S5G$_2$, S5). However, knock-down of *sNPFR* in GABAergic neurons using *dvgat*-GAL4 (Fei et al., 2010), a GAL4 line under the control of drosophila vesicular GABA transporter (*dvgat*) promoter, significantly attenuated the decrease in bitter sensitivity caused by starvation (Fig. S5J$_2$, red line), without any effect on sugar sensitivity (Figure S5J$_1$). Thus, sNPF likely modulates bitter sensing GRNs indirectly, perhaps by stimulating inhibitory neurons (Figure 5H).

Interoceptive AKH$^+$ neuroendocrine cells act upstream of sNPF to control bitter sensitivity

We next searched for interoceptive neurons that might act upstream of the sNPF pathway to regulate bitter sensitivity during starvation. The corpora cardiaca (CC) contains interoceptive neuroendocrine cells that release the peptide adipokinetic hormone (AKH), a fly analog of glucagon, during starvation (Kim and Rulifson, 2004). Genetic ablation of

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these cells using *akh-GAL4* and *UAS-hid* attenuated the starvation-dependent decrease in bitter sensitivity, without affecting sugar sensitivity (Figure 6A$_{1–3}$, B$_{1–2}$, and S6A$_{1–3}$; ablation was confirmed; see Figure S6B$_{1–2}$). Consistent with this result, a hypomorphic mutation in the *AKH receptor* gene (*akhr*) (Lee et al., 2006; Shohat-Ophir et al., 2012), *akhr*^{EY11371} (Bharucha et al., 2008) (Figure S6C), also attenuated the starvation-dependent decrease in bitter sensitivity relative to genetic background-matched wild-type controls (Figure 6C$_{1–3}$, 6D$_2$). In contrast, bitter sensitivity under fed conditions (Figure 6C$_1$ and 6D$_2$), and sugar sensitivity regardless of starvation level (Figure 6D$_1$ and S6D$_{1–3}$) were not affected. Normalized-sugar PER assay comparing 1-day WS flies and unstarved flies revealed that *akhr*^{EY11371} flies showed no change in bitter sensitivity during starvation (Figure S6E$_{1–2}$).

As an independent approach to investigating the role of AKH$^+$ cells, we asked whether thermogenetic activation of these neuroendocrine cells, using dTrpA1, would suffice to decrease bitter sensitivity. We performed this experiment in 18 hr wet starved flies, which exhibit increased sugar sensitivity, but which do not yet exhibit decreased bitter sensitivity (Figure 1E$_{1–2}$). Flies expressing dTrpA1 in AKH$^+$ neurons were pre-incubated at 30 °C for 30 minutes, after which gustatory sensitivity was tested at 18 °C. Indeed, thermogenetic activation of AKH$^+$ cells significantly decreased bitter sensitivity (Figure 6E$_2$ and 6F$_2$, red lines and boxes), relative to controls pre-incubated at 18°C (blue lines and boxes). This effect was not observed in genetic control flies subjected to 30°C pretreatment (Figure 6E$_1$ and S6F$_{1–2}$) and did not affect sugar sensitivity (Figure 6F$_1$, and S6G$_{1–4}$). Therefore activation of AKH$^+$ neuroendocrine cells is sufficient to specifically decrease bitter sensitivity in partially starved flies.

Because both AKH and sNPF regulate bitter sensitivity in the same direction, we investigated whether these neuropeptides act in a common pathway. Antibody staining experiments have indicated that AKH$^+$ neuroendocrine cells in the CC (Kim and Rulifson, 2004; Lee and Park, 2004) do not co-express sNPF (Kahsai et al., 2010). Consistent with this, expression of sNPF in AKH$^+$ cells did not rescue the *sNPF* mutant phenotype (Figure S6H). To test whether sNPF functions genetically downstream of AKH$^+$ cells, we asked whether the *sNPF* mutation would suppress the effect of activating AKH$^+$ cells. Indeed, in a heterozygous *sNPF*^{c00448} background thermogenetic activation of AKH$^+$ cells was unable to reduce bitter sensitivity (cf. Figure 6E$_2$ vs. 6E$_{3–4}$, 6F$_{1–2}$ and S6F$_{1–2}$). Thus, haploinsufficiency of *sNPF* is epistatic to artificial activation of AKH$^+$ neuroendocrine cells. This suggests that sNPF-expressing neurons may mediate the effect of AKH$^+$ cells to control bitter sensitivity (Fig. 7). However, an indirect, permissive role for sNPF in AKH action is not excluded by these data (Figure 7A, dashed curved arrow).

DISCUSSION

We have used starvation in *Drosophila* as a model system to understand how changes in internal states influence behavioral decisions. Starved animals exhibit enhanced sugar sensitivity and decreased bitter sensitivity, allowing them to accept food resources they would otherwise reject as insufficiently caloric or potentially toxic. Here we provide behavioral, cellular and genetic evidence that bitter sensitivity is independently modulated

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during food deprivation, in the opposite direction as sugar sensitivity. We identify parallel neuromodulatory pathways that control these changes, and show that they are recruited at different levels of energy deficit (Figure 7). This independent and reciprocal control of sweet vs. bitter taste sensitivity during starvation affords a greater dynamic range of control over feeding decisions. More generally, our data illustrate how different components of a time-varying, scalable multi-dimensional internal state can be independently regulated to achieve robust state-dependent changes in behavior.

Sugar and bitter sensitivities are independently modulated in starved flies

The idea that changes in gustatory sensitivity during starvation in flies exclusively reflect an increase in sugar sensitivity has been the dominant view in the field, based on behavioral studies in the blowfly *Phormia* (Moss and Dethier, 1983). Here we present several lines of evidence that bitter sensitivity decreases during starvation in *Drosophila*, independently of the increase in sugar sensitivity. First, we measured the sensitivity of PER behavior to inhibition by bitter compounds at different times of starvation, using progressively lower fixed concentrations of sucrose to offset the increase in sugar sensitivity. Such “normalized PER assays” revealed a decrease in behavioral sensitivity to bitter tastants during starvation, even when compensating for increased sugar sensitivity (Fig. 1D, E).

Second, we identified two independent neuromodulatory pathways (Fig. 7A), loss- and gain-of-function genetic manipulations of which repeatedly revealed a double-dissociation of the control of sugar- and bitter-taste sensitivity during starvation, at multiple levels of regulation (Fig. 7B). Sugar sensitivity is increased by a pathway in which $dNPF^+$ neurons act genetically upstream of DA^+ neurons, which increase their release of DA onto sweet-sensitive GRNs, thereby enhancing their physiological responsiveness to sugars (Inagaki et al., 2012; Marella et al., 2012). Bitter sensitivity is decreased, conversely, by AKH^+ interoceptive neuroendocrine cells, which act genetically upstream of $sNPF^+$ LNCs; in turn LNCs indirectly (perhaps via inhibitory interneurons) reduce the physiological responsiveness of Gr66a GRNs to bitter tastants. In each of close to two dozen genetic manipulations of these different pathway components, changes were observed in either bitter or sugar sensitivity, but not both.

Moreover, the phase of these genetic phenotypes was opposite: gain-of-function manipulations of the dNPF→DA pathway increased sugar sensitivity, while in the AKH→sNPF pathway they decreased bitter sensitivity, and vice-versa (Fig. 7B).

Orthogonal regulation of gustatory sensitivity by NPF family members

Previous studies have implicated dNPF and sNPF in the control of starvation dependent changes in physiology and behavior (Beshel and Zhong, 2013; Hergarden et al., 2012; Krashes et al., 2009; Lee et al., 2004; Root et al., 2011). Here we show that these neuropeptides control reciprocal changes in sweet and bitter sensitivity during starvation. $dNPF^+$ neurons promote increased sugar sensitivity, while sNPF promotes decreased bitter sensitivity. While may be coincidental that these neuropeptides and their receptors are genetically related, it is tempting to speculate that this reciprocal regulation arose in

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evolution by duplication and modification of a single neuromodulatory pathway that originally regulated a single taste modality.

That said, the functions of dNPF and sNPF in controlling starvation-dependent changes in gustatory sensitivity may not necessarily be analogous. dNPF is expressed by very few neurons in the brain (Krashes et al., 2009), while sNPF is expressed in several hundred cells (Nassel et al., 2008). It is possible that this difference reflects a qualitative distinction in the type of function that these two neuropeptides perform (e.g., state control vs. signal amplification). Alternatively, it may simply reflect a difference in their frequency of utilization in different circuits. Whatever the case, both dNPF and sNPF have been implicated in additional functions besides hunger modulation (Kahsai et al., 2010; Kapan et al., 2012; Kim et al., 2013; Knapek et al., 2013; Lee et al., 2006; Shohat-Ophir et al., 2012), indicating that their effects on behavior are context-dependent.

Our studies identify a subset of neurons, called lateral neurosecretory cells (LNCs), in which sNPF acts to regulate bitter sensitivity. Although LNCs are known to express other neuropeptides (Kahsai et al., 2010; Kapan et al., 2012), our loss- and gain-of-function genetic experiments indicate that sNPF in LNCs is necessary and sufficient for the starvation-dependent decrease in bitter sensitivity. In addition to this function, however, sNPF expressed in LNCs has been reported to exert multiple influences during food-deprivation: LNCs regulate IPCs, sensitivity to starvation and metabolism (Kahsai et al., 2010; Kapan et al., 2012). Interestingly, LNCs project not only to the SEZ, but also to other brain areas and the CC (where AKH is produced (Kapan et al., 2012)). It is possible that sNPF and AKH form a feedback circuit to regulate starvation-driven behavioral changes.

Our studies leave unanswered a number of questions regarding the function and site of action of NPF family members in the starvation-dependent control of gustatory sensitivity. For example, it is not yet clear whether dNPF peptides themselves, or rather $dNPF^+$ neurons acting through some other neurotransmitter or neuromodulator, control changes in sugar sensitivity. It is also not clear whether $dNPF^+$ neurons act directly on DA neurons that control Gr5a GRN sugar sensitivity, or indirectly via intermediate connections. Interestingly previous research has shown that $dNPF^+$ neurons have a direct inhibitory effect on dopaminergic MB-MP1 neurons to regulate starvation driven memory (Krashes et al., 2009). Experiments to knock down NPFR expression in DA neurons, however, did not affect sugar sensitivity (data not shown), implying an indirect effect. Similarly, the site at which sNPF reduces the sensitivity of Gr66 GRNs to bitter compounds remains unclear. Elucidation of these missing details may clarify the extent to which dNPF and sNPF exert analogous but orthogonal roles in controlling gustatory sensitivity during starvation.

Food deprivation recruits neuromodulatory cascades that modify the sensitivities of primary gustatory neurons

Our earlier (Inagaki et al., 2012) and present findings suggest that, in *Drosophila*, hunger modulates the sensitivity of two orthogonal classes of primary gustatory neurons to regulate feeding choices. Modulation of primary sensory neurons enables the state-dependent tuning of each sensory modality before the signal is integrated with other inputs at higher levels in the brain. Interestingly, in mice, it has been reported that multiple neuromodulators and

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hormones modulate the sensitivity of taste cells (Elson et al., 2010; Kawai et al., 2000), although whether this modulation causes starvation-dependent behavioral changes is not clear (Sternson et al., 2013).

Risk vs. benefit may determine the sequence of state-dependent changes recruited during food-deprivation

Animals continuously compare potential gains and risks to determine their behaviors (Dethier, 1976; Gillette et al., 2000; Itskov and Ribeiro, 2013). Because the need to feed increases with starvation, it is reasonable that the decision to accept or reject a potential food resource is modulated by energy deficit. A comparison of fed vs. 2 days starved flies revealed that sugar sensitivity alone changes 4.6 fold (Figure $1B_2$) and bitter sensitivity alone changes 4.8 fold (Figure $1D_2$); while the relative preference for food containing both sugar and bitter increases 10.2 fold (Figure $1C_2$). The increased acceptance of unpalatable food during starvation reflects both the “masking” effect of increased sugar sensitivity on the detection of bitter compounds (Moss and Dethier, 1983), and, as shown here, an independent decrease in bitter taste sensitivity. This reciprocal tuning of both sugar and bitter sensitivity contributes to a dramatic increase in the acceptance of food resources containing both reduced caloric value and potentially toxic compounds, by a starving fly.

Food-deprivation creates a type of “global organismal state change” (Kim et al., 2013; LeDoux, 2012) that is multi-dimensional: it involves multiple physiological and behavioral changes. Interestingly, these different changes occur with different kinetics during food deprivation. Some of them, such as the increase in sugar sensitivity (6 hours of starvation; present results), feeding amount (6–12 hours of starvation: (Farhadian et al., 2012; Hergarden et al., 2012), and food-related olfactory sensitivity (several hours of starvation: (Root et al., 2011)) are initiated during mild starvation, while others, such as the decrease in bitter sensitivity (1–2 days of starvation; present results) and increase in locomotion (2 days of starvation: (Isabel et al., 2005; Lee and Park, 2004))) are recruited during severe starvation just before death.

Interestingly, changes occurring during mild starvation appear to be low-risk, in that their premature implementation is unlikely to kill the animal, whereas changes accompanying severe starvation place the animal at higher-risk for damage or death: e.g., the decrease in bitter sensitivity allows intake of potentially toxic substances. A similar increase in risky behaviors has been observed in other settings involving state-dependent escalation (Blanchard and Blanchard, 1989; Potegal, 2012). These considerations may explain why the brain has evolved multiple mechanisms for the adaptive control of behavior in response to organismal state changes. One mechanism first activates lower risk responses, when energy demands are mild, while the other recruits higher risk responses when energy demands are severe and no other options are available.

The results presented here outline two parallel, orthogonal pathways that translate changing energy needs into the decision to accept a food resource. These data add to a growing body of evidence that neuromodulatory cascades serve as key mediators of state-dependent control (Bargmann, 2012; Flavell et al., 2013; Komuniecki et al., 2014; Taghert and Nitabach, 2012). The widespread projections of neuromodulatory neurons, and the specific

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expression of their receptors, allows them to coordinate the activity of multiple, behaviorally distinct sub-circuits in parallel. This property, and the ability of such modulators to alter the response properties of neurons and circuits (Marder and Bucher, 2007) are well suited to a mediating function in state control. Cascades of neuromodulators, moreover, afford multiple regulation points, allowing dynamic state control with potential feedback and/or feedforward regulation (Taghert and Nitabach, 2012). Our results may provide entry points to study in more detail the dynamics of neuromodulatory cascades and their impact on organismal physiology and behavior.

EXPERIMENTAL PROCEDURES

Fly Strains

Adult female *Drosophila melanogaster* were used for all experiments. Since genetic background affects the basal sugar and bitter sensitivities, all the comparisons were made within the same genetic background. Flies were backcrossed for at least 6 generations to ensure the same genetic background. Descriptions of detail genotypes are in the Supplemental Experimental Procedures.

PER Assays

For PER assays, 3–7 day-old female flies were wet-starved or fed in vials. Wet starvation was performed by keeping flies in a vial with a water-soaked filter paper. PER was tested as described previously (Inagaki et al., 2012). Detail procedures are in the Supplemental Experimental Procedures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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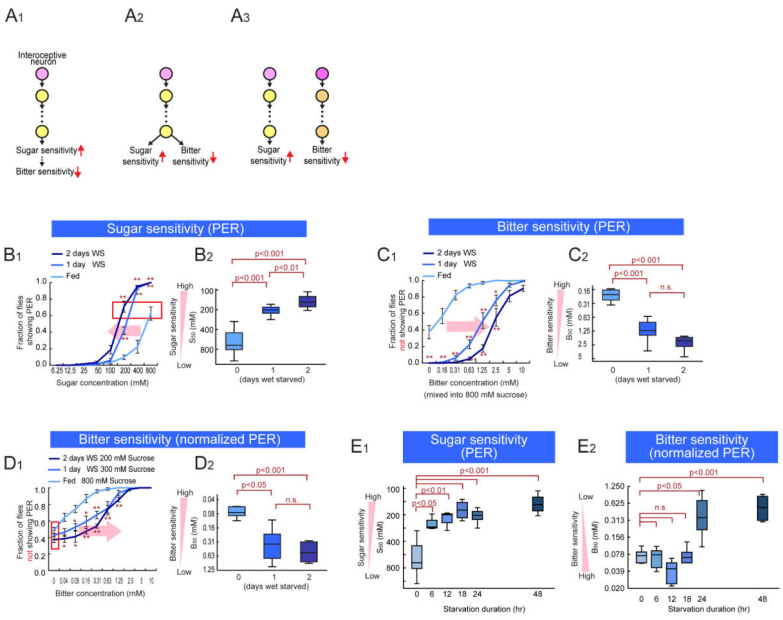

Figure 1. Modulation of Sugar and Bitter Sensitivity During Starvation

(A) Schematics illustrating different models to explain the reciprocal control of sugar and bitter sensitivity during starvation. (B) Fraction of flies showing PER to different concentration of sucrose at different starvation levels. (B_1) Average responses. Error bars represent SEM. Two-way ANOVA followed by post hoc t-test with Bonferroni correction at each sugar concentration. *p<0.05; **p<0.005. n>5 for each experimental group. (B_2) S_{50} (the sugar concentration at which 50% of flies show PER) plotted as a function of starvation duration. One-way ANOVA followed by post hoc t-test with Bonferroni correction. The same plotting and statistical analysis of PER assay are used throughout this paper. Red box indicates the sucrose concentrations that yield the equivalent PER responses at different starvation levels. (C, D) Fraction of flies not showing PER to different concentration of lobeline mixed into 800mM sucrose (C) or different concentrations of sucrose (D). n>5 for each experimental group. (E) S_{50} and B_{50} measured and plotted as a function of starvation duration. One-way ANOVA followed by post hoc t-test with Bonferroni correction (n>5 for each experimental group). Panels B_1 and B_2 are independent replications of results previously reported in (Inagaki et al, 2012) and are presented here for purposes of comparison. See also Figure S1.

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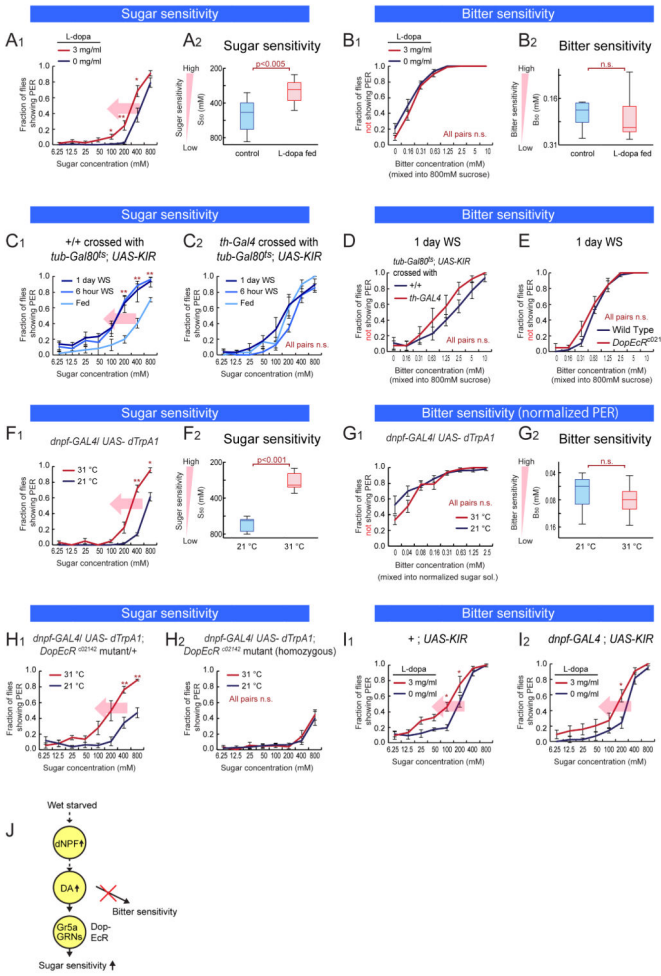

Figure 2. Neuronal Pathway Regulating Sugar Sensitivity During Starvation

(A–B) Sugar and bitter sensitivity of non-starved wild type flies fed with L-dopa. (C–E) Sugar and bitter sensitivity of flies with genetic perturbation of dopaminergic signal. (F–G) Sugar and bitter sensitivity of flies with thermogenetic activation of dNPF neurons (w-; *dnpf-GAL4* (II) crossed with w-; *UAS-dTrpA1* (II); *UAS-dTrpA1* (III)). For 31 °C experiments, flies were pre-incubated in 31 °C for 30 min. Bitter sensitivity was measured using normalized-sugar PER assay (sucrose concentration used: 800 mM for 21 °C and 400 mM for 31 °C). Data from non-normalized PER responses are shown in Figure S2B$_1$. (H) Sugar sensitivity of flies with thermogenetic activation of dNPF neurons combined with *DopEcR* mutation (w-; *dnpf-GAL4* (II); *DopEcR*^{c02142} crossed with w-; *UAS-dTrpA1* (H_1) or w-; *UAS-dTrpA1* (II); *DopEcR*^{c02142} (H_2)). (I) Sugar sensitivity of flies with L-dopa feeding combined with genetic silencing of NPF neurons. (J) Schematic illustrating neuromodulatory pathway regulating sugar sensitivity but not affecting bitter sensitivity. n>5 for all experimental groups. Panels $A_{1–2}$ are independent replications of results previously reported in (Inagaki et al, 2012), and are presented here for purposes of comparison. See also Figure S2.

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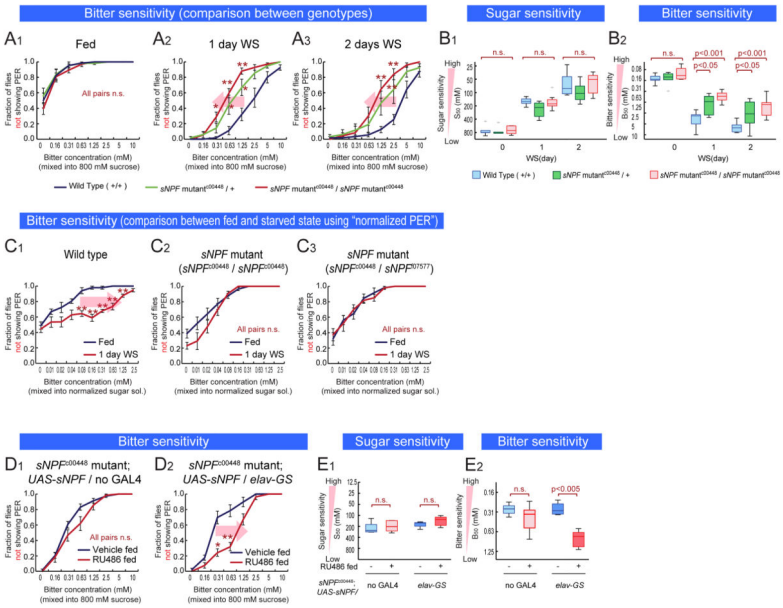

Figure 3. sNPF is Necessary and Sufficient for Bitter Sensitivity Control During Starvation

(A–B) Sugar and bitter sensitivity of wild type and *sNPF*^{c00448} mutant flies in the same genetic background. (C) Bitter sensitivity measured with normalized-sugar PER assays in wild type flies (C_1), *sNPF* mutant flies (w-; *sNPF*^{c00448} (C_2) and w-; *sNPF*^{c00448}/ *sNPF*^{f07577} (C_3)). Lobeline was mixed into 800 mM sucrose solution for fed flies, or 200 mM sucrose solution for 1-day WS flies. (D–E) Sugar and bitter sensitivity of *sNPF* mutant flies with pan-neuronal, adult rescue of sNPF expression (w-; *sNPF*^{c00448}; *UAS-sNPF* crossed with w-; *sNPF*^{c00448}; + (D_1) or w-; *sNPF*^{c00448}; *elav-GeneSwitch* (D_2)). Sucrose solution with or wihout 0.5 mM RU486 was fed to flies for 2days before experiments. n>5 for all experimental groups. See also Figure S3.

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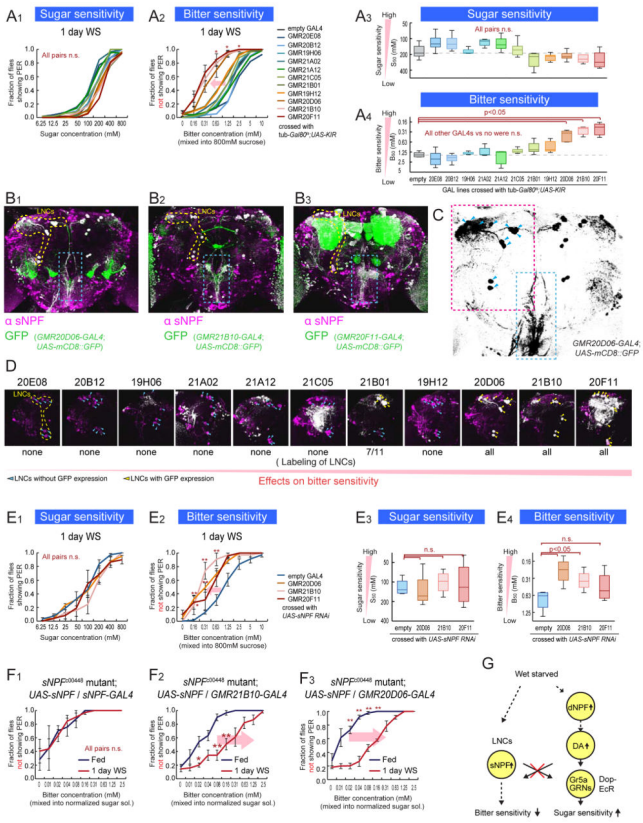

Figure 4. Subsets of sNPF Neurons Regulate Bitter Sensitivity During Starvation

(A) Sugar and bitter sensitivity of flies with genetic silencing of different subsets of sNPF neurons. For this experiment, w-; *UAS-KIR2.1; tub-Gal80^{ts}* flies were crossed with the indicated GAL4 lines or promoterless *BDP-GA*L4 flies (empty-GAL4). Flies were incubated at 31 °C for 2 days to inactivate Gal80^{ts} before experiments. (B) Representative confocal projections of whole mount brains of sNPF promoter GAL4 lines crossed with *UAS-mCD8::GFP* flies and stained with anti-sNPF precursor antibody. Overlap of signals are shown in white color. LNCs are surrounded by yellow dotted lines. Axonal projection of LNCs are surrounded by blue dotted boxes. (C) Structure of LNCs. Blue arrowheads indicate cell bodies of LNCs. (D) Enlarge representative confocal projections of dorso-posterior side of the sNPF promoter GAL4 lines crossed with *UAS-mCD8::GFP*. LNCs are surrounded by yellow dotted line in the left panel. White color indicates the locations with overlap of GFP and anti-sNPF signals (Raw GFP signals in green are not shown to clarify the locations with the overlap. See Figure S4 for raw data). Blue arrowheads and yellow arrowheads indicate LNCs without and with GFP expression, respectively. (E) Sugar and bitter sensitivity of flies with *UAS-sNPFR* RNAi driven under the control of sNPF promoter GAL4 lines or *BDP-GA*L4 flies (No-GAL4). **(F)** Bitter sensitivity measured with normalized-sugar PER assays in *sNPF* mutant flies with genetic rescue of sNPF expression in different subsets of neurons (w-; *sNPF^{c00448}*; *UAS-sNPF* crossed with w-; *sNPF^{c00448}*; *sNPF-GAL4* (F_2) or w-; *sNPF^{c00448}*; *GMR21B10-GAL4* (F_3)). See also Figure $3C_{2–3}$ for comparison. n>5 for all experimental groups. (G) Schematic summarizing results. See also Figure S4.

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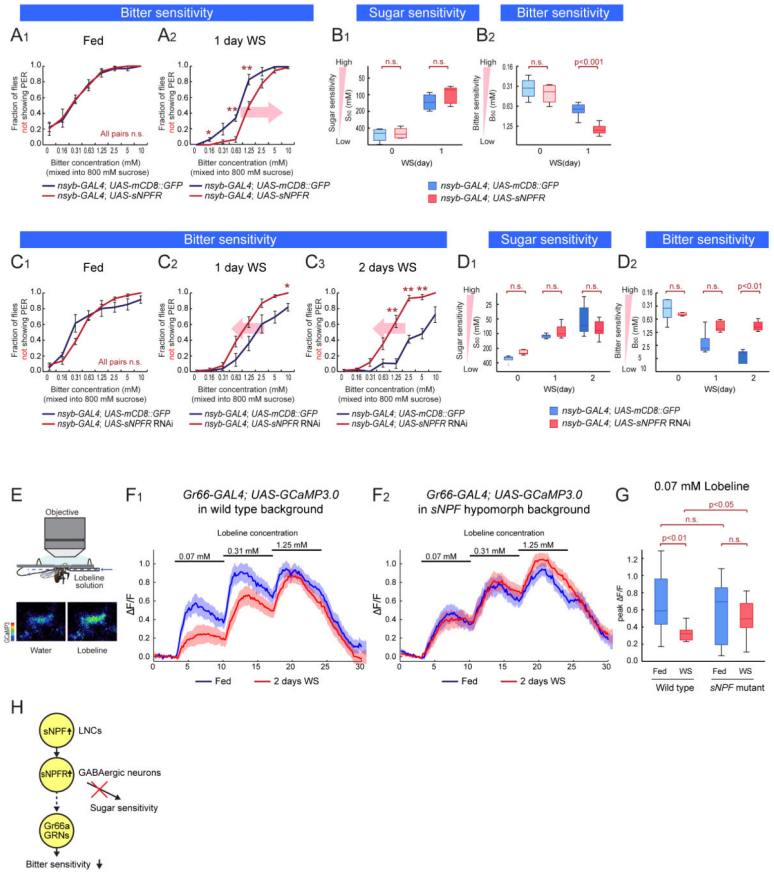

Figure 5. Modulation Target of sNPF Pathway

(A–B) Sugar and bitter sensitivity of flies with genetic over-expression of *sNPFR* (w-;; *nsyb-GAL4* crossed with *UAS-mCD8::GFP* or *UAS-sNPFR. UAS-mCD8::GFP* and *UAS-sNPFR* flies are in the same genetic background). (C–D) Sugar and bitter sensitivity of flies with genetic knock-down of *sNPFR* (UAS-Dicer2;; *nsyb-GAL4* crossed with *UAS-mCD8::GFP* or *UAS-sNPFR* RNAi. *UAS-mCD8::GFP* and *UAS-sNPFR* RNAi flies are in the same genetic background). n>5 for each experimental group in A–D. (E) The experimental setup for calcium imaging of bitter-sensing GRNs. Blue arrow indicates direction of flow of bitter solution. The two images below the diagram are representative fields of view showing the GCaMP response of Gr66 GRNs. The fluorescent intensity of GCaMP3 is shown in pseudo-color (scale bar on left). (F) Responses (ΔF/F) to different concentrations of lobeline solution in the central projections of bitter sensing GRNs. The solid lines represent average traces, and envelopes indicate SEM (n>12 for each condition). w-; *Gr66-GAL4; UAS-GCaMP3.0* (F_1) and w-; *Gr66-GAL4/sNPF*^{c00448}; *UAS-GCaMP3.0* (F_2) were used. (G) Quantification of peak fluorescent changes (ΔF/F) in response to 0.07 mM lobeline solution. One-way ANOVA followed by post hoc t-test with Bonferroni correction. (H) Schematic illustrating neuronal pathway regulating bitter sensitivity. See also Figure S5.

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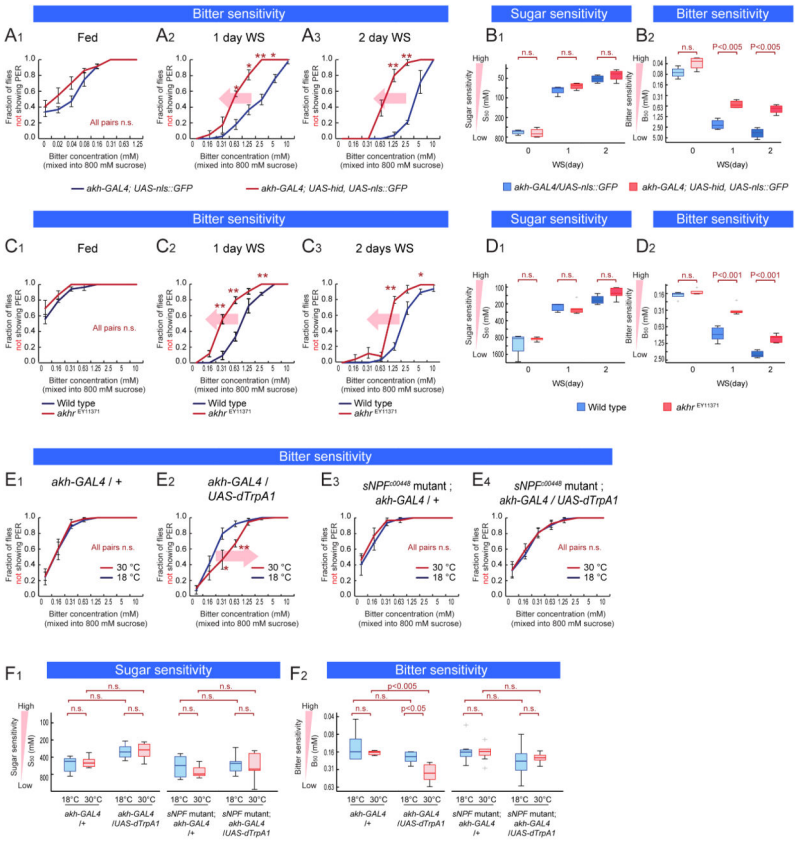

Figure 6. AKH Acts Genetically Upstream of the sNPF Pathway

(A–B) Sugar and bitter sensitivity of flies with or without genetic ablation of AKH neuroendocrine cells (w-; *akh-GAL4* (III) crossed with w-; *UAS-nls::GFP* or w-;*UAS-nls::GFP*, *UAS-hid*). (C–D) Sugar and bitter sensitivity of wild type and $AKHR^{EY11371}$ mutant flies in the same genetic background. (E–F) Sugar and bitter sensitivity of flies with genetic thermoactivation of AKH-producing cells (w-; +; *akh-GAL4* (III) crossed with w-; +; + (E_1) or w-; *UAS-dTrpA1* (II); *UAS-dTrpA1* (III) (E_2). w-; $sNPF^{c00448}$; *akh-GAL4* (III) crossed with w-; +; + (E_3) or w-; *UAS-dTrpA1* (II); *UAS-dTrpA1* (III) (E_4)). Flies were preincubated in 30 °C or 18 °C for 30 min and PER was performed in 18 °C. n>5 for all experimental groups. See also Figure S5.

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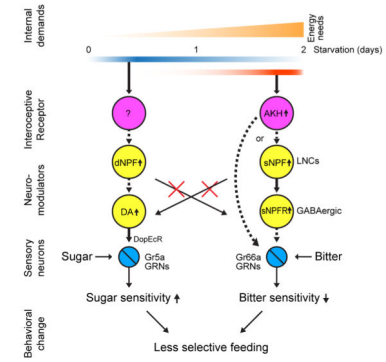
A



B

	Starvation	DA		NPF		sNPF/sNPFR		AKH/AKHR	
		GOF (L-dopa feeding)	LOF (KIR2.1, *DopEcR* mutant)	GOF (dTrpA1)	LOF (KIR2.1)	GOF (sNPFR over-expression)	LOF (KIR2.1, *sNPF* mutant, RNAi)	GOF (dTrpA1)	LOF (hid, *akhr* mutant)
Sugar sensitivity	↑ increase	↑	↓	↑	↓	∅	∅	∅	∅
Bitter sensitivity	↓ decrease	∅ no change	∅	∅	∅	↓	↑	↓	↑

Figure 7. Distinct Neuronal Pathways Modulating Sugar and Bitter Sensitivity During Starvation

(A) Schematic illustrating the two distinct neuronal pathways we identified to control sugar and bitter sensitivity in an independent manner. Dashed arrows indicate genetic interactions that we have not shown to be direct. (B) Table summarizing findings in this paper.