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Sexual Failure Decreases Sweet Taste Perception in Male *Drosophila* via Dopaminergic Signaling

Gaohang Wang, Wei Qi, Rui Huang, Liming Wang

Institute of Molecular Physiology, Shenzhen Bay Laboratory, Shenzhen, China • Department of Biology, Stanford University, Stanford, United States • Guangyang Bay Laboratory, Chongqing Institute for Brain and Intelligence, Chongqing, China • School of Public Health, Capital Medical University, Beijing, China • Chinese Institutes for Medical Research, Beijing, China

eLife Assessment

This study provides **valuable** findings on the effects of mating experience on sweet taste perception. The data as presented provide **convincing** evidence that the dopaminergic signaling-mediated reward system underlies this mating state-dependent behavioral modulation. The work will interest neuroscientists and particularly biologists working on neuromodulation and the effects of internal states on sensory perception.

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Abstract

Sweet taste perception, a critical aspect of the initiation of feeding behavior, is primarily regulated by an animal's internal metabolic state. However, non-metabolic factors, such as motivational and emotional states, can also influence peripheral sensory processing and hence feeding behavior. While mating experience is known to induce motivational and emotional changes, its broader impact on other innate behaviors, such as feeding, remains largely uncharacterized. In this study, we demonstrated that mating failure of male fruit flies suppressed sweet taste perception via dopamine signaling in specific neural circuitry. Upon repetitive failure in courtship, male flies exhibited a sustained yet reversible decline of sweet taste perception, as measured by the proboscis extension reflex (PER) towards sweet tastants as well as the neuronal activity of sweet-sensing $Gr5a^+$ neurons in the proboscis. Mechanistically, we identified a small group of dopaminergic neurons projecting to the subesophageal zone (SEZ) and innervating with $Gr5a^+$ neurons as the key modulator. Repetitive sexual failure decreased the activity of these dopaminergic neurons and in turn suppressed $Gr5a^+$ neurons via Dop1R1 and Dop2R receptors. Our findings revealed a critical role for dopaminergic signaling in integrating reproductive experience with appetitive sensory processing, providing new insights into the complex interactions between different innate behaviors and the role of brain's reward systems in regulating internal motivational and emotional states.

Introduction

As an innate behavior, feeding is essential for the survival and reproduction of all animal species, regulated by a dynamic interplay of internal and external factors such as nutritional status, circadian rhythms, food quality, and emotional states (Atasoy et al., 2012; Hergarden et al., 2012; Lim et al., 2012; Morton et al., 2006; Park et al., 2016; Shiu et al., 2022; Xu et al., 2008; Yapici et al., 2016; Zhan et al., 2016). Beyond nutritive value, palatable food also serves as a powerful reward signal that regulates feeding behavior by influencing various aspects of learning, memory, and motivation (Dayan & Balleine, 2002; Wise, 2004). The function of dopamine system, a key regulator in reward processing, is evolutionarily conserved across species. Studies in both fruit flies and rodents demonstrate that the consumption of sugar or other highly appealing foods triggers a robust dopamine response. The neural substrates of these responses, such as the mushroom bodies in fruit flies and the ventral tegmental area in rodents, underscore the universality of this reward circuit (Burke et al., 2012; Heymann et al., 2020; Liu et al., 2012; Takahashi et al., 2016; Yokel & Wise, 1975).

Mating behavior, as another critical driver of the survival and reproduction of animal species, is also influenced by external stimuli and internal states (Clowney et al., 2015; Kallman et al., 2015; Kohatsu et al., 2011). Failure in mating behavior leads to various behavioral adaptations and even abnormalities. For example, male fruit flies exhibit courtship conditioning, where unsuccessful mating attempts reduce subsequent courtship toward inappropriate mates, demonstrating behavioral plasticity aimed at enhancing future reproductive success (Keleman et al., 2012). Meanwhile, the effect of mating failure extends beyond reproductive behavior. It has been shown that mating failure increases alcohol consumption in fruit flies (Shohat-Ophir et al., 2012), a behavior that mirrors reward-seeking tendencies such as substance abuse seen in higher organisms under stress. Recent studies also demonstrate that mating-related sensory cues can recalibrate female odor-driven preferences through a dopamine-gated learning circuit in the mushroom body (Boehm et al., 2022). In males, dopamine dynamically adjusts the temporal integration window of copulation decision neurons to regulate mating persistence (Gautham et al., 2024). Additionally, mating itself has a rewarding nature. In male flies, approaching copulation activates dopaminergic neurons that suppress visual threat responses, suggesting that the reward system can override aversive cues to promote mating behavior (Cazale-Debat et al., 2024; Zhang et al., 2016). In male mice, after interaction with females, chemical signals are detected by the accessory olfactory bulb and transmitted to neural circuits that regulate mating behavior (Decoster et al., 2024). These signals are then projected to the ventral tegmental area, where serotonin neurons play a crucial role in encoding both reward acquisition and anticipation (Hashikawa et al., 2016).

Given the established impact of mating experience on behavioral plasticity, we aimed to investigate whether it also affected reward perception and processing in fruit flies.

Specifically, we examined how courtship failure influenced sweet sensitivity and feeding behavior, both of which were tightly linked to reward. In our study, we found that sexual failure led to decreased sweet sensitivity and feeding behavior in male fruit flies, highlighting a close link between two important innate behaviors. Furthermore, we identified a small group of dopaminergic neurons that projected to the SEZ of the fly brain, modulating the activity of sweet-sensing gustatory neurons in a manner dependent on sexual experience. These dopaminergic neurons were functionally epistatic to sweet-sensing neurons and promoted behavioral responses to appetitive cues, but their activity decreased following courtship failure. As a result, the activity of $Gr5a^{+}$ neurons in the proboscis was reduced via signaling through Dop1R1 and Dop2R receptors, leading to reduced sweet sensitivity. These findings suggest that courtship failure inhibits the dopamine-mediated reward system in the brain, directly influencing sweet perception

and feeding behavior. This provides key insights into how reproductive stressors, such as mating failure, can affect broader behavioral output, expanding our understanding of the complex interplay between instinctive drives.

Results

Courtship failure reduced sweet sensitivity in male flies

To investigate whether mating experience influenced feeding behavior in *Drosophila melanogaster*, we employed a modified courtship conditioning paradigm (McBride et al., 1999; Shohat-Ophir et al., 2012) (**Figure 1A**) and examined three groups of males with different mating experiences *in prior*. In *Drosophila*, mated females typically refuse further copulation, leading males exposed to mated females to experience repeated sexual rejection. In contrast, males paired with multiple virgin females could engage in multiple rounds of successful copulations. In our experiments, virgin males were isolated from females for 4 days, after which they were divided into three groups on the 5th day: the Naïve group (continued isolation for 4.5 hours), the Failed group (each male exposed to 25 mated females for 4.5 hours), and the Satisfied group (each male exposed to 25 virgin females for 4.5 hours). Following the treatment, we measured sucrose intake using the MAFE (MAual FEeding) assay we developed previously (Qi et al., 2015). Despite the fact that the amount of sucrose consumed by individual flies was similar across different groups (**Figure 1-figure supplement 1A**), the proportion of males ever consuming any sucrose was significantly lower in the Failed group (**Figure 1B**), suggesting a reduced likelihood of meal initiation among these males. Notably, mating failure also compromised the flies' ability to withstand starvation, as Failed males died significantly faster under food deprivation (**Figure 1-figure supplement 1B**).

Since taste perception is closely linked to the initiation of feeding behavior, we next examined whether sexual experience affected sweet sensitivity using the PER assay. This well-established method quantified the tendency to initiate feeding behavior towards different gustatory stimuli (Scott, 2005, 2018). While three groups of male flies all exhibited dose-dependent responses to varying sucrose concentrations, males in the Failed group demonstrated a rightward shift of the concentration-response curve, indicating significantly reduced sweet sensitivity (**Figure 1C** and **Figure 1-figure supplement 1C**). Consistently, the Mean Acceptance Threshold (MAT), the sucrose concentration at which 50% of the population displayed a PER response, was significantly elevated in the Failed group (**Figure 1C** and **Figure 1-figure supplement 1C** right). Furthermore, we found that diminished sweet sensitivity extended to other sweet compounds such as fructose and glucose (**Figure 1-figure supplement 1D-E**), indicating a general reduction in sweet sensitivity and an overall elevation in the threshold to initiate feeding upon appetitive cues.

To determine the time course of this effect, we monitored PER responses in male flies at 24, 48, and 72 hours post-treatment. Sweet sensitivity remained suppressed for up to 48 hours in Failed males but returned to baseline levels by 72 hours (**Figure 1D**). To explore the mechanisms underlying this lasting change, we examined whether it could be attributed to the exposure to cis-vaccenyl acetate (cVA), a pheromone present on the cuticle of mated females but absent from virgin females (Ejima et al., 2007). Conditioning males with either mated females or decapitated virgin females produced similar reductions in sweet sensitivity (**Figure 1E**), suggesting that cVA is not essential for this behavioral effect. We then tested whether the reduced sweet sensitivity was a result of failed copulation. Failed males were then divided into two groups for additional treatments: one group remained untreated, while the other was allowed to copulate with virgin females. We found that the reduction in sweet sensitivity caused by sexual failure could be reversed through subsequent successful copulation. Such males showed significantly increased sugar sensitivity compared to those that did not have a chance to copulate at all (**Figure 1F**). In contrast, when

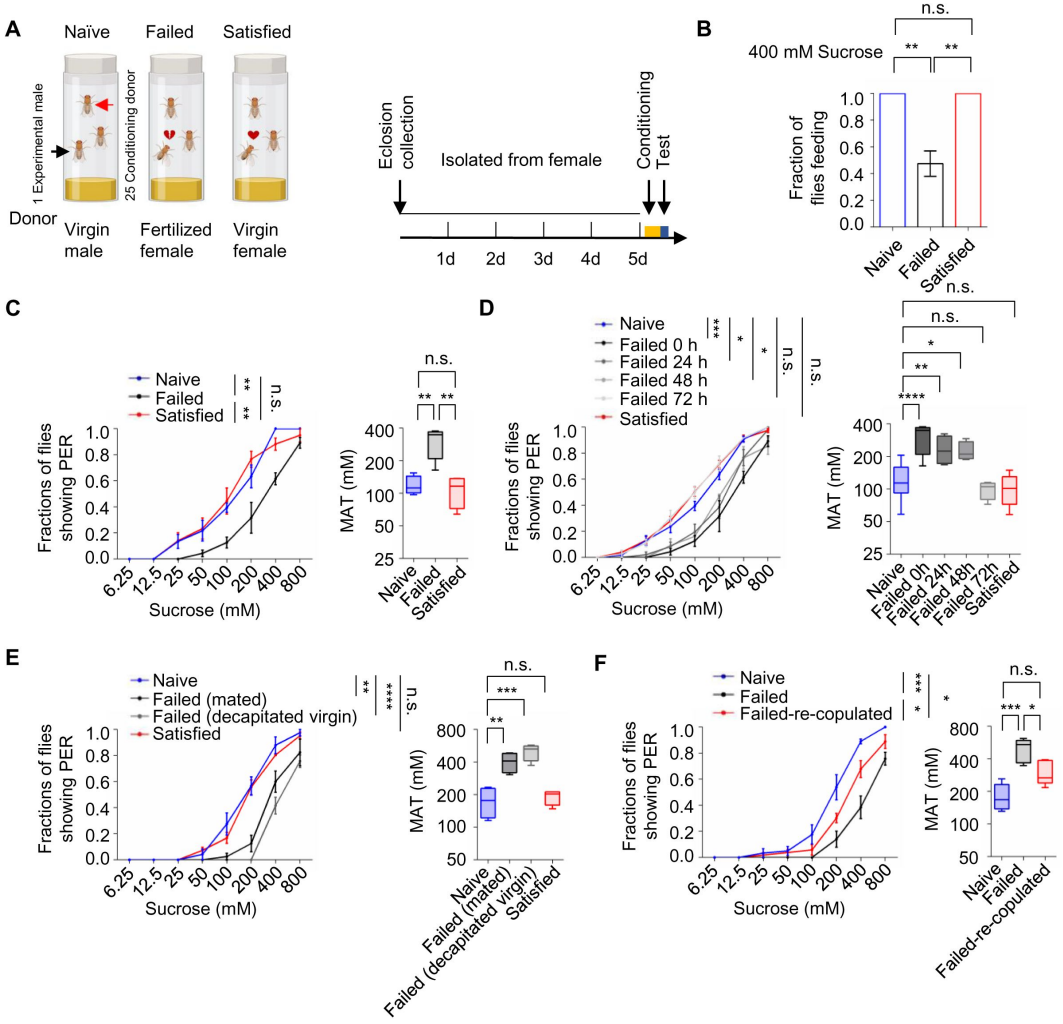

Figure 1

Sexual failure decreased sweet sensitivity.

(A) Schematic illustrating courtship conditioning strategy. (B) Percentage of males that performed feeding to 400 mM sucrose. (C) Fraction of Naïve, Failed and Satisfied male flies showing PER responses to different concentrations of sucrose (n=60-90). The PER experiment was performed immediately after conditioning unless otherwise indicated. Left: average PER responses of Naïve (blue), Failed (black), and Satisfied (red) flies. Right: MAT (mean acceptance threshold; the sucrose concentration where 50% of the flies show PER), plotted as a function of sexual state. (D) Fraction of male flies showing PER responses to different concentrations of sucrose (n=48-60). The PER experiment was performed immediately, 24 h, 48 h, and 72 h after conditioning. Naïve and Satisfied showed pooled data from 0 to 72 hours post-treatment (E) Fraction of male flies showing PER responses to different concentrations of sucrose (n=48-60). Failed male flies were generated by two methods: one group was subjected to mated females (with cVA), and the other was subjected to decapitated virgin females (without cVA). (F) Fraction of male flies showing PER responses to different concentrations of sucrose (n=48-60). Failed-re-copulated males were generated by placing individual failed males (within 30 minutes post-treatment) with 25 virgin females in a food vial for 4.5 h. Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$. One-way ANOVA (B, C-F MAT data) and two-way ANOVA (C-F PER data) were applied for statistical analysis.

previously mated flies experienced courtship failure, their sweet sensitivity was also significantly decreased, indicating that prior successful mating did not prevent the alteration in sweet sensitivity induced by subsequent courtship failure (**Figure 1-figure supplement 1F**). Overall, our findings reveal that sexual failure exerted a long-lasting yet reversible effect to reduce sweet sensitivity in male flies.

Dopamine signaling mediated the suppression of sweet sensitivity following sexual failure

The long-lasting and reversible effect of sexual failure hinted certain changes in flies' internal motivational state. Dopamine was reported to regulate PER behavior in a metabolic state-dependent manner (Inagaki et al., 2012; Marella et al., 2012). We thus hypothesized that dopamine might also play a key role in the taste plasticity triggered by sexual failure. To test this, we first used a pharmacological approach to reduce dopamine signaling in male flies by feeding them 3-iodotyrosine (3IY), a tyrosine hydroxylase-specific inhibitor that blocked dopamine synthesis (Inagaki et al., 2012) and then measured their PER responses. Flies treated with 3IY showed comparable sweet responses across all three experimental groups, with an overall reduction in sweet sensitivity compared to the control group, effectively abolishing the changes induced by sexual failure (**Figure 2A**). To further confirm the necessity of dopamine in modulating sexual experience-dependent sweet sensitivity, we employed a genetic approach by using $Th^{-/-}$ mutant flies, in which tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, was eliminated (Deng et al., 2019; Friggi-Grelin et al., 2003; Marella et al., 2012). As expected, $Th^{-/-}$ mutant flies in the Failed group exhibited sweet sensitivity indistinguishable from those in the Naïve and Satisfied groups (**Figure 2-figure supplement 1A**).

Next, we investigated whether elevating dopamine levels could restore diminished sweet sensitivity in males from the Failed group. We first tested the effect of exogenous dopamine on sweet sensitivity by injecting dopamine into the base of the flies' proboscis. Consistent with previous findings (Inagaki et al., 2012; Marella et al., 2012), increasing dopamine levels enhanced sweet sensitivity (**Figure 2-figure supplement 1B**). We then measured PER behavior in Naïve, Failed, and Satisfied flies injected with either dopamine or ddH_2O (control). While ddH_2O-treated males from the Failed group displayed reduced sweet sensitivity compared to Naïve and Satisfied males, dopamine-treated Failed flies did not show such reduction (**Figure 2B**), further suggesting that dopamine signaling is necessary for sexual failure to suppress sweet sensitivity.

Besides dopamine, serotonin, another important biogenic amine, has been reported to modulate motivational states and influence feeding behavior (Owald & Waddell, 2015; Ries et al., 2017; Srinivasan et al., 2008). Therefore, we examined whether serotonin also played a role in the sexual experience-dependent changes in sweet sensitivity. Feeding male flies with DL-p-chlorophenylalanine (pCPA) or methysergide, both blocking serotonin signaling (Engelman et al., 1967; McCorvy et al., 2018), did not affect the reduction in sweet sensitivity upon sexual failure (**Figure 2-figure supplement 1C-D**).

To further validate the involvement of dopamine signaling in sexual failure-induced behavioral changes, we used *TH-Gal4* to drive *UAS-dTRPA1* or *UAS-Shibire^{ts}* (*Shi^{ts}*) to transiently activate or inhibit dopaminergic neurons, respectively (Deng et al., 2019). Activation of dopaminergic neurons through the temperature-sensitive cation channel dTRPA1 (Pulver et al., 2009) increased sugar sensitivity in all male flies and eliminated differences among groups with different sexual experiences. Meanwhile, control genotypes expressing only *TH-Gal4* or *UAS-dTRPA1* still showed significantly reduced sugar sensitivity in the Failed group (**Figure 2C**). Additionally, males from the Failed group showed decreased sweet sensitivity across all genotypes at the permissive temperature (20 °C) (**Figure 2-figure supplement 1E**). In contrast, blocking dopaminergic neurons via the temperature-sensitive dominant-negative Dynamin *Shibire^{ts}* (*Shi^{ts}*)

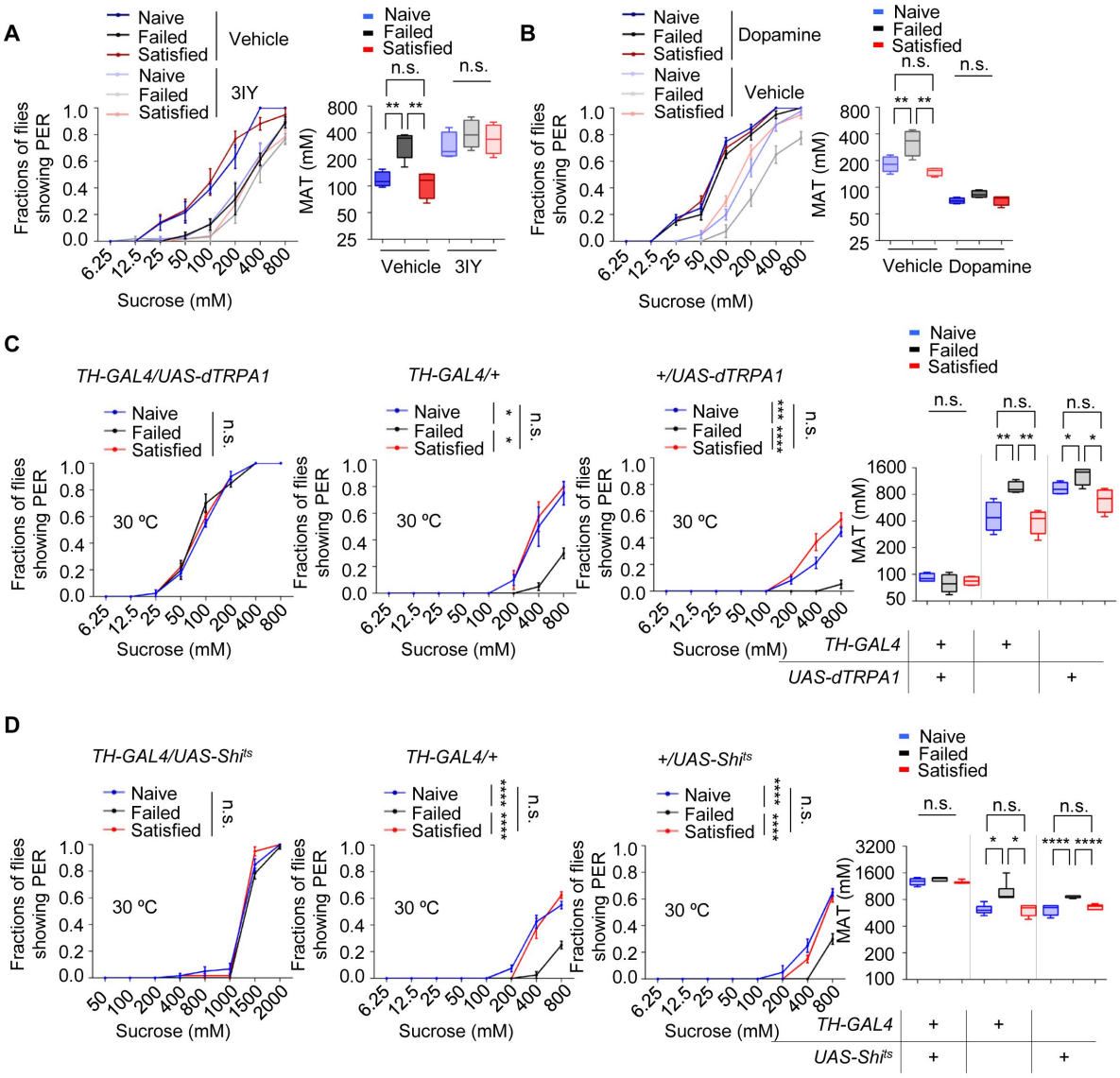

Figure 2

Dopamine signaling modulated the effect of sexual experience on sweet sensation.

(A) PER responses in male flies fed with 3IY, compared to male flies fed with vehicle (n=48-60). (B) PER responses in male flies with dopamine injection, compared to male flies injected with vehicle (n=48-60). (C-D) PER responses in male flies with indicated genotypes at 30°C (n=48-60). Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$. Two-way ANOVA (A-D PER data) and one-way ANOVA (A-D MAT data) were applied for statistical analysis.

(**Figure 2-figure supplement 1E**). In contrast, blocking dopaminergic neurons via the temperature-sensitive dominant-negative Dynamin *Shibire*^{ts} (*Shi*^{ts}) (Kitamoto, 2001) decreased sugar sensitivity across all three groups and also completely suppressed the differences among groups (**Figure 2D**). In comparison, in the two control genotypes and under permissive temperature (20 °C), sexual failure could still suppress sweet sensitivity (**Figure 2D**, **Figure 2-figure supplement 1F**). These findings demonstrate that reduced sweet sensitivity in sexually failed males is mediated by dopaminergic neurons.

Dopamine signaling directly modulated the activity of Gr5a$^+$ gustatory neurons upon sexual failure

We next asked how dopamine signaling modulated taste sensitivity. Gustatory receptor neurons (GRNs) respond to specific taste stimuli, with Gr5a$^+$ neurons, which express sweet taste receptors, playing a key role in sweet perception (Dahanukar et al., 2007; Thorne et al., 2004; Wang et al., 2004). We hypothesized that behavioral changes following sexual failure might be linked to altered activities of sweet-sensing GRNs. To test this, we expressed calcium indicator GCaMP6m (Chen et al., 2013) in Gr5a$^+$ neurons and conducted *in vivo* calcium imaging. Gr5a$^+$ neurons project to SEZ in the fly brain, where we recorded calcium signals in response to sucrose stimulation. Sexual failure significantly reduced the responsiveness of Gr5a$^+$ neurons to sucrose, consistent with our behavioral observations (**Figure 3A-D**).

Given that dopamine signaling has been shown to modulate sweet sensitivity (Inagaki et al., 2012; Marella et al., 2012), it was plausible that dopamine signaling directly modulated the activity of Gr5a$^+$ neurons upon sexual failure. We thus examined projection patterns of dopaminergic neurons relative to those of Gr5a$^+$ neurons. Using *TH-Gal4* and *Gr5a-LexA*, we expressed *UAS-mCD8GFP* and *LexAop-rCD2RFP* in the same fly brains, respectively. The brain imaging data revealed that dopaminergic projections were closely aligned with Gr5a$^+$ GRNs in the SEZ (**Figure 3-figure supplement 1A**), suggesting possible synaptic connections of Gr5a$^+$ neurons and dopaminergic neurons.

We confirmed this idea using enhanced trans-synaptic reconstruction (nSyb-GRASP) (Feinberg et al., 2008; Gordon & Scott, 2009; Macpherson et al., 2015), which could visualize direct and active synaptic innervations between two groups of neurons. The nSyb-GRASP system relies on the expression of two split-GFP fragments on membranes of two neuronal populations, which fluoresce only upon the reassembly of these two fragments at active synapses. By employing the nSyb-GRASP system in Gr5a$^+$ neurons and dopaminergic neurons, we observed GFP signals in the SEZ (**Figure 3-figure supplement 1B**). Furthermore, in males that experienced mating failure, synaptic connections between dopaminergic neurons and Gr5a$^+$ GRNs were significantly weakened (**Figure 3E-F**). These results suggest that dopaminergic neurons directly innervate Gr5a$^+$ neurons and modulate their activity upon sexual failure.

This idea was further assessed by other complementary approaches. We expressed the photosensitive protein CsChrimson (Klapoetke et al., 2014) in dopaminergic neurons and GCaMP6m in Gr5a$^+$ neurons. Light activation of dopaminergic neurons significantly enhanced the calcium responses of Gr5a$^+$ neurons to sugar stimuli both in Naïve and Failed groups (**Figure 3-figure supplement 1C-D**). Furthermore, by using a CaLexA reporter system (Masuyama et al., 2012), we assessed the impact of sexual experience on neural activity in male fruit flies. The results showed that sexual failure led to a significant reduction in CaLexA-mediated GFP signals in dopaminergic neurons in the SEZ but not in the antennal lobes (AL) (**Figure 3G-I**). In summary, these findings indicate that mating experience modulates sweet taste perception in flies by inhibiting the activity of specific dopamine neurons in the SEZ and reducing synaptic transmission between dopaminergic neurons and sweet-sensing Gr5a$^+$ GRNs.

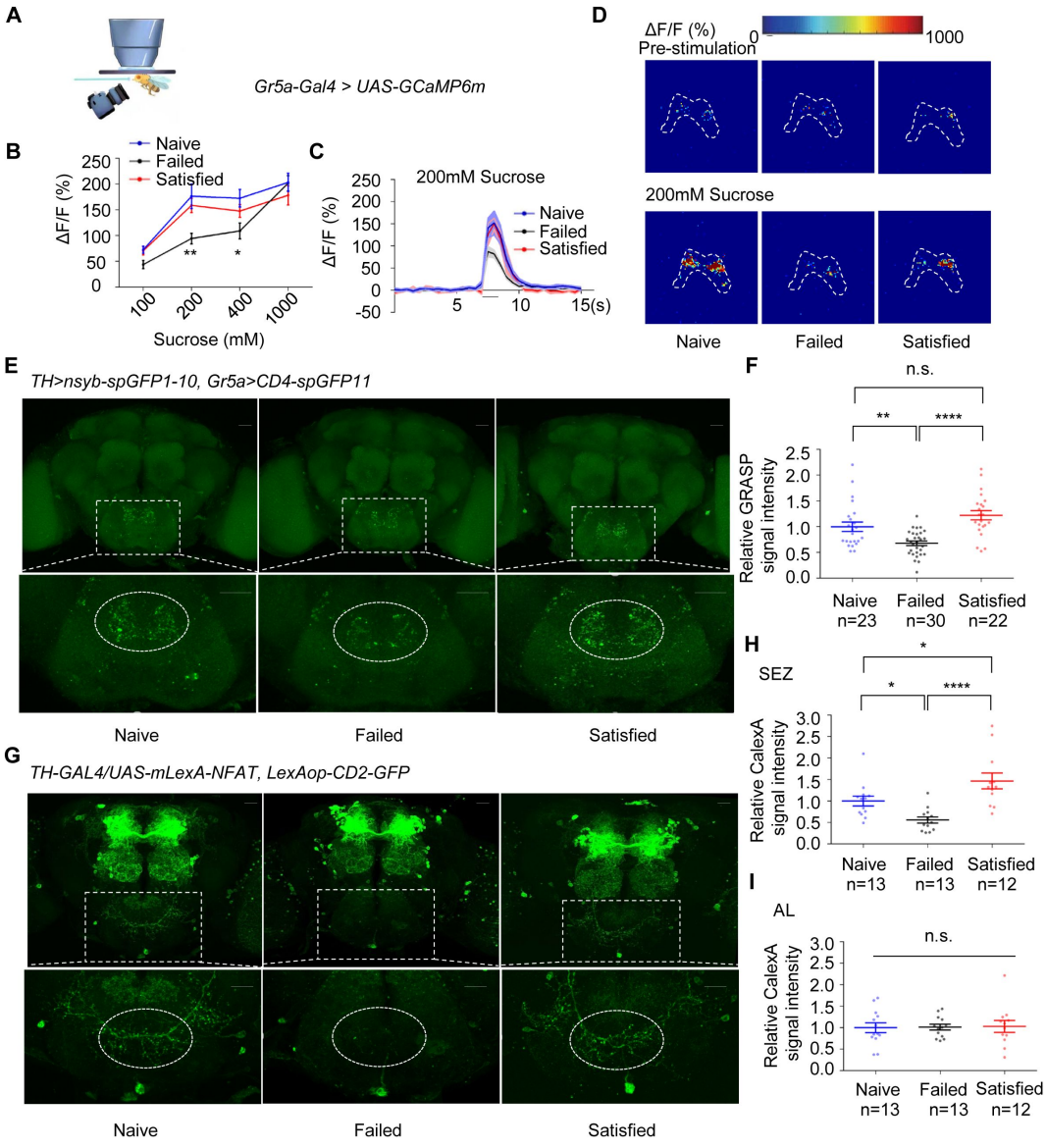

Figure 3

Dopamine neurons modulated the activity of Gr5a$^+$ neurons upon sexual failure.

(A) Schematic diagram of *in vivo* calcium imaging paradigm. (B) Quantification of the *in vivo* calcium responses of Gr5a$^+$ neurons to the indicated concentrations of sucrose (n=6-22). (C) Averaged traces of the calcium responses to 200 mM sucrose. The horizontal black bar represents sucrose application. The solid lines represent the average trace, and shaded regions represent ±SEM. (D) Representative pseudocolor images of the calcium responses to 200mM sucrose. The dashed area was quantified to measure the response. (E) Top: Representative images from the GRASP experiment between dopaminergic neurons and sweet GRNs. Bottom: Enlarged images of the SEZ region seen on the top. Scale bars, 20 μm. (F) Average fluorescence reported by *TH>nsyb-spGFP1-10, Gr5a>CD4-spGFP11* (normalized to Naive) in the SEZ. Regions of interest (ROIs) are circled by dashed lines in (E). Lines represent mean ± SEM, and dots indicate values for individual flies. (G) Top: Representative images of CaLexA-induced GFP reporter in dopaminergic neurons. Bottom: Enlarged images of the SEZ region was shown on top. Scale bars, 20 μm. (H-I) Average fluorescence reported by *TH-GAL4/UAS-mLexA-NFAT, LexAop-CD2-GFP* (normalized to Naive) in the SEZ(H), in the AL (I). Regions of interest (ROIs) are circled by dashed lines in (G). Lines represent mean ± SEM, and dots indicate values for individual flies. Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$. Two-way ANOVA (B) and one-way ANOVA (F, H, and I) were applied for statistical analysis.

Two dopamine receptors, Dop1R1 and Dop2R, mediated the effect of sexual failure

We next explored the molecular mechanisms involved in the reduction of sweet sensitivity upon sexual failure. Four dopamine receptors (Dop1R1, Dop1R2, Dop2R, and DopEcR) have been identified in *Drosophila* (Deng et al., 2019; Feng et al., 1996; Hearn et al., 2002; Srivastava et al., 2005; Sugamori et al., 1995). Through screening of these receptors, we found that mutations in *Dop1R1* or *Dop2R* abolished the effect of sexual failure on sweet sensitivity, while mutations in *Dop1R2* or *DopEcR* did not prevent the decline in sweet sensitivity following sexual failure (**Figure 4A-F**). Intriguingly, although Dop2R and DopEcR are both required for starvationLJdriven enhancement of sugar sensitivity (Inagaki et al., 2012; Marella et al., 2012), Dop2R and Dop1R1 (but not DopEcR) were necessary for the suppression of sweet sensitivity after courtship failure. This dissociation implies that reproductive experience and metabolic state engage distinct dopaminergic circuits, possibly via different neuronal subsets, dopamine release dynamics, or receptor recruitment, to modulate peripheral sensitivity.

To further confirm the roles of Dop1R1 and Dop2R, we used RNA interference (RNAi) to knock down these receptors in flies' nervous system. In line with the above results, knockdown of Dop1R1 or Dop2R blocked the influence of sexual experience on sweet sensitivity, while knockdown of Dop1R2 or DopEcR had no significant effect (**Figure 4-figure supplement 1A-D**). These results suggest that reductions in sweet sensitivity induced by sexual failure are mediated by dopamine signaling acting on Dop1R1 and Dop2R receptors.

Next, we activated the neurons expressing Dop1R1 or Dop2R by expressing the bacterial sodium channel NaChBac (Nitabach et al., 2006). Constitutive activation via NaChBac not only prevented courtship failure-induced decline in sucrose responsiveness but also enhanced PER in Naïve flies compared to the GAL4/+ controls (**Figure 5A-B**). Meanwhile, activating Dop1R2$^+$ or DopEcR$^+$ neurons did exhibit such effect (**Figure 5-figure supplement 1A-C**).

We also tried to silence Dop1R1$^+$ or Dop2R$^+$ neurons using the hyperpolarizing potassium channel (Kir2.1) (Baines et al., 2001). Kir2.1 - mediated inhibition suppressed sweet sensitivity across Naïve, Satisfied, and Failed groups and abolished any additional reduction in the Failed cohort (**Figure 5C-D**). These findings demonstrate that dopaminergic input through Dop1R1/Dop2R acts as a positive drive to Gr5aLJ sweetLJsensing neurons and that sexual failure suppresses sweet sensitivity by attenuating this dopaminergic tone.

Gr5a$^+$ neurons received dopaminergic modulation through Dop1R1 and Dop2R

The above discoveries raised a possibility that sweet-sensing Gr5a$^+$ neurons might receive direct dopaminergic modulation via Dop1R1 and Dop2R receptors, and that such mechanism might be critical for the modulation of sweet sensitivity upon sexual failure. To investigate this possibility, we first examined the expression of Dop1R1 and Dop2R in Gr5a$^+$ neurons. Immunostaining confirmed that both receptors were expressed in the labellum (**Figure 6-figure supplement 1A**). Moreover, co-labeling experiments revealed a significant overlap of Dop1R1/Dop2R expression in Gr5a$^+$ neurons (**Figure 6A-B**).

Next, we assessed whether the expression of Dop1R1 and Dop2R in Gr5a$^+$ neurons was functionally important for the reduction of sweet sensitivity upon sexual failure. By using RNAi to reduce Dop1R1 or Dop2R expression in Gr5a$^+$ neurons, we found that males experiencing sexual failure no longer exhibited a reduction in sweet sensitivity compared to Naïve or successfully mated males (**Figures 6C-D**). In contrast, reducing the expression of another dopamine receptor, DopEcR, which was also partially expressed in Gr5a$^+$ neurons (**Figure 6-figure**

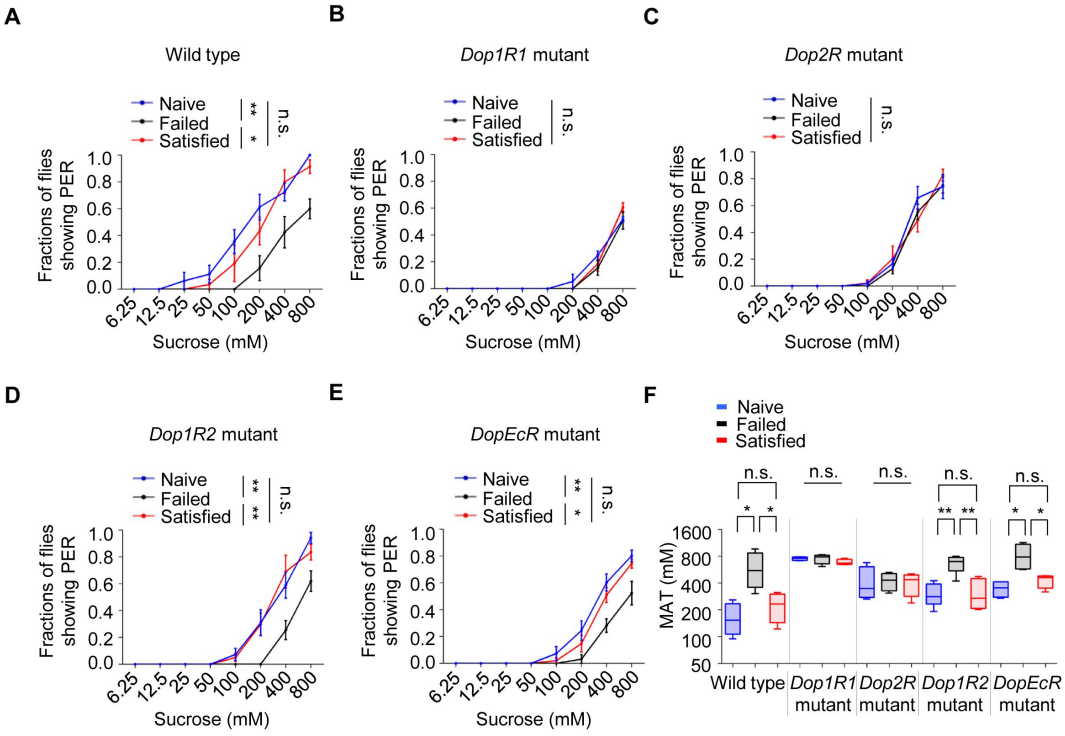

Figure 4

The effect of sexual failure was modulated through two dopamine receptors, Dop1R1 and Dop2R.

(A-E) PER responses in male flies with indicated genotypes (n=48-75). (F) MAT calculation for the PER data from (A-E), plotted as a function of sexual state and type of flies. Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$. Two-way ANOVA (A-E) and one-way ANOVA (F) were applied for statistical analysis.

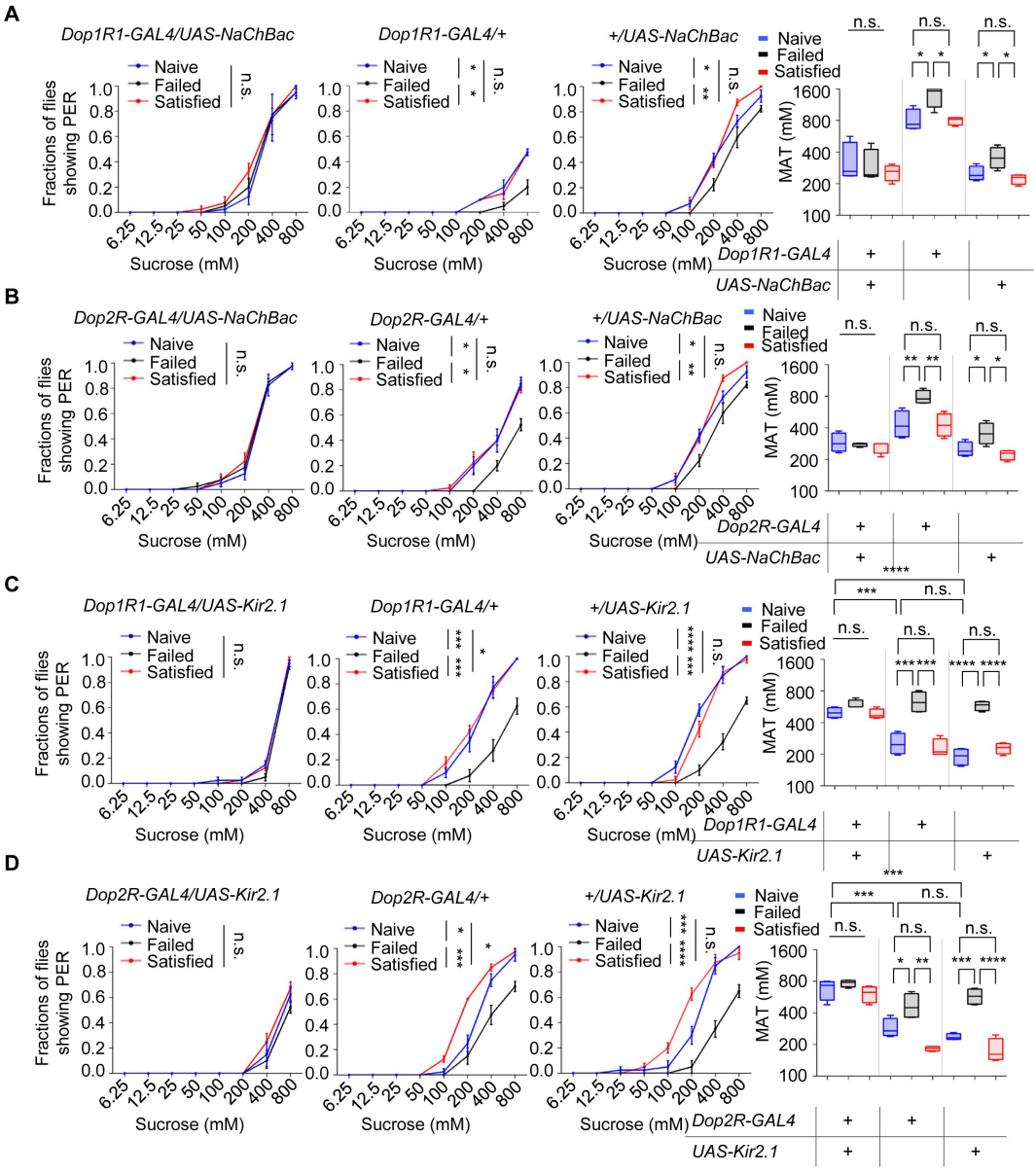

Figure 5

Dop1R1^{+} and Dop2R^{+} neurons regulated sexual experience-dependent sweet sensitivity.

(A-D) PER responses in male flies with indicated genotypes (n=48-60). Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$. Two-way ANOVA (A-D PER data) and one-way ANOVA (A-D MAT data) were applied for statistical analysis.

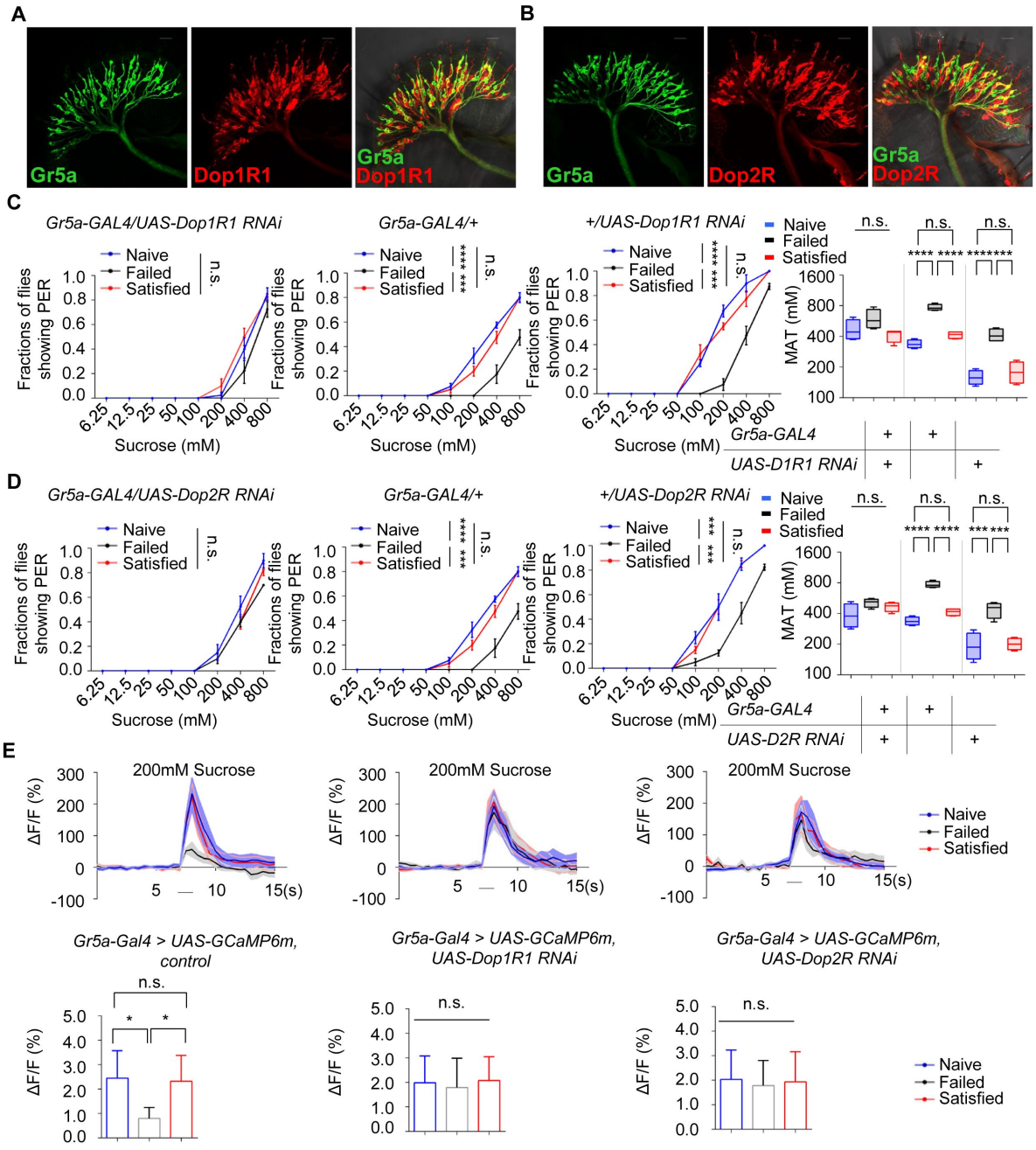

Figure 6

Gr5a^{+} neurons received dopaminergic modulation through Dop1R1 and Dop2R.

(A-B) The expression of membrane-bound GFP (mCD8GFP, Green) in Gr5a^{+} neurons driven by *Gr5a-LexA* and membrane-bound RFP (mCD8RFP, Red) in dopamine receptor neurons driven by *Dop1R1-Gal4* (A), and *Dop2R-Gal4* (B). Scale bars, 20 μm. (C-D) PER responses in male flies with indicated genotypes (n=60-75). (E) Averaged traces of the calcium responses to 200mM sucrose in male flies with indicated genotypes was shown on top. Quantification of the calcium responses was shown on the bottom (n=6-10). The horizontal black bars represent sucrose application. The solid lines represent the average trace, and shaded regions represent ±SEM. Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$. Two-way ANOVA (C-D PER data) and one-way ANOVA (C-D MAT data, E) were applied for statistical analysis.

supplement 1B), did not prevent the reduction of sweet sensitivity upon sexual failure (**Figure 6-figure supplement 1C**). These data suggest that Dop1R1 and Dop2R in $Gr5a^+$ neurons are indeed essential for the observed behavioral changes.

We then investigated whether Dop1R1 and Dop2R were required for the modulation of neuronal activity of $Gr5a^+$ neurons. Following sexual failure, the calcium responses of $Gr5a^+$ neurons to sugar stimuli were suppressed (**Figure 3C**). But this effect was eliminated when Dop1R1 or Dop2R expression was knocked down in $Gr5a^+$ neurons (**Figures 6E**). In summary, our results indicate that Dop1R1 and Dop2R in sweet-sensing $Gr5a^+$ neurons mediate the sexual experience-dependent regulation of sweet sensation by modulating the activity of these neurons.

Discussion

Various innate behaviors are crucial for the survival and reproduction of animals, such as feeding, mating, aggression, sleep, and running away from predators (Amir et al., 2015; Decoster et al., 2024; Hall, 1994; Hashikawa et al., 2016; Hergarden et al., 2012; Nair et al., 2023; Weber & Dan, 2016; Yapici et al., 2016). However, when animals are exposed to competing and even conflicting needs of these innate behaviors, they have to adjust their behavioral output to balance these needs in a timely and precise manner. In the present study, we explored the potential link between two innate behaviors, mating and feeding. We found that failed experiences in sexual interactions suppressed sweet taste perception and hence feeding behavior in male fruit flies (**Figure 7**). The evolutionary significance of this behavioral plasticity remains unclear. It may represent an adaptive response where reproductive failure shifts time allocation away from feeding to conserve resources or seek alternative mating opportunities. Alternatively, it may indicate a maladaptive state of disrupted reward processing. Previous research has demonstrated that mating failure diminishes animals' motivational state, leading to widespread behavioral changes (Beckwith et al., 2017; Jung et al., 2020; Ryvkin et al., 2024; Shen et al., 2023; Shohat-Ophir et al., 2012). Our study extended this knowledge by showing that mating failure could also affect reward-related feeding behavior. This suggests that different instinctive behaviors might share a common reward mechanism, which exerts broader effects to influence animals' behavioral choices and physiological states.

Feeding provides energy critical for animals' survival and it is not surprising that feeding is under tight and complex regulations (Atasoy et al., 2012; Morton et al., 2006; Xu et al., 2008; Yapici et al., 2016; Zhan et al., 2016). While a high volume of studies focus on mechanisms of how metabolic states modulate feeding behavior (Ganguly et al., 2021; Inagaki et al., 2012; Marella et al., 2012; Pool & Scott, 2014; Shiu et al., 2022), our study suggests the motivational drive of feeding, especially that influenced by mating experience. Previous investigations have shown that males failed in sexual behaviors increase their ethanol preference (Shohat-Ophir et al., 2012), have an accelerated aging process (Gendron et al., 2014), and exhibit a sexual experience-induced preference behavior (Shen et al., 2023). Here we demonstrated that sexual failure suppressed feeding behavior, one of the most basic homeostatic systems critical for animal fitness. These results collectively suggest that there may be a common neural mechanism that underlies the crosslink between mating failure and other innate behaviors.

Notably, we found that this behavioral coordination persisted for an extended duration and gradually faded over time, yet could be quickly reversed by successful mating. This suggests that the regulatory mechanism may modulate the internal state of the animal. A likely mechanism for this is through the reward system, as both successful mating and feeding are known to activate the reward system (Burke et al., 2012; Dai et al., 2022; Schultz, 2010; Stuber & Wise, 2016; Zer-Krispil et al., 2018). To support this idea, our study demonstrated that dopamine, a key neurotransmitter associated with reward (Peters et al., 2021; Wise, 2004), plays an integral role in mediating this behavioral regulation. Dopamine plays a central role in regulating internal

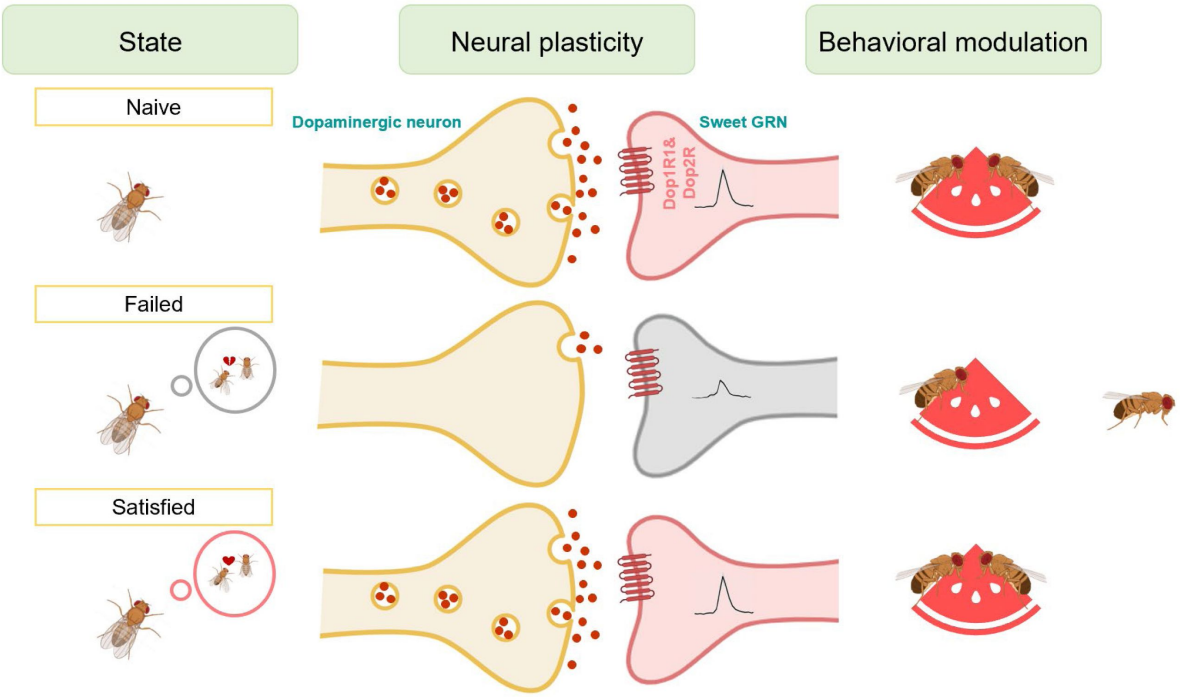

Figure 7

Working model for the sexual failure-dependent modulation of sweet perception.

In the SEZ of the fly brain, dopaminergic neurons form synaptic connections with sweet-sensing Gr5a^{+} neurons, which express the dopamine receptors Dop1R1 and Dop2R. This connectivity enables Gr5a^{+} neurons to receive dopaminergic modulation, enhancing the flies' sweet sensitivity. However, after mating failure, the activity of these SEZ dopaminergic neurons is selectively inhibited, and their synaptic connections with Gr5a^{+} neurons are weakened. This reduction in dopaminergic signaling ultimately decreases the flies' sweet taste perception. This mechanism highlights a specific pathway by which unsuccessful mating experiences influence sensory processing through neural plasticity in dopamine-associated circuits.

motivational states. It has been shown that factors like social interactions, mating, hunger, and vigilance influence behavioral output through dopamine signaling (Dai et al., 2022; de Jong et al., 2019; Inagaki et al., 2012; Marella et al., 2012; Schultz, 2010; Watabe-Uchida & Uchida, 2018). Dopamine has been implicated in coding the valence of mating and feeding experiences (Burke et al., 2012; Dai et al., 2022; Stuber & Wise, 2016; Zhang et al., 2019). Thus, it is possible that dopamine system acts as a motivation/reward center, allocating limited energy toward different behaviors in an internal state-dependent manner. This raises a critical question: does dopamine also mediate interactions among various innate behaviors, such as sleep, aggression, courtship, fear, and feeding, especially when these behaviors are in conflict? Additionally, it remains to be determined whether a single group of dopamine neurons mediates the crosslink amongst these behaviors, or whether distinct neuronal populations regulate separate behavioral interactions, and the mechanisms by which sexual failure selectively impacts different dopaminergic subgroups warrant further investigation.

Disturbances in innate behaviors have widespread and lasting effects on animals' overall behavioral patterns and physiological states (Goldstein et al., 2018; Grzelka et al., 2023; Jourjine et al., 2016; Sun et al., 2023). This cross-behavioral influence is likely connected to the concept of primal emotions. Emotions are a fundamental mechanism for behavioral regulation across species, not exclusive to more complex animals. In *Drosophila*, previous studies have shown that the flies have emotion-like states such as stress and fear (Anderson & Adolphs, 2014; Gu et al., 2019). Our research suggests that mating failure may act as a trigger for negative emotions, affecting other behaviors like feeding and reward responses. This finding provides a new perspective on how emotions influence behavioral adaptation through neural mechanisms and offers a model for future research on emotion and behavior.

In summary, this study reveals the neural mechanisms through which mating failure suppresses sweet taste perception in *Drosophila* and establishes a foundation for investigating the complex interactions between emotions and the regulation of innate behaviors. Future research will explore how the dopamine system modulates the output of various innate behaviors under different emotional states and whether such regulatory mechanism is conserved across species.

Materials and methods

Key resources table

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Genetic reagent (D. melanogaster)	TH-GAL4	PMID: 30799021		Gift from Yi Rao
Genetic reagent (D. melanogaster)	UAS-dTRPA1	other		Gift from David Anderson
Genetic reagent (D. melanogaster)	UAS-Shi^{ts}	other		Gift from David Anderson
Genetic reagent (D. melanogaster)	Gr5a-GAL4	Bloomington Drosophila Stock Center	Cat: #57592	
Genetic reagent (D. melanogaster)	UAS-GCaMP6m	Bloomington Drosophila Stock Center	Cat: #42748	
Genetic reagent (D. melanogaster)	Gr5a-LexA	Bloomington Drosophila Stock Center	Cat: #93014	
Genetic reagent (D. melanogaster)	UAS-nSyb-spGFP1-10, lexAop-CD4-spGFP11	Bloomington Drosophila Stock Center	Cat: #64314	
Genetic reagent (D. melanogaster)	UAS-CalexA	Bloomington Drosophila Stock Center	Cat: #66542	
Genetic reagent (D. melanogaster)	Dop1R1-/- mutant	PMID: 30799021		Gift from Yi Rao
Genetic reagent (D. melanogaster)	Dop2R-/- mutant	PMID: 30799021		Gift from Yi Rao
Genetic reagent (D.	Dop1R2-/- mutant	PMID: 30799021		Gift from Yi Rao

melanogaster)				
Genetic reagent (D. melanogaster)	DopEcR-/- mutant	PMID: 30799021		Gift from Yi Rao
Genetic reagent (D. melanogaster)	Dop1R1-GAL4	PMID: 30799021		Gift from Yi Rao
Genetic reagent (D. melanogaster)	Dop2R-GAL4	PMID: 30799021		Gift from Yi Rao
Genetic reagent (D. melanogaster)	UAS-NaChBac	Bloomington Drosophila Stock Center	Cat: #9469	
Genetic reagent (D. melanogaster)	UAS-Kir2.1	Bloomington Drosophila Stock Center	Cat: #6595	
Genetic reagent (D. melanogaster)	10×UAS-IVS-mCD8::RFP, 13×LexAop2-mCD8::GFP	Bloomington Drosophila Stock Center	Cat: #32229	
Genetic reagent (D. melanogaster)	UAS-GCaMP6m	Bloomington Drosophila Stock Center	Cat: #42750	
Genetic reagent (D. melanogaster)	UAS-Dop1R1 RNAi	Tsinghua Fly Center	Cat: #4278	
Genetic reagent (D. melanogaster)	UAS-Dop2R RNAi	Tsinghua Fly Center	Cat: #2141	
Genetic reagent (D. melanogaster)	Th-/- mutant	PMID: 30799021		Gift from Yi Rao
Genetic reagent (D. melanogaster)	LexAop-rCD2RFP, UAS-mCD8GFP	Bloomington Drosophila Stock Center	Cat: #67093	
Genetic reagent (D. melanogaster)	LexAop-GCaMP6m	other		Gift from Yufeng Pan
Genetic reagent (D. melanogaster)	UAS-CsChrimson	other		Gift from Yufeng Pan
Genetic reagent	elav-GAL4	Bloomington	Cat: #25750	

(D. melanogaster)		Drosophila Stock Center		
Genetic reagent (D. melanogaster)	UAS-Dop1R2 RNAi	Tsinghua Fly Center	Cat: #5285	
Genetic reagent (D. melanogaster)	UAS-DopEcR RNAi	Tsinghua Fly Center	Cat: #3275	
Genetic reagent (D. melanogaster)	Dop1R2-GAL4	PMID: 30799021		Gift from Yi Rao
Genetic reagent (D. melanogaster)	DopEcR-GAL4	PMID: 30799021		Gift from Yi Rao
Genetic reagent (D. melanogaster)	UAS-mCD8::GFP	Bloomington Drosophila Stock Center	Cat: #5137	
Antibody	anti-GFP (mouse)	Sigma-Aldrich	Cat: # G6539	
Antibody	anti-GFP (mouse)	Abcam	Cat: #ab1218	
Antibody	anti-DsRed (rabbit)	Takara Bio	Cat: # 632496	
Antibody	anti-GFP (rabbit)	Thermo Fisher Scientific	Cat: #A11122	
Antibody	Goat anti-mouse Alexa Fluor 488	Thermo Fisher Scientific	Cat: #A11029	
Antibody	Goat anti-rabbit Alexa Fluor 546	Thermo Fisher Scientific	Cat: # A11010	
Antibody	Goat anti-rabbit Alexa Fluor 488	Thermo Fisher Scientific	Cat: # A11034	
Chemical compound	Sucrose	Sigma-Aldrich	Cat: #S0389	
Chemical compound	3-Iodo-L-tyrosine	Sigma-Aldrich	Cat: #I8250	
Chemical compound	Dopamine	Aladdin	Cat: #A303863	
Chemical compound	NaCl	Sigma-Aldrich	Cat: #S3014	
Chemical	$MgCl_2$	Sigma-Aldrich	Cat:	

compound			#M4880	
Chemical compound	$NaHCO_3$	Sigma-Aldrich	Cat: #S5761	
Chemical compound	NaH_2PO_4	Sigma-Aldrich	Cat: #S3139	
Chemical compound	KCl	Sigma-Aldrich	Cat: #P9541	
Chemical compound	$CaCl_2$	Sigma-Aldrich	Cat: #C5670	
Chemical compound	HEPES	Sigma-Aldrich	Cat: #54457	
Chemical compound	Paraformaldehyde	Electron Microscopy	Cat: #15713	
Chemical compound	Calf serum	Thermo Fisher Scientific	Cat: #16010159	
Chemical compound	Triton X-100	Sigma-Aldrich	Cat: #T8787	
Chemical compound	D-Fructose	Tokyo Chemical Industry	Cat: #F0060	
Chemical compound	D-Glucose	Tokyo Chemical Industry	Cat: #G0048	
Chemical compound	DL-p-chlorophenylalanine	Sigma-Aldrich	Cat: # c6506	
Chemical compound	Methysergide maleate	Abcam	Cat: #ab120530	
Chemical compound	All trans-retinal	Sigma-Aldrich	Cat: #R2500	
Commercial assay or kit	Graduated glass capillary	VWR	Cat: #53432-604	
Software, algorithm	Fiji/ImageJ	NIH		
Software, algorithm	GraphPad Prism 9	GraphPad Software		

Flies

Flies were maintained in vials with standard cornmeal food at 25°C and 60% humidity in a 12 h:12 h light: dark cycle unless otherwise indicated. For temperature-sensitive flies (Shibire^{ts} and dTRPA1), male flies were kept at 20°C before the behavioral tests. Fly strains used in the manuscript:

elav-GAL4 (#25750), *UAS-mCD8::GFP* (#5137), *LexAop-rCD2RFP*, *UAS-mCD8GFP* (#67093), *10×UAS-IVS-mCD8::RFP, 13×LexAop2-mCD8::GFP* (#32229), *UAS-GCaMP6m* (#42748), *UAS-GCaMP6m* (#42750), *UAS-CalexA* (#66542), *UAS-NaChBac* (#9469), *UAS-Kir2.1*(#6595), *Gr5a-GAL4* (#57592), *Gr5a-LexA* (#93014), *UAS-nSyb-spGFP1-10, lexAop-CD4-spGFP11*(#64314) were obtained from the Bloomington *Drosophila* Stock Center at Indiana University. *UAS-Shi^{ts}* and *UAS-dTRPA1* were kindly shared by David Anderson (California Institute of Technology). *Dop1R1$^{-/-}$*mutant, *Dop1R2$^{-/-}$*

mutant, *Dop2R*$^{-/-}$ mutant, *DopEcR*$^{-/-}$ mutant, *Th*$^{-/-}$ mutant, *Dop1R1-GAL4*, *Dop1R2-GAL4*, *Dop2R-GAL4*, *DopEcR-GAL4*, *TH-GAL4* were kindly shared by Yi Rao (Peking University). *LexAop-GCaMP6m* and *UAS-CsChrimson* were kindly shared by Yufeng Pan (Southeast University). *UAS-Dop1R1 RNAi* (#4278), *UAS-Dop1R2 RNAi* (#5285), *UAS-Dop2R RNAi* (#2141), and *UAS-DopEcR RNAi* (#3275) were obtained from the Tsinghua Fly Center.

Conditioning protocols

We used a modified courtship conditioning protocol (McBride et al., 1999; Shohat-Ophir et al., 2012) to generate Naïve, Failed, and Satisfied males. The Failed group was generated by exposing virgin males to either mated females or decapitated virgin females. Individual virgin males were placed with either 25 mated females or 25 decapitated virgin females in a food vial for 4.5 h. To generate mated females for the conditioning, 50 males were added to the 25 virgin females for 16 h before the conditioning. The decapitated female flies were prepared freshly before assays. The Satisfied group was generated by exposing virgin males to virgin females. Individual virgin males were placed with 25 virgin females in a food vial for 4.5 h. The Naïve group consisted of the age-matched virgin males kept isolated from females until the assays.

The MAFE assay

The MAFE assay was carried out as described previously (Qi et al., 2015). Briefly, individual male flies were gently introduced and immobilized in a 200 µl pipette tip. The end of each pipette tip was shaped to expose the fly's proboscis. Flies were first sated with sterile water and then presented with 400 mM sucrose filled in a graduated glass capillary (VWR, #53432-604). The sucrose was presented until the flies no longer responded to a series of 10 sucrose stimuli. The ingested volume was calculated based on the volume change before and after the feeding.

PER assay

PER was assayed as described previously (Qi et al., 2015). Individual male flies were mounted into a 200 µl pipette tip. Flies were first sated with sterile water and then subjected to different sugar solutions, each tested 3 times. Flies showing PER responses to at least 1 of 3 trials were considered positive to that sugar concentration. For drug treatment experiments, flies were maintained on food containing a drug for 2 days. Drugs used for treatment included 3-Iodo-L-tyrosine (3IY) (Sigma-Aldrich, 10 mg/ml), DL-p-chlorophenylalanine (pCPA) (Sigma-Aldrich, 10 mg/ml), Methysergide maleate (Abcam, 25mg/ml). For the dopamine injection experiment, Dopamine (Aladdin 0.1-10 ng/µl) was injected into the proboscis of each male fly using a glass micropipette. The standard PER assay was carried out at 25°C. For Shibire^{ts} and dTRPA1 experiments, flies were raised at 20°C, and assays were performed at either 20°C or 30°C. For PER assays performed at 24, 48, and 72 hours post-treatment, male flies were conditioned and then transferred into vials containing standard food before the behavioral assays. For Failed-re-copulated experiments, individual Failed male flies (within 30 minutes post-treatment) were allowed to mate with 25 virgin females in a food vial for 4.5 h. For Satisfied-Failed experiments, individual Satisfied male flies (72 hours post-treatment) were exposed to 25 mated females for 4.5 h. All PER assays had n>4 replicates with 10-15 flies per replicate unless otherwise stated.

MAT calculation

The method to calculate MAT has been thoroughly described in previous papers (Inagaki et al., 2012; Inagaki et al., 2014). In general, sigmoid interpolation was performed to fit the results into sigmoidal curves. The sigmoid curves for sugar were defined as follows:

$$F_S = \frac{1}{1 + e\left(-\alpha_s \log_2 \frac{S_{con}}{S_{50}}\right)}$$

F_S: Fraction of flies showing the PER on different sugar concentrations. Scon: Sugar concentration.

S_{50}: Sucrose concentration where 50% of flies show PER

α_S: Slope of the sigmoid curve

Based on the experimentally obtained results (F_S, Scon), S_{50} and α_S were chosen to fit the results. For all experimental results, fitting based on nonlinear regression was calculated with Python.

Starvation resistance assay

Starvation resistance of individual flies was monitored using DAMS (Trikinetics). Briefly, individual male flies were lightly anesthetized and introduced into 5 × 65 mm polycarbonate tubes (Trikinetics). One end of these tubes was filled with 2% agar or 5% sucrose, and the other end was blocked with cotton wool. These polycarbonate tubes were then inserted into DAMS monitors and kept in fly incubators during the course of the experiments.

Immunohistochemistry

Fly brains or labella were dissected in PBS on ice and fixed in 4% paraformaldehyde at room temperature for 60 min. To examine GRASP signals, the fly brains were dissected 20 hours post-treatment. After rinsing with PBS for 3 times, the fixed brains or labella were blocked in Penetration/Blocking Buffer (10% Calf Serum and 0.5% Triton X-100 in PBS) at room temperature for 90 min and incubated with primary antibodies at 4LJ for 24 h. The samples were then rinsed with Washing Buffer (0.5% Triton X-100 in PBS) at room temperature for 3 × 30 min and incubated with secondary antibodies at 4°C for 24 h. After another 3 rinses with Washing Buffer, the brains or labella were mounted in Fluoroshield (Sigma-Aldrich) for imaging. Images were acquired with Nikon C2 confocal microscope (40 × /0.80w DIC N2).

The antibodies were used as follows: rabbit anti-GFP (1:1000, Thermo Fisher Scientific), mouse anti-GFP (1: 1000, Abcam), mouse anti-GFP (1: 500, Sigma-Aldrich), rabbit anti-DsRed (1:1000, Takara Bio), Goat anti-rabbit Alexa Fluor 488 (1:1000, Thermo Fisher Scientific), Goat anti-mouse Alexa Fluor 488 (1:1000, Thermo Fisher Scientific), Goat anti-rabbit Alexa Fluor 546 (1:1000, Thermo Fisher Scientific).

Calcium imaging

The in vivo calcium imaging experiment was performed as previously described (Qi et al., 2021). Flies were briefly anesthetized on ice and mounted onto transparent plastic tape. The cuticle part around the $Gr5a^+$ region of the brain was gently removed with forceps, and the brain was bathed in sugar-free adult hemolymph-like solution (AHL, 108 mM NaCl, 8.2 mM $MgCl_2$, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, 5 mM KCl, 2 mM $CaCl_2$, 5 mM HEPES, pH7.3). A micro manipulator delivered sucrose to the proboscis of the fly, and the actual feeding bouts were imaged by a digital camera (FLIR USB 2.0, Point Gray). The calcium signals of $Gr5a^+$ neurons were collected by a Nikon C2 confocal microscope, with a water immersion objective lens (40 × /0.80w DIC N2).

For CsChrimson experiments, newly enclosed flies were maintained in vials with standard cornmeal food for 3 days and transferred to standard cornmeal food supplemented with 400 μM all-trans-retinal (Sigma-Aldrich) for 2 days before experiments. CsChrimson activation was provided by a fiber-coupled 625 nm LED (M625L3, Thorlabs). Image analyses were performed in ImageJ and plotted in GraphPad Prism6 or MATLAB. The ratio changes were calculated using the following formula: $\Delta F/F = [F - F_0]/F_0$, where F was the peak intensity, and F_0 was the average intensity at baseline.

Statistical analysis

Data presented in this study were all verified for normal distribution by D'Agostino-Pearson omnibus test. In one-way ANOVA (for comparisons among three or more groups) tests, statistical significance was corrected using post hoc Tukey's test. The two-way ANOVA (for comparisons with more than one variant) tests use post hoc Bonferroni correction.

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Additional information

Author contributions

Gaohang Wang, Resources, Data curation, Formal analysis, Validation, Investigation, Methodology, Writing -original draft; Wei Qi, Investigation; Rui Huang, Resources, Formal analysis, Supervision, Funding acquisition, Investigation, Methodology, Writing - original draft, Project administration, Writing - review and editing; Liming Wang, Conceptualization, Resources, Supervision, Funding acquisition, Investigation, Writing - original draft, Project administration, Writing - review and Editing.

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National Natural Science Foundation of China (32271008)

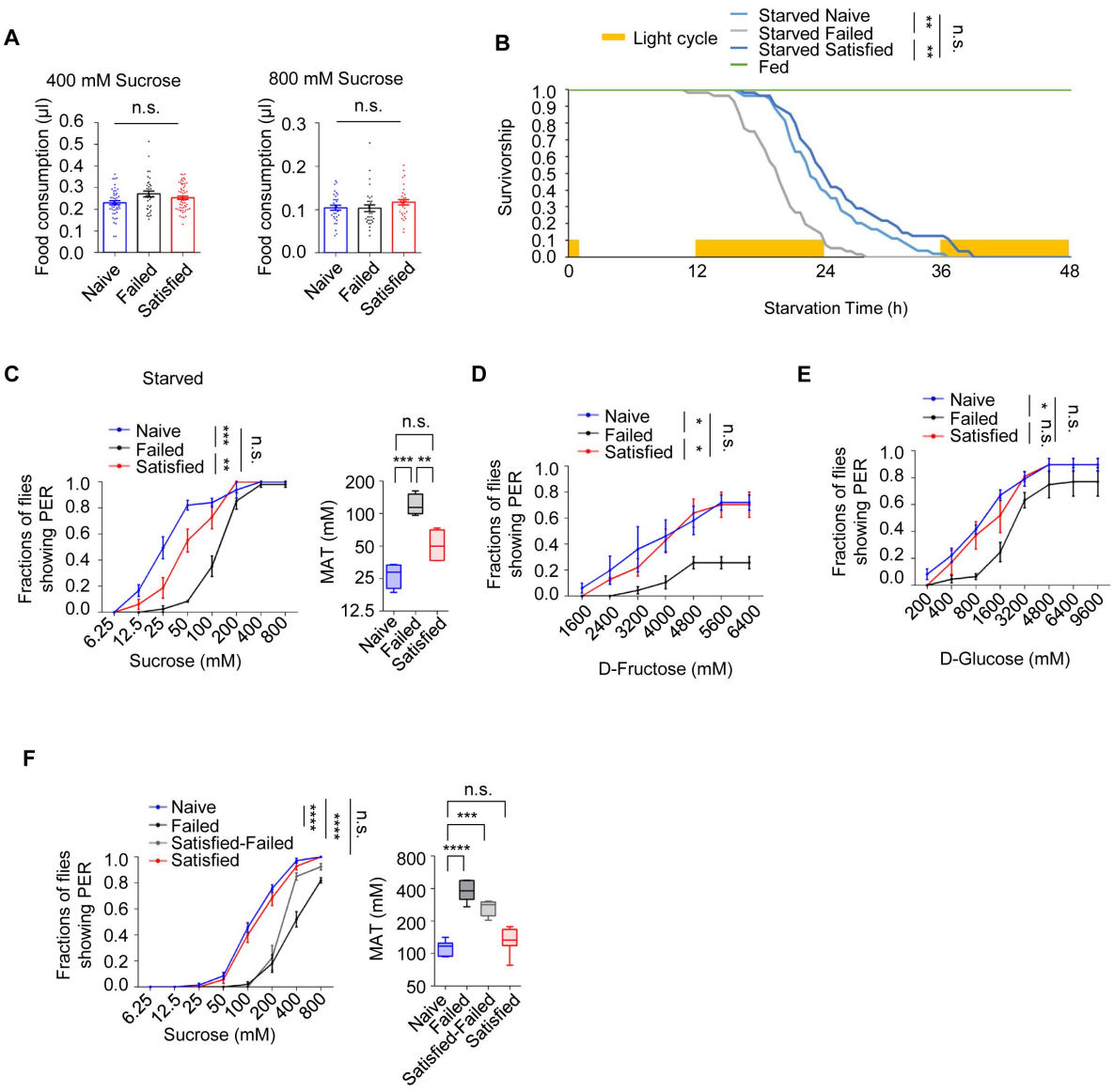

Figure 1-supplement 1

Sexual failure decreased sweet sensitivity.

(A) Volume of 400mM sucrose (left) and 800mM (right) consumed by males fed *ad libitum* (n = 35-60). (B) Starvation resistance of males was assayed in the presence of 5% sucrose (Fed) or 2% agar (Starved) (n = 32-64). Yellow bars represent 12 hr light-on period. (C) Fraction of starved Naïve, Failed, and Satisfied male flies showing PER responses to different concentrations of sucrose (n=40-60). (D) Fraction of Naïve, Failed, and Satisfied male flies showing PER responses to different concentrations of D-Fructose (n=48-60). (E) Fraction of male flies showing PER responses to different concentrations of D-Glucose (n=48-60). (F) Fraction of male flies showing PER responses to different concentrations of sucrose (n=48-60). Satisfied-Failed males were generated by placing individual Satisfied males (72 hours post-treatment) with 25 mated females in a food vial for 4.5 h. Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$. One-way ANOVA (A, and C, F MAT data) and two-way ANOVA (B, D, E, and C, F PER data) were applied for statistical analysis.

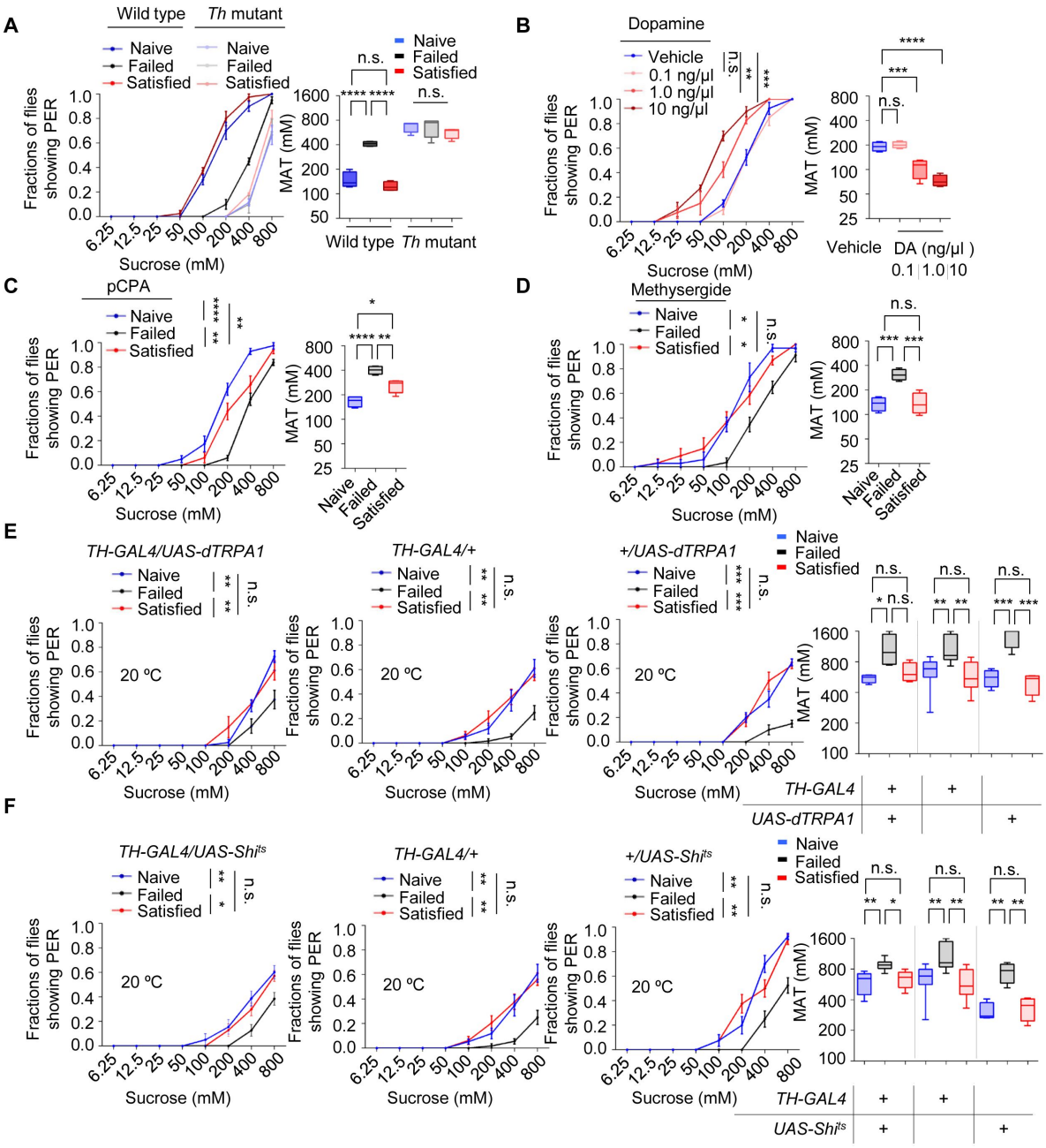

Figure 2-supplement 1

Dopamine but not serotonin signaling modulated sexual experience-dependent sweet sensitivity.

(A) PER responses in *Th* mutant male flies (n=48-60). (B) PER responses in male flies injected with various doses of dopamine (n=48-60). (C) PER responses in male flies fed with DL-p-chlorophenylalanine (pCPA) (n=48-60). (D) PER responses in male flies fed with methysergide (n=48-60). (E-F) PER responses in male flies with indicated genotypes at 20°C(n=48-60). Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$. Two-way ANOVA (A-F PER data) and one-way ANOVA (A-F MAT data) were applied for statistical analysis.

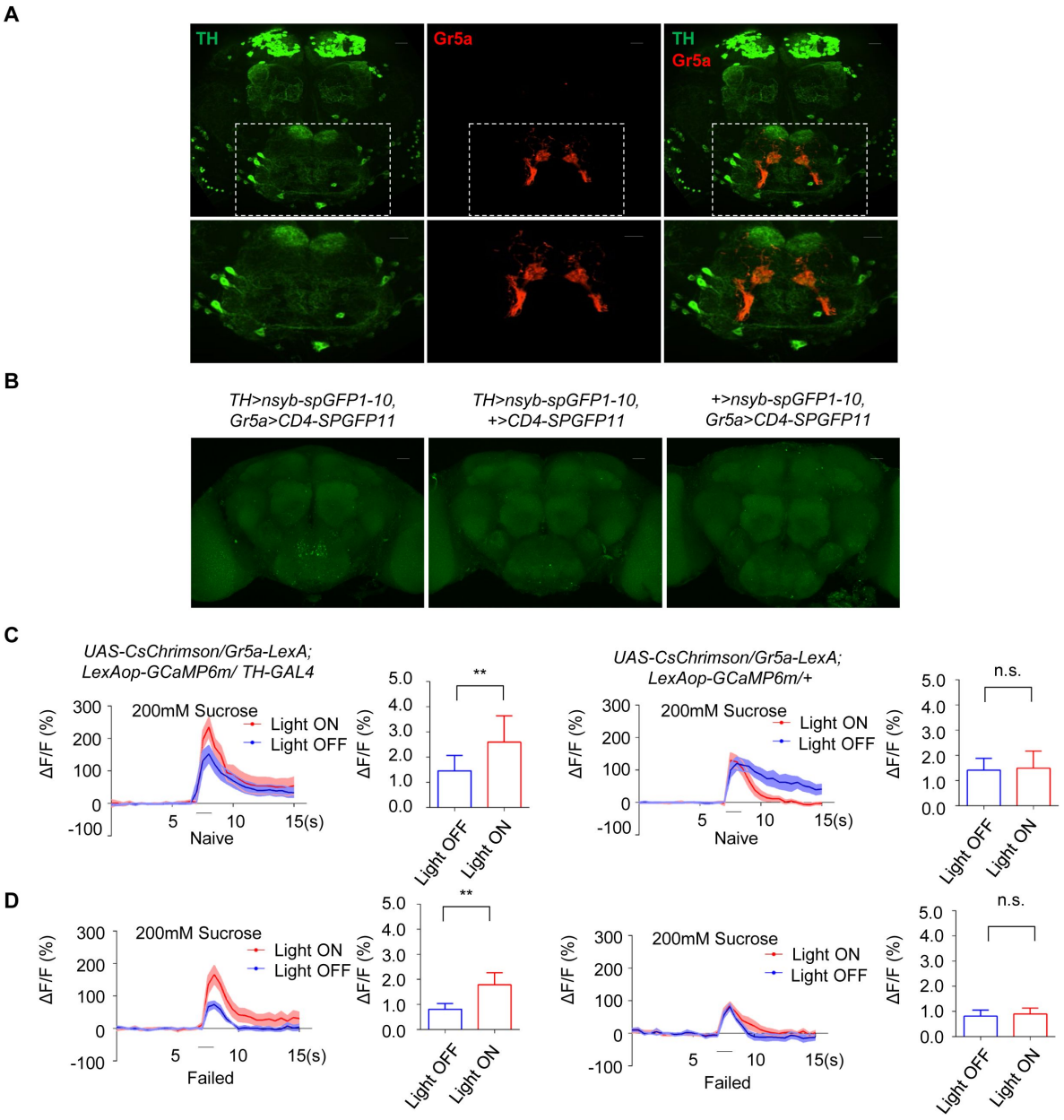

Figure 3-supplement 1

Dopaminergic neurons had functional connections with sweet GRNs.

(A) The expression of membrane-bound GFP (mCD8GFP, Green) in dopaminergic neurons driven by *TH-Gal4* and membrane-bound RFP (rCD2RFP, Red) in Gr5a^{+} neurons driven by *Gr5a-LexA.* Scale bars, 20 μm. (B) Representative image of the GRASP experiment to show connections between dopaminergic neurons and sweet GRNs in *TH>nsyb-spGFP1-10, Gr5a>CD4-spGFP11* flies (left). Genetic controls of GRASP experiments were shown on the center and right. Scale bars, 20 μm. (C-D) Averaged traces of the calcium responses to 200mM sucrose in male flies with indicated genotypes was shown on the left. Quantification of the calcium responses was shown on the right (n=6-13). Top: Naïve. Bottom: Failed. The horizontal black bars represent sucrose application. The solid lines represent the average trace, and shaded regions represent ±SEM. One-way ANOVA (C and D) was applied for statistical analysis.

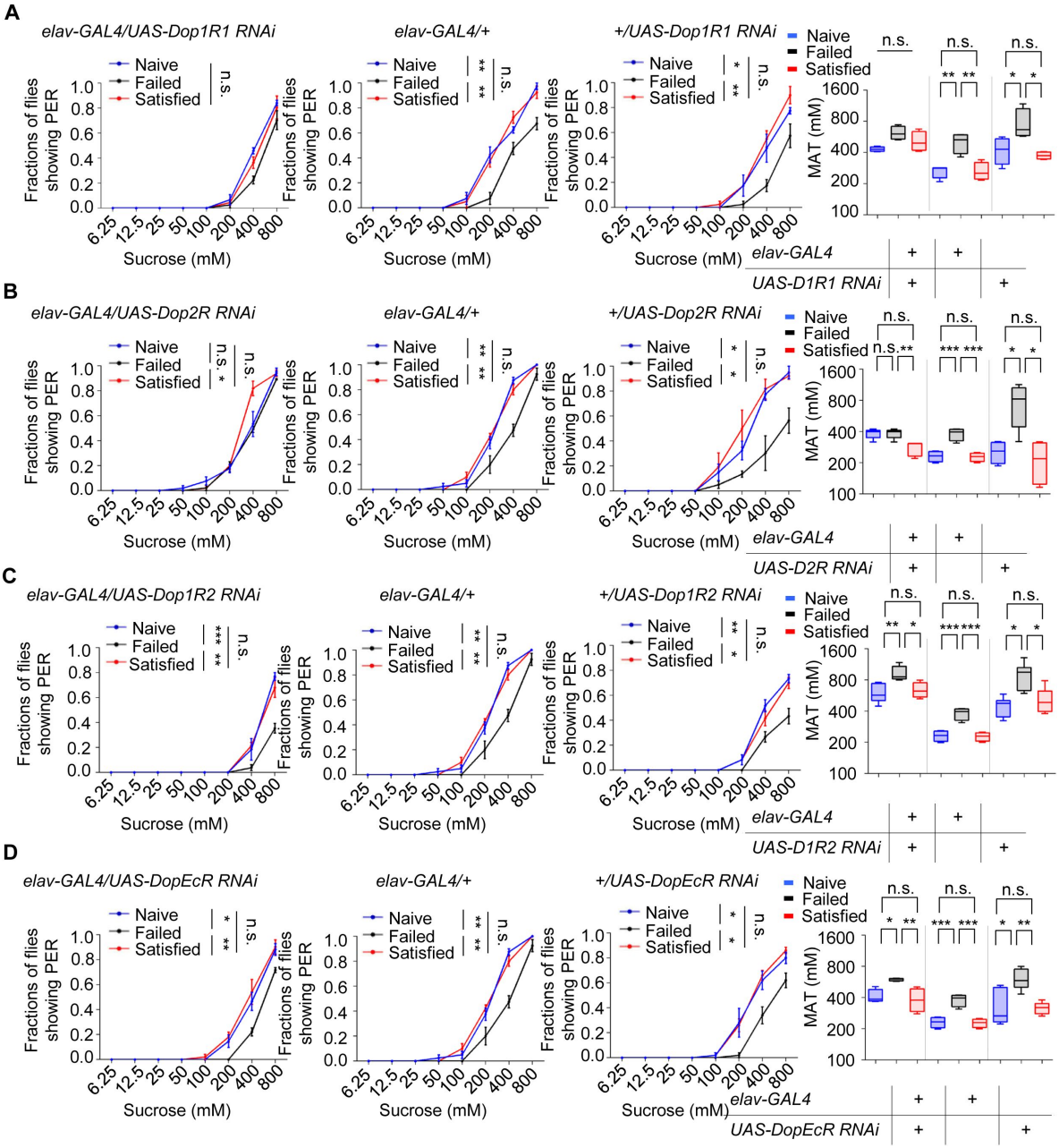

Figure 4-supplement 1

Dop1R1 and Dop2R were necessary for sexual failure-induced change in sweet sensitivity.

(A-D) PER responses in male flies with indicated genotypes (n=48-60). Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$. Two-way ANOVA (A-D PER data) and one-way ANOVA (A-D MAT data) were applied for statistical analysis.

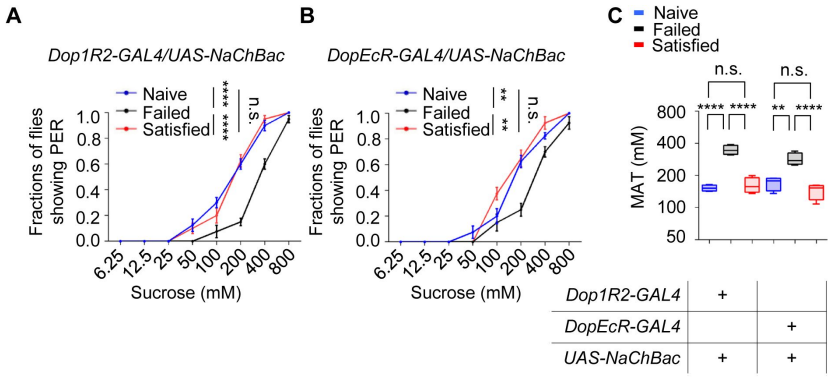

Figure 5-supplement 1

Dop1R2^{+} or DopEcR^{+} neurons did not affect sweet sensitivity following sexual failure.

(A-B) PER responses in male flies with indicated genotypes (n=48-60). (C) MAT, plotted as a function of sexual state and type of flies. Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$. Two-way ANOVA (A and B) and one-way ANOVA (C) were applied for statistical analysis.

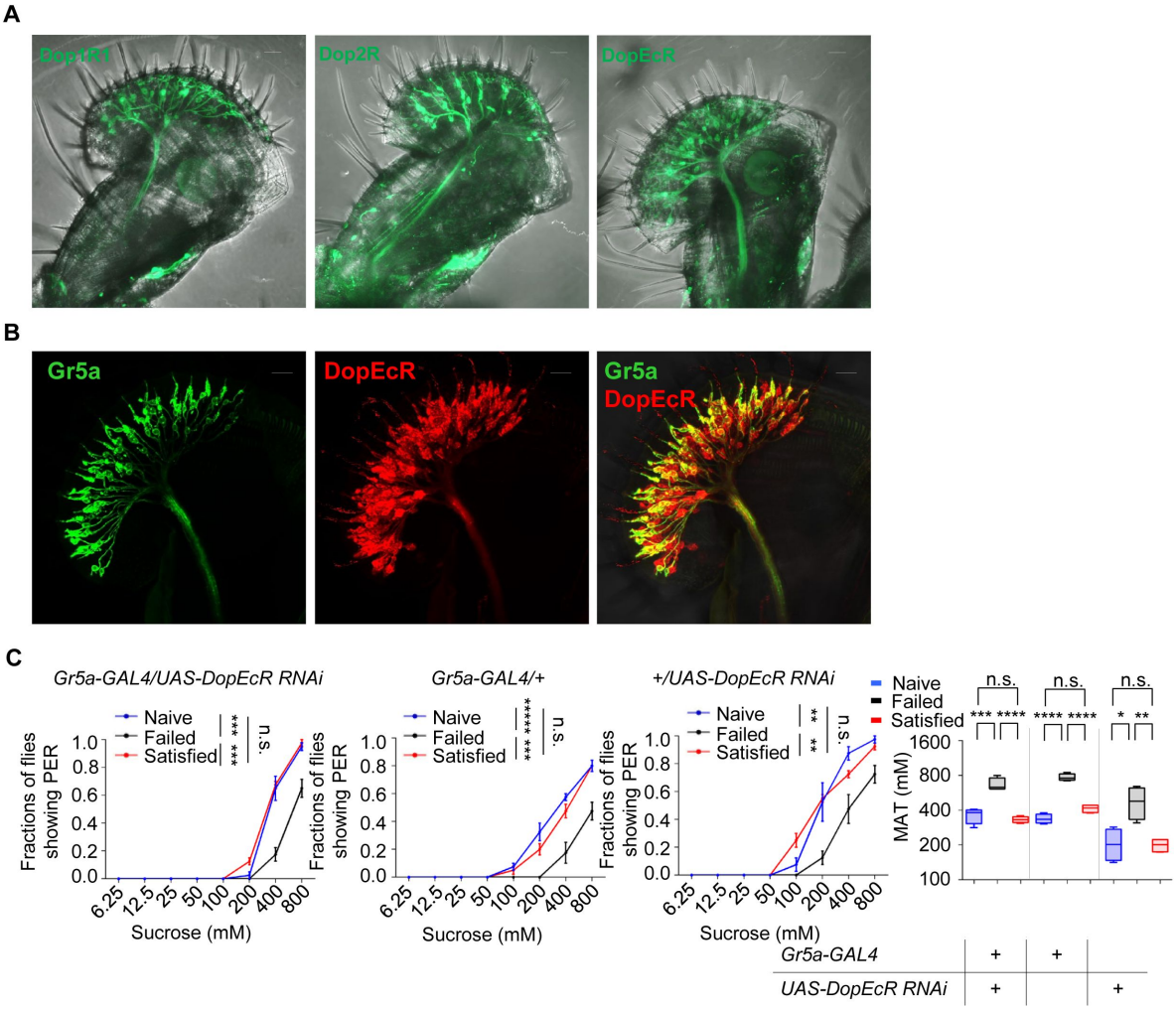

Figure 6-supplement 1

Dopaminergic modulation of Gr5a^{+} neurons following sexual failure did not require DopEcR.

(A) The expression of membrane-bound GFP (mCD8GFP) in dopamine receptor neurons driven by *Dop1R1-Gal4* (left), *Dop2R-Gal4* (center), and *DopEcR-Gal4* (right). Scale bars, 20 μm. (B) The expression of membrane-bound GFP (mCD8GFP, Green) in Gr5a^{+} neurons driven by *Gr5a-LexA* (left) and membrane-bound RFP (mCD8RFP, Red) in dopamine receptor neurons driven by *DopEcR-Gal4*. Scale bars, 20 μm. (C) PER responses in male flies with indicated genotypes (n=60-75). Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$. Two-way ANOVA (C PER data) and one-way ANOVA (C MAT data) were applied for statistical analysis.

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Author information

Gaohang Wang

Institute of Molecular Physiology, Shenzhen Bay Laboratory, Shenzhen, China

Wei Qi
Department of Biology, Stanford University, Stanford, United States

Rui Huang
Guangyang Bay Laboratory, Chongqing Institute for Brain and Intelligence, Chongqing, China
ORCID iD: 0000-0003-4656-1682

For correspondence: huangrui0716@sina.com

Liming Wang
Institute of Molecular Physiology, Shenzhen Bay Laboratory, Shenzhen, China, School of Public Health, Capital Medical University, Beijing, China, Chinese Institutes for Medical Research, Beijing, China
ORCID iD: 0000-0002-7256-8776

For correspondence: lmwang83@cimrbj.ac.cn

Editors

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Reviewer #1 (Public review):

Wang et al. investigated how sexual failure influences sweet taste perception in male Drosophila. The study revealed that courtship failure leads to decreased sweet sensitivity and feeding behavior via dopaminergic signaling. Specifically, the authors identified a group of dopaminergic neurons projecting to the subesophageal zone that interact with sweet-sensing Gr5a+ neurons. These dopaminergic neurons positively regulate the sweet sensitivity of Gr5a+ neurons via DopR1 and Dop2R receptors. Sexual failure diminishes the activity of these dopaminergic neurons, leading to reduced sweet taste sensitivity and sugar feeding behavior in the male flies. These findings highlight the role of dopaminergic neurons in integrating reproductive experiences to modulate appetitive sensory responses.

Previous studies have explored the dopaminergic-to-Gr5a+ neuronal pathways in regulating sugar feeding under hunger conditions. Starvation has been shown to increase dopamine release from a subset of TH-GAL4 labeled neurons, known as TH-VUM, in the subesophageal zone. This enhanced dopamine release activates dopamine receptors in Gr5a+ neurons, heightening their sensitivity to sugar and promoting sucrose acceptance in flies. Since the function of the dopaminergic-to-Gr5a+ circuit motif has been well established, the primary contribution of Wang et al. is to show that mating failure in male flies can also engage this circuit to modulate sugar feeding behavior. This contribution is valuable because it highlights the role of dopaminergic neurons in integrating diverse internal state signals to inform behavioral decisions.

An intriguing discrepancy between Wang et al. and earlier studies lies in the involvement of dopamine receptors in Gr5a+ neurons. Prior research has shown that Dop2R and DopEcR, but not DopR1, mediate starvation-induced enhancement of sugar sensitivity in Gr5a+ neurons.

In contrast, Wang et al. report that DopR1 and Dop2R, but not DopEcR, are involved in the mating failure-induced suppression of sugar sensitivity in these neurons. Further investigation is needed to clarify how dopamine selectively engages different receptor types depending on internal state.

The data in this revised version are largely convincing and support the authors' conclusions. However, I remain concerned about the results shown in Figure 6E. The authors show that knocking down DopR1 or Dop2R in Gr5a+ neurons restores sucrose-evoked activity in Failed flies to levels seen in Naive and Satisfied animals. This appears to contradict the proposed model, in which these receptors positively modulate Gr5a+ activity through dopaminergic input. If dopamine signaling is reduced in Failed flies, further receptor knockdown should have no effect or further reduce activity-not restore it. I encourage the authors to clarify this apparent inconsistency and, if possible, provide a mechanistic explanation.

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Reviewer #2 (Public review):

Summary:

The authors exposed naïve male flies to different groups of females, either mated or virgin. Male flies can successfully copulate with virgin females; however, they are rejected by mated females. This rejection reduces sugar preference and sensitivity in males. Investigating the underlying neural circuits, the authors show that dopamine signaling onto GR5a sensory neurons is required for reduced sugar preference. GR5a sensory neurons respond less to sugar exposure when they lack dopamine receptors.

Strengths:

The findings add another strong phenotype to the existing dataset about brain-wide neuromodulatory effects of mating. The authors use several state-of-the-art methods, such as activity-dependent GRASP, to decipher the underlying neural circuitry. They further perform rigorous behavioral tests and provide convincing evidence for the local labellar circuit.

Weaknesses:

The authors focus on the circuit connection between dopamine and gustatory sensory neurons in the male SEZ. Therefore, it is still unknown how mating modulates dopamine signaling and what possible implications on other behaviors might result from a reduced sugar preference.

The authors updated missing literature in the manuscript and performed additional experiments regarding behavior, but also to further prove the functional connectivity between TH neurons and GR5a neurons.

I have no further recommendations.

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Reviewer #3 (Public review):

Summary

This study by Wang et al. explores a compelling link between two fundamental innate behaviors in Drosophila melanogaster, mating and feeding, demonstrating that repeated sexual failure in male flies leads to a transient yet reversible decrease in sweet taste

perception. The authors show that this modulation is mediated by dopamine signaling from a specific subset of dopaminergic neurons in the subesophageal zone (SEZ) that directly influence Gr5a$^+$ sweet-sensing neurons.

Aims of the Study

The authors aimed to understand whether unsuccessful mating attempts could affect sensory processing of sweet stimuli and thus feeding behavior in male fruit flies. They further sought to dissect the neural circuitry and molecular pathways underlying this behavioral plasticity, with a particular focus on dopaminergic modulation.

Major Strengths and Weaknesses

Strengths:

- Novelty: The idea that reproductive experience modulates gustatory perception adds a new dimension to our understanding of cross-modal behavioral integration.
- Experimental approach: The study uses a broad array of genetic, pharmacological, imaging, and behavioral assays to demonstrate a causal relationship between sexual failure and reduced sweet perception, mediated by specific dopaminergic pathways.
- Methodological design: The authors link behavioral outcomes (reduced proboscis extension reflex) with neural activity (calcium imaging of Gr5a$^+$ neurons) and molecular specificity (dopamine receptor subtype roles), providing a robust multi-level framework.

Weaknesses:

- Ecological relevance: While the laboratory conditions are well controlled, the adaptive value or natural context of this taste modulation following mating failure remains speculative.

Achievement of Aims and Support for Conclusions

The authors have convincingly achieved their central aim. The results support the conclusion that sexual failure reduces sweet taste sensitivity through dopamine signaling. The reduced activity in Gr5a$^+$ neuron after courtship rejection, its rescue by dopamine or successful copulation, and the requirement of specific dopamine receptors support the proposed model.

Impact and Utility

This work advances the field's understanding of how motivational states shaped by social experiences can directly influence sensory perception and behavior. It underscores the role of the dopaminergic system not only in reward but in integrating internal states across distinct behavioral responses. The experimental approach, including courtship conditioning paradigms and in vivo imaging methods, provides a valuable foundation for related studies in sensory modulation and behavioral plasticity.

Additional Context

This study supports a growing body of literature suggesting that insects possess emotion-like internal states that influence their behavior across contexts. The findings resonate with prior work on how stressors like social isolation or courtship failure lead to compensatory changes in other reward-seeking behaviors (e.g., ethanol consumption). Moreover, the concept that neural systems underlying basic drives like hunger and mating are dynamically

interconnected may be conserved across phyla, suggesting broader relevance to understanding internal state-dependent modulation of behavior.

The authors addressed all the comments of previous reviews. The changes increased the clarity of the manuscript, the interpretation of the results and reinforce the conclusion.

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Author response:

The following is the authors' response to the original reviews.

> ***Reviewer #1 (Public review):***
>
> *Wang et al. investigated how sexual failure influences sweet taste perception in male Drosophila. The study revealed that courtship failure leads to decreased sweet sensitivity and feeding behavior via dopaminergic signaling. Specifically, the authors identified a group of dopaminergic neurons projecting to the suboesophageal zone that interacts with sweet-sensing Gr5a+ neurons. These dopaminergic neurons positively regulate the sweet sensitivity of Gr5a+ neurons via DopR1 and Dop2R receptors. Sexual failure diminishes the activity of these dopaminergic neurons, leading to reduced sweet-taste sensitivity and sugar-feeding behavior in male flies. These findings highlight the role of dopaminergic neurons in integrating reproductive experiences to modulate appetitive sensory responses.*
>
> *Previous studies have explored the dopaminergic-to-Gr5a+ neuronal pathways in regulating sugar feeding under hunger conditions. Starvation has been shown to increase dopamine release from a subset of TH-GAL4 labeled neurons, known as TH-VUM, in the suboesophageal zone. This enhanced dopamine release activates dopamine receptors in Gr5a+ neurons, heightening their sensitivity to sugar and promoting sucrose acceptance in flies. Since the function of the dopaminergic-to-Gr5a+ circuit motif has been well established, the primary contribution of Wang et al. is to show that mating failure in male flies can also engage this circuit to modulate sugar-feeding behavior. This contribution is valuable because it highlights the role of dopaminergic neurons in integrating diverse internal state signals to inform behavioral decisions.*
>
> *An intriguing discrepancy between Wang et al. and earlier studies lies in the involvement of dopamine receptors in Gr5a+ neurons. Prior research has shown that Dop2R and DopEcR, but not DopR1, mediate starvation-induced enhancement of sugar sensitivity in Gr5a+ neurons. In contrast, Wang et al. found that DopR1 and Dop2R, but not DopEcR, are involved in the sexual failure-induced decrease in sugar sensitivity in these neurons. I wish the authors had further explored or discussed this discrepancy, as it is unclear how dopamine release selectively engages different receptors to modulate neuronal sensitivity in a context-dependent manner.*

Our immunostaining experiments showed that three dopamine receptors, Dop1R1, Dop2R, and DopEcR were expressed in $Gr5a^{+}$ neurons in the proboscis, which was consistent with previous findings by using RT-PCR (Inagaki et al 2012). As the reviewer pointed out, we found that Dop1R1 and Dop2R were required for courtship failure-induced suppression of sugar sensitivity, whereas Marella et al 2012 and Inagaki et al 2012 found that Dop2R and DopEcR were required for starvation-induced enhancement of sugar sensitivity. These results may suggest that different internal states (courtship failure vs. starvation) modulate the peripheral sensory system via different signaling pathways (e.g. different subsets of dopaminergic neurons; different dopamine release mechanisms; and different dopamine receptors). We have discussed these possibilities in the revised manuscript.

> *The data presented by Wang et al. are solid and effectively support their conclusions. However, certain aspects of their experimental design, data analysis, and interpretation warrant further review, as outlined below.*
>
> *(1) The authors did not explicitly indicate the feeding status of the flies, but it appears they were not starved. However, the naive and satisfied flies in this study displayed high feeding and PER baselines, similar to those observed in starved flies in other studies. This raises the concern that sexually failed flies may have consumed additional food during the 4.5-hour conditioning period, potentially lowering their baseline hunger levels and subsequently reducing PER responses. This alternative explanation is worth considering, as an earlier study demonstrated that sexually deprived males consumed more alcohol, and both alcohol and food are known rewards for flies. To address this concern, the authors could remove food during the conditioning phase to rule out its influence on the results.*

This is an important consideration. To rule out potential confound from food intake during courtship conditioning, we have now also conducted courtship conditioning in vials absent of food. In the absence of any feeding opportunity over the 4.5-hour courtship conditioning period, sexually rejected males still exhibited a robust decrease in sweet taste sensitivity compared with Naïve and Satisfied controls (Figure 1-supplement 1C). These data confirm that the suppression of PER is driven by courtship failure per se, rather than by differences in feeding during the conditioning phase.

> *(2) Figure 1B reveals that approximately half of the males in the Failed group did not consume sucrose yet Figure 1-S1A suggests that the total volume consumed remained unchanged. Were the flies that did not consume sucrose omitted from the dataset presented in Figure 1-S1A? If so, does this imply that only half of the male flies experience sexual failure, or that sexual failure affects only half of males while the others remain unaffected? The authors should clarify this point.*

Our initial description of the experimental setup might be a bit confusing. Here is a brief clarification of our experimental design and we have further clarified the details in the revised manuscript, which should resolve the reviewer's concerns:

After the behavioral conditioning, male flies were divided for two assays. On the one hand, we quantified PER responses of individual flies. As shown in Figure 1C, Failed males exhibited decreased sweet sensitivity (as demonstrated by the right shift of the dose-response curve). On the other hand, we sought to quantify food consumption of individual flies by using the MAFE assay (Qi et al 2005).

In the initial submission, we used 400 mM sucrose for the MAFE assay. When presented with 400 mM sucrose, approximately 100% of the flies in the Naïve and Satisfied groups, and 50% of the flies in the Failed group, extended their proboscis and started feeding, as a natural consequence of decreased sugar sensitivity (Figure 1B). We were able to quantify the actual volume of food consumed of these flies showing PER responses towards 400 mM sucrose and observed no change (Figure 1-supplement 1A, left). To avoid potential confusion, we have now repeated the MAFE assay with 800 mM sucrose, which elicited feeding in ~100% of flies among all three groups, as shown in Figure 1C. Again, we observed no change in food intake (Figure 1-supplement 1A, right).

These experiments in combination suggest that sexual failure suppresses sweet sensitivity of the Failed males. Meanwhile, as long as they still responded to a certain food stimulus and initiated feeding, the volume of food consumption remained unchanged. These results led us

to focus on the modulatory effect of sexual failure on the sensory system, the main topic of this present study.

> *(3) The evidence linking TH-GAL4 labeled dopaminergic neurons to reduced sugar sensitivity in Gr5a+ neurons in sexually failed males could be further strengthened. Ideally, the authors would have activated TH-GAL4 neurons and observed whether this restored GCaMP responses in Gr5a+ neurons in sexually failed males. Instead, the authors performed a less direct experiment, shown in Figures 3-S1C and D. The manuscript does not describe the condition of the flies used in this experiment, but it appears that they were not sexually conditioned. I have two concerns with this experiment. First, no statistical analysis was provided to support the enhancement of sucrose responses following activation of TH-GAL4 neurons. Second, without performing this experiment in sexually failed males, the authors lack direct evidence to confirm that the dampened response of Gr5a+ neurons to sucrose results from decreased activity in TH-GAL4 neurons.*

We have now quantified the effect of TH^+ neuron activation on $Gr5a^+$ neuron calcium responses. in Naïve males, dTRPA1-mediated activation of TH^+ cells significantly enhanced sucrose-induced calcium responses (Figure 3-supplement 1C); while in Failed males, the baseline activity of $Gr5a^+$ neurons was lower (Figure 3C), the same activation also produced significant (even slightly larger) effect on the calcium responses of $Gr5a^+$ neurons (Figure 3-supplement 1D).

Taken together, we would argue that these experiments using both Naïve and Failed males were adequate to show a functional link between TH^+ neurons and $Gr5a^+$ neurons. Combining with the results that these neurons form active synapses (Figure 3-supplement 1B) and that the activity of TH^+ neurons was dampened in sexually failed males (Figure 3G-I), our data support the notion that sexual failure suppresses sweet sensitivity via TH-Gr5a circuitry.

> *(4) The statistical methods used in this study are poorly described, making it unclear which method was used for each experiment. I suggest that the authors include a clear description of the statistical methods used for each experiment in the figure legends. Furthermore, as I have pointed out, there is a lack of statistical comparisons in Figures 3-S1C and D, a similar problem exists for Figures 6E and F.*

We have added detailed information of statistical analysis in each figure legend.

> *(5) The experiments in Figure 5 lack specificity. The target neurons in this study are Gr5a+ neurons, which are directly involved in sugar sensing. However, the authors used the less specific Dop1R1- and Dop2R-GAL4 lines for their manipulations. Using Gr5a-GAL4 to specifically target Gr5a+ neurons would provide greater precision and ensure that the observed effects are directly attributable to the modulation of Gr5a+ neurons, rather than being influenced by potential off-target effects from other neuronal populations expressing these dopamine receptors.*

We agree with the reviewer that manipulating Dop1R1 and Dop2R genes (Figure 4) and the neurons expressing them (Figure 5) might have broader impacts. For specificity, we have also tested the role of Dop1R1 and Dop2R in $Gr5a^+$ neurons by RNAi experiments (Figure 6). As shown by both behavioral and calcium imaging experiments, knocking down Dop1R1 and Dop2R in $Gr5a^+$ neurons both eliminated the effect of sexual failure to dampen sweet sensitivity, further confirming the role of these two receptors in $Gr5a^+$ neurons.

> *(6) I found the results presented in Fig. 6F puzzling. The knockdown of Dop2R in Gr5a+ neurons would be expected to decrease sucrose responses in naive and satisfied flies,*

> *given the role of Dop2R in enhancing sweet sensitivity. However, the figure shows an apparent increase in responses across all three groups, which contradicts this expectation. The authors may want to provide an explanation for this unexpected result.*

We agree that there might be some potential discrepancies. We have now addressed the issues by re-conducting these calcium imaging experiments again with a head-to-head comparison with the controls (Gr5a-GCaMP, +/- Dop1R1 and Dop2R RNAi).

In these new experiments, Dop1R1 or Dop2R knockdown completely prevented the suppression of $Gr5a^+$ neuron responsiveness by courtship failure (Figure 6E), whereas the activities of $Gr5a^+$ neurons in Naïve/Satisfied groups were not altered. These results demonstrate that Dop1R1 and Dop2R are specifically required to mediate the decrease in sweet sensitivity following courtship failure.

> *(7) In several instances in the manuscript, the authors described the effects of silencing dopamine signaling pathways or knocking down dopamine receptors in Gr5a neurons with phrases such as 'no longer exhibited reduced sweet sensitivity' (e.g., L269 and L288), 'prevent the reduction of sweet sensitivity' (e.g., L292), or 'this suppression was reversed' (e.g. L299). I found these descriptions misleading, as they suggest that sweet sensitivity in naive and satisfied groups remains normal while the reduction in failed flies is specifically prevented or reversed. However, this is not the case. The data indicate that these manipulations result in an overall decrease in sweet sensitivity across all groups, such that a further reduction in failed flies is not observed. I recommend revising these descriptions to accurately reflect the observed phenotypes and avoid any confusion regarding the effects of these manipulations.*

We have changed the wording in the revised manuscript. In brief, we think that these manipulations have two consequences: suppressing the overall sweet sensitivity, and eliminating the effect of sexual failure on sweet sensitivity.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *The authors exposed naïve male flies to different groups of females, either mated or virgin. Male flies can successfully copulate with virgin females; however, they are rejected by mated females. This rejection reduces sugar preference and sensitivity in males. Investigating the underlying neural circuits, the authors show that dopamine signaling onto GR5a sensory neurons is required for reduced sugar preference. GR5a sensory neurons respond less to sugar exposure when they lack dopamine receptors.*
>
> *Strengths:*
>
> *The findings add another strong phenotype to the existing dataset about brain-wide neuromodulatory effects of mating. The authors use several state-of-the-art methods, such as activity-dependent GRASP to decipher the underlying neural circuitry. They further perform rigorous behavioral tests and provide convincing evidence for the local labellar circuit.*
>
> *Weaknesses:*
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> *The authors focus on the circuit connection between dopamine and gustatory sensory neurons in the male SEZ. Therefore, it is still unknown how mating modulates dopamine signaling and what possible implications on other behaviors might result from a reduced sugar preference.*

We agree with the reviewer that in the current study, we did not examine the exact mechanism of how mating experience suppressed the activity of dopaminergic neurons in the SEZ. The current study mainly focused on the behavioral characterization (sexual failure suppresses sweet sensitivity) and the downstream mechanism (TH-Gr5a pathway). We think that examining the upstream modulatory mechanism may be more suitable for a separate future study.

We believe that a sustained reduction in sweet sensitivity (not limited to sucrose but extend to other sweet compounds Figure 1-supplement 1D-E) upon courtship failure suggests a generalized and sustained consequence on reward-related behaviors. Sexual failure may thus resemble a state of “primitive emotion” in fruit flies. We have further discussed this possibility in the revised manuscript.

> ***Reviewer #3 (Public review):***
>
> *Summary*
>
> *In this work, the authors asked how mating experience impacts reward perception and processing. For this, they employ fruit flies as a model, with a combination of behavioral, immunostaining, and live calcium imaging approaches.*
>
> *Their study allowed them to demonstrate that courtship failure decreases the fraction of flies motivated to eat sweet compounds, revealing a link between reproductive stress and reward-related behaviors. This effect is mediated by a small group of dopaminergic neurons projecting to the SEZ. After courtship failure, these dopaminergic neurons exhibit reduced activity, leading to decreased Gr5a+ neuron activity via Dop1R1 and Dop2R signaling, and leading to reduced sweet sensitivity. The authors therefore showed how mating failure influences broader behavioral outputs through suppression of the dopamine-mediated reward system and underscores the interactions between reproductive and reward pathways.*
>
> *Concern*
>
> *My main concern regarding this study lies in the way the authors chose to present their results. If I understood correctly, they provided evidence that mating failure induces a decrease in the fraction of flies exhibiting PER. However, they also showed that food consumption was not affected (Fig. 1, supplement), suggesting that individuals who did eat consumed more. This raises questions about the analysis and interpretation of the results. Should we consider the group as a whole, with a reduced sensitivity to sweetness, or should we focus on individuals, with each one eating more? I am also concerned about how this could influence the results obtained using live imaging approaches, as the flies being imaged might or might not have been motivated to eat during the feeding assays. I would like the authors to clarify their choice of analysis and discuss this critical point, as the interpretation of the results could potentially be the opposite of what is presented in the manuscript.*

Please refer to our responses to the Public Review (Reviewer 1, Point 2) for details.

> ***Recommendations for the authors:***
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> ***Reviewer #1 (Recommendations for the authors):***
>
> *(1) The label for the y-axis in Figure 1B should be "fraction", not "percentage".*

We have revised the figure as suggested.

> *(2) I suggest that the authors indicate the ROIs they used to quantify the signal intensity in Figure 3E and G.*

We have revised the figures as suggested.

> *(3) There is a typo in Figure 4A: it should be "Wilde type", not "Wide type".*

We have revised the figure as suggested.

> *(4) The elav-GAL4/+ data in Figure 4-S1B, C, and D appears to be reused across these panels. However, the number of asterisks indicating significance in the MAT plots differs between them (three in panels B and C, and four in panel D). Is this a typo?*

It is indeed a typo, and we have revised the figure accordingly.

> ***Reviewer #2 (Recommendations for the authors):***
>
> *Additional comments:*
>
> *The authors should add this missing literature about dopamine and neuromodulation in courtship:*
>
> *Boehm et al., 2022 (eLife) - this study shows that mating affects olfactory behavior in females.*
>
> *Cazalé-Debat et al., 2024 (Nature) - Mating proximity blinds threat perception.*
>
> *Gautham et al., 2024 (Nature) - A dopamine-gated learning circuit underpins reproductive state-dependent odor preference in Drosophila females.*

We have added these references in the introduction section.

> *Has the mating behavior been quantified? How often did males copulate with mated and virgin females?*

We tried to examine the copulation behavior based on our video recordings. In the “Failed” group (males paired with mated females), we observed virtually no successful copulation events at all, confirming that nearly 100% of those males experienced sexual failure. In contrast, males in the “Satisfied” group (paired with virgin females) mated on average 2-3 times during the 4.5-hour conditioning period. We have added some explanations in the manuscript.

> *Do the rejected males live shorter? Is the effect also visible when they are fed with normal fly food, or is it only working with sugar?*

We did not directly measure the lifespan of these males. But we conducted a relevant assay (starvation resistance), in which “Failed” males died significantly faster than both Naïve and Satisfied controls, indicating a clear reduction in their ability to endure food deprivation (Figure 1-supplement 1B). Since sweet taste is a primary cue for food detection in *Drosophila*, and sugar makes up a large portion of their standard diet, the drop in sugar sensitivity we observed in Failed males could likewise impair their perception and consumption of regular fly food, hence their resistance to starvation.

> *Also, the authors mention that the reward pathway is affected, this is probably the case as sugar sensation is impaired. One interesting experiment would be (and maybe has*

> *been done?) to test rejected males in normal odor-fructose conditioning. The data would suggest that they would do worse.*

We have already measured how courtship failure affected fructose sensitivity (Figure 1 supplement 1D), and we found that the reduction in fructose perception was even more profound than for sucrose. We have not yet tested whether Failed males showed deficits in odor-fructose associative conditioning. That was indeed a very interesting direction to explore. But olfactory reward learning relies on molecular and circuit mechanisms distinct from those governing taste. We therefore argue such experiments would be more suitable in a separate, follow up study.

> *The authors could have added another group where males are exposed to other males. It would be interesting if this is also a "stressful" context and if it would also reduce sugar preference - probably beyond the scope of this paper.*

In our experiments, all flies, including those in the Naïve, Failed, and Satisfied groups, were housed in groups of 25 males per vial before the conditioning period (and the Naïve group remained in the same group housing until PER testing). This means every cohort experienced the same level of “social stress” from male-male interactions. While it would indeed be interesting to compare that to solitary housing or other male-only exposures, isolation itself imposes a different kind of stress, and disentangling these effects on sugar preference would require a separate, dedicated study beyond the scope of the present work.

> *Would the behavior effect also show up with experienced males? Maybe this has been tested before. Does mating rejection in formerly successful males have the same impact?*

As suggested by the reviewer, we performed an additional experiment in which males that had previously mated successfully were subsequently subjected to courtship rejection. As shown in Figure 1 supplement 1F, prior successful mating did not prevent the decline in sweet sensitivity induced by subsequent mating failure, indicating that even experienced males exhibit the reduction in sugar sensitivity after rejection.

> *Is the same circuit present and functioning in females? Does manipulating dopamine receptors in GR5a neurons in females lead to the same phenotype? This would suggest that different internal states in males and females could lead to the same phenotype and circuit modulations.*

This is indeed a very interesting suggestion. In male flies, Gr5a-specific knockdown of dopamine receptors did not alter baseline sweet sensitivity, but it selectively prevented the reduction in sugar perception that followed mating failure (Figure 6C-D), indicating that this dopaminergic pathway is engaged only in the context of courtship rejection. By extension, knocking down the same receptors in female GR5a neurons would likewise be expected to leave their basal sugar sensitivity unchanged. Moreover, because there is currently no established paradigm for inducing mating failure in female flies, we cannot yet test whether sexual rejection similarly modulates sweet taste in females, or whether it operates via the same circuit.

> ***Reviewer #3 (Recommendations for the authors):***
>
> *Suggestions to the authors:*
>
> *Introduction, line 61. I suggest the authors add references in fruit flies concerning the rewarding nature of mating. For example, the paper from Zhang et al, 2016 "Dopaminergic Circuitry Underlying Mating Drive" demonstrates the role of the*

> *dopamine rewarding system in mating drive. There is a large body of literature showing the link between dopamine and mating.*

We have added this literature in the introduction section.

> *Figure 1B and Figure Supplement 1: If I understood correctly, Figure Supplement 1A shows that the total food consumption across all tested flies remains unchanged. However, fewer flies that failed to mate consumed sucrose. I would be curious to see the results for sucrose consumption per individual fly that did eat. According to their results, individual flies that failed to mate should consume more sucrose. This would change the conclusion. The authors currently show that a group of flies that failed to mate consumed less sucrose overall, but since fewer males actually ate, those that failed to mate and did eat consumed more sucrose. The authors should distinguish between failed and satisfied flies in two groups: those that ate and those that did not.*

Please see our responses to the Public Review for details (Reviewer 1, Point 2).

> *Figure 1C, right: For a better understanding of all the "MAT" figures, I suggest the authors start the Y axis with the unit 25 and increase it to 400. This would match better the text (line 114) saying that it was significantly elevated in the failed group. As it is, we have the impression of a decrease in the graph.*

We have revised the figures accordingly.

> *Line 103: When suggesting a reduced likelihood of meal initiation of these males, do these males take longer to eat when they did it? In other words, is the latency to eat increased in failed males? That would be a good measure of motivational state.*

We tried to analyze feeding latency in the MAFE assay by measuring the time from sucrose presentation to the first proboscis extension, but it was too short to be accurately accounted. Nevertheless, when conducting the experiments, we did not feel/observe any significant difference in the feeding latency between Failed males and Naïve or Satisfied controls.

> *Line 117. I don't understand which results the authors refer to when writing "an overall elevation in the threshold to initiate feeding upon appetitive cues". Please specify.*

This phrase refers to the fact that for every sweet tastant we tested, including sucrose (Figure 1C), fructose and glucose (Figure 1 supplement 1D-E), the concentration-response curve in Failed males shifted to the right, and the Mean Acceptance Threshold (MAT) was significantly higher. In other words, for these different appetitive cues, mating failure raised the concentration of sugar required to trigger a proboscis extension, indicating a general elevation in the threshold to initiate feeding upon an appetitive cue.

> *Figure 1D. Please specify the time for the satisfied group.*

For clarity, the Naïve and Satisfied groups in Figure 1D each represent pooled data from 0 to 72 hours post-treatment, as their sweet sensitivity remained stable throughout this period. Only the Failed group was shown with time-resolved data, since it was the only group exhibiting a dynamic change in sugar sensitivity over time. We have now specified this in the figure legend.

> *Figure 1F. The phenotype was not totally reversed in failed-re-copulated males. Could it be due to the timing between failure and re-copulation? I suggest the authors mention in the figure or in the text, the time interval between failure and re-copulation.*

We'd like to clarify that the interval between the initial treatment ("Failed") and the opportunity for re copulation was within 30 minutes. The incomplete reversal in the Failed-re-copulated group indeed raised interesting questions. One possible explanation is that mating failure reduces synaptic transmissions between the SEZ dopaminergic neurons and $Gr5a^+$ sweet sensory neurons (Figure 3), and the regeneration of these transmissions takes a longer time. We have added this information to the figure legend and the Method section.

> *Line 227-228 and Figure 3E. The authors showed that the synaptic connections between dopaminergic neurons and Gr5a+ GRNs were significantly weakened. I am wondering about the delay between mating failure and the GFP observation. It would be informative to know this timing to interpret this decrease in synaptic connections. If the timing is relatively long, it is possible that we can observe a neuronal plasticity. However, if this timing is very short, I would not expect such synaptic plasticity.*

The interval between the behavioral treatment and the GRASP-GFP experiment was approximately 20 hours. We chose this time window because it was sufficient for both GFP expression and accumulation. Therefore, the observed reduction in synaptic connections between dopaminergic neurons and $Gr5a^+$ GRNs likely reflects a genuine, experience-induced structural and functional change rather than an immediate, transient effect. We have added this information to the revised manuscript for clarity in the Method section.

> *Line 240-243: The authors demonstrated that there is a reduction of CaLexA-mediated GFP signals in dopaminergic neurons in the SEZ after mating failure, but not a reduction in Gr5a+ GRNs. I suggest replacing "indicate" with "suggest' in line 240.*

We have made the change accordingly. Meanwhile, we would like to clarify that while we observed a reduction of NFAT signal in SEZ dopaminergic neurons (Figure 3G), we did not directly test NFAT signal in $Gr5a^+$ neurons. Notably, the results that the synaptic transmissions from SEZ dopaminergic neurons to $Gr5a^+$ neurons were weakened (Figure 3E-F), and the reduction of NFAT signal in SEZ dopaminergic neurons (Figure 3G-I), were in line with a reduction in sweet sensitivity of $Gr5a^+$ neurons upon courtship failure (Figure 3B-D).

> *Line 243: replace "consecutive" with "constitutive".*

We have revised it accordingly.

> *Figure 5: I have trouble understanding the results obtained in Figure 5. Both constitutive activation and inhibition of Dop1R1 and Dop2R neurons lead to the same results, knowing that males who failed mating no longer exhibit decreased sweet sensitivity. I would have expected contrary results for both experimental conditions. I suggest the author to discuss their results.*

Both activation and inhibition of Dop1R1 and Dop2R neurons eliminated the effect of courtship failure on sweet sensitivity (Figure 5). These results are in line with our hypothesis that courtship failure leads to changes in dopamine signaling and hence sweet sensitivity. If dopamine signaling via Dop1R1 and Dop2R was locked, either to a silenced or a constitutively activated state, the effect of courtship failure on sweet sensitivity was eliminated.

Nevertheless, as the reviewer pointed out, constitutive activation/inhibition should in principle lead to the opposite effect on Naïve flies. In fact, when $Dop1R1^+$/$Dop2R^+$ neurons were silenced in Naïve flies, PER to sucrose was significantly reduced (Figure 5C-D), confirming that these neurons normally facilitate sweet sensation. Meanwhile, while neuronal activation by NaChBac did show a trend towards enhanced PER compared to the GAL4/+ controls, it did not exhibit a difference compared to +>UAS-NaChBac controls that

showed a high PER level, likely due to a potential ceiling effect. We have added the discussions to the manuscript.

> *Figure 7: I suggest the authors modify their figure a bit. It is not clear why in failed mating, the red arrow in "behavioral modulation" goes to the fly. The authors should find another way to show that mating failure decreased the percentage of flies that are motivated to eat sugar.*

We have modified the figure as suggested.

> *Overall, I would suggest the authors be precautious with their conclusion. For example, line 337= "sexual failure suppressed feeding behavior". This is not what is shown by this study. Here, the study shows that mating failure decreases the fraction of flies to eat sucrose. Unless the authors demonstrate that this decrease is generalizable to other metabolites, I suggest the authors modify their conclusion.*

While we primarily used sucrose as the stimulant in our experiments, we also tested responses to two other sugars: fructose and glucose (Figure 1 supplement 1D-E). In all three cases, mating failure led to a significant reduction in sweet perception, suggesting that the effect of courtship failure is not limited to a single metabolite but rather reflects a general decrease in sweet sensitivity. Meanwhile, reduced sweet sensitivity indeed led to a reduction of feeding initiation (Figure 1).

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