Neuroscience

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

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eLife Assessment

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

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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. Research has 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. Additionally, mating itself has a rewarding nature. 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 similar consumption volumes across 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.

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**, left). 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**, right). Furthermore, we found that the diminished sweet sensitivity extended to other sweet compounds such as fructose and glucose (**Figure 1-figure supplement 1B-C**), 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**). Overall, our findings reveal that sexual failure exerted a long-lasting yet reversible effect to reduce sweet sensitivity in male flies.

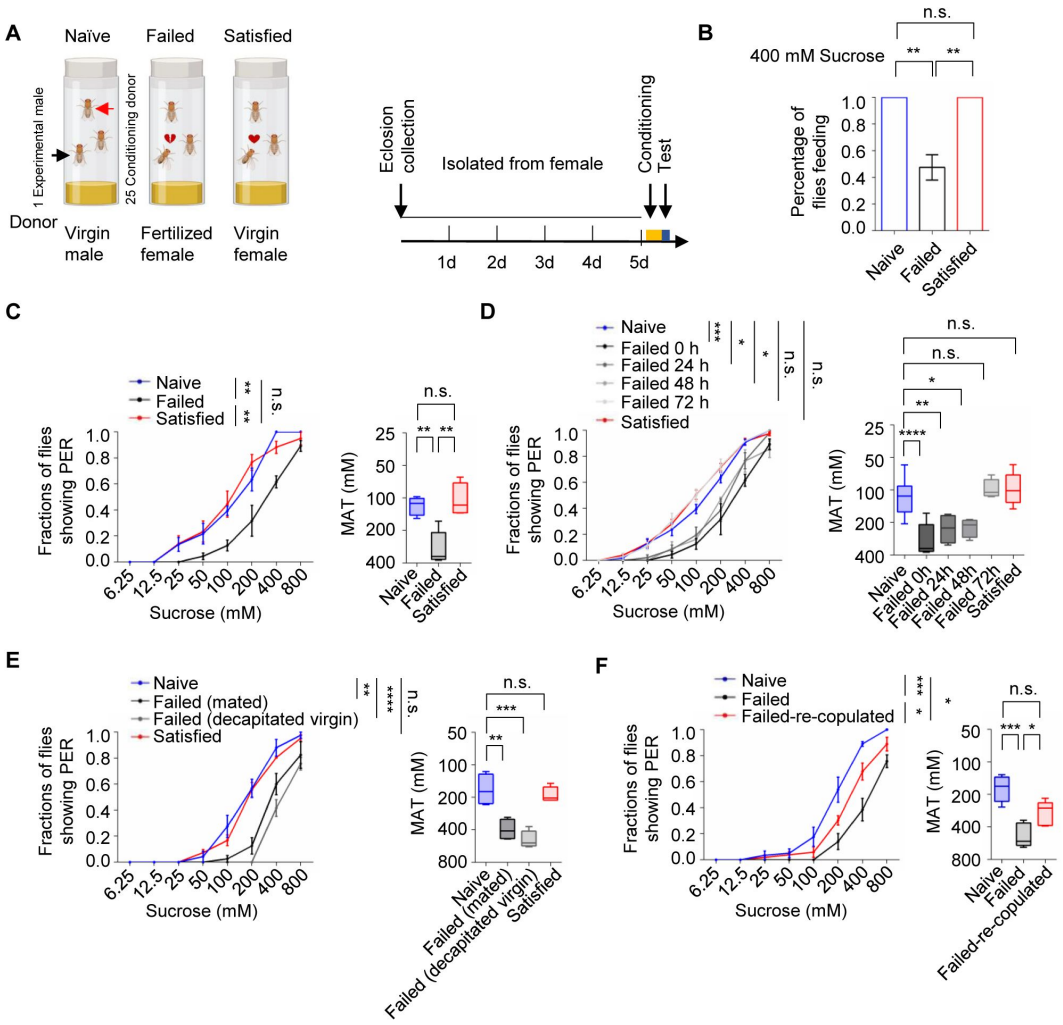

Figure 1

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. The PER experiment was performed immediately after conditioning unless otherwise indicated. Left: average PER responses of Naïve (blue), Failed (black), and Satisfied (red) flies (n=60-90). 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. The PER experiment was performed immediately, 24 h, 48 h, and 72 h after conditioning (n=48-60). (E) Fraction of male flies showing PER responses to different concentrations of sucrose. Failed male flies were generated by two methods: one group was subjected to mated females (with cVA), and the other was subject to decapitated virgin females (without cVA) (n=48-60). (F) Fraction of male flies showing PER responses to different concentrations of sucrose. Failed-re-copulated males were generated by placing individual failed males with 25 virgin females in a food vial for 4.5 h. Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

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 as 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}*) (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.

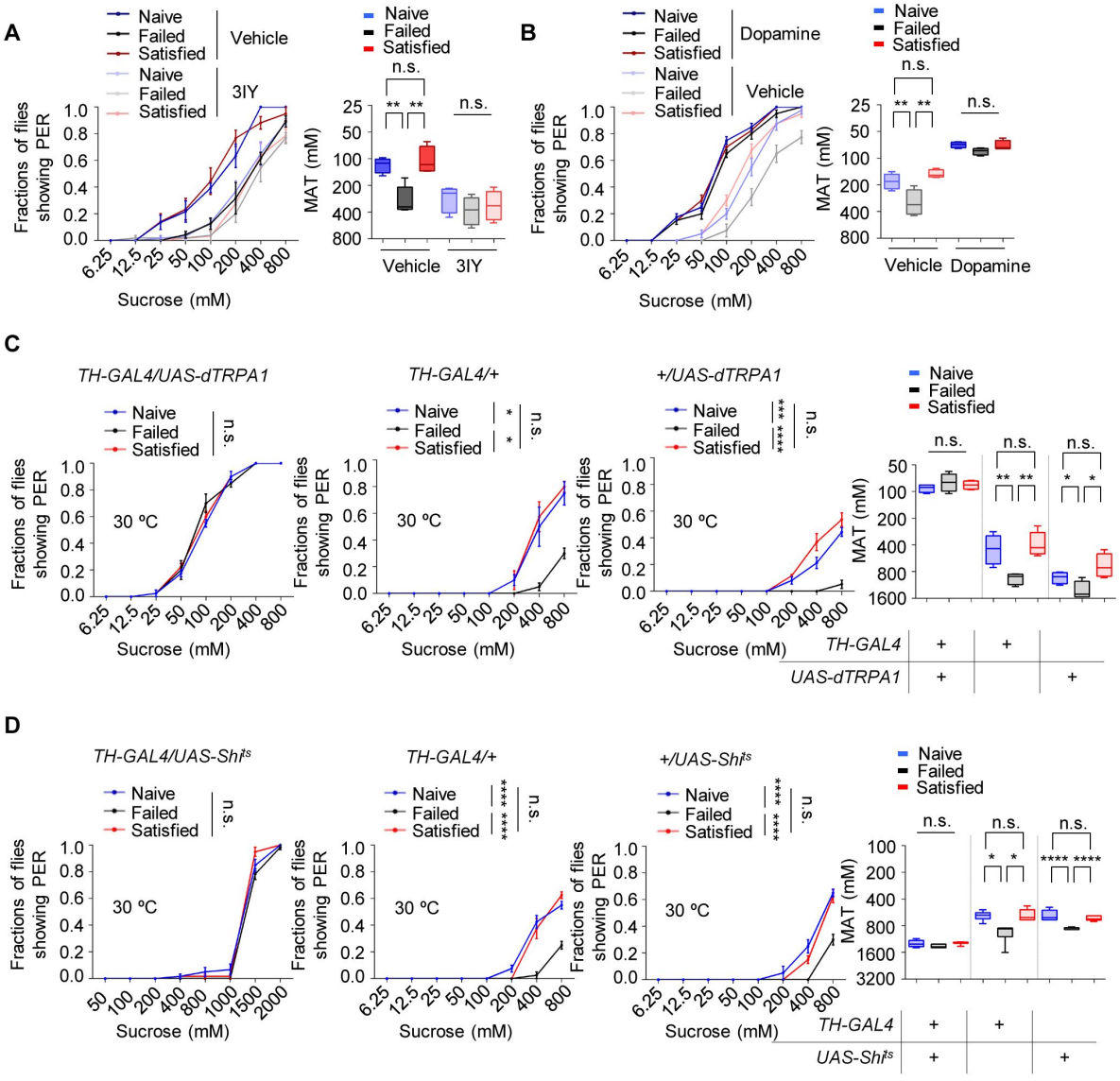

Figure 2

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., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

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 fluoresces 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 with 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 (**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.

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

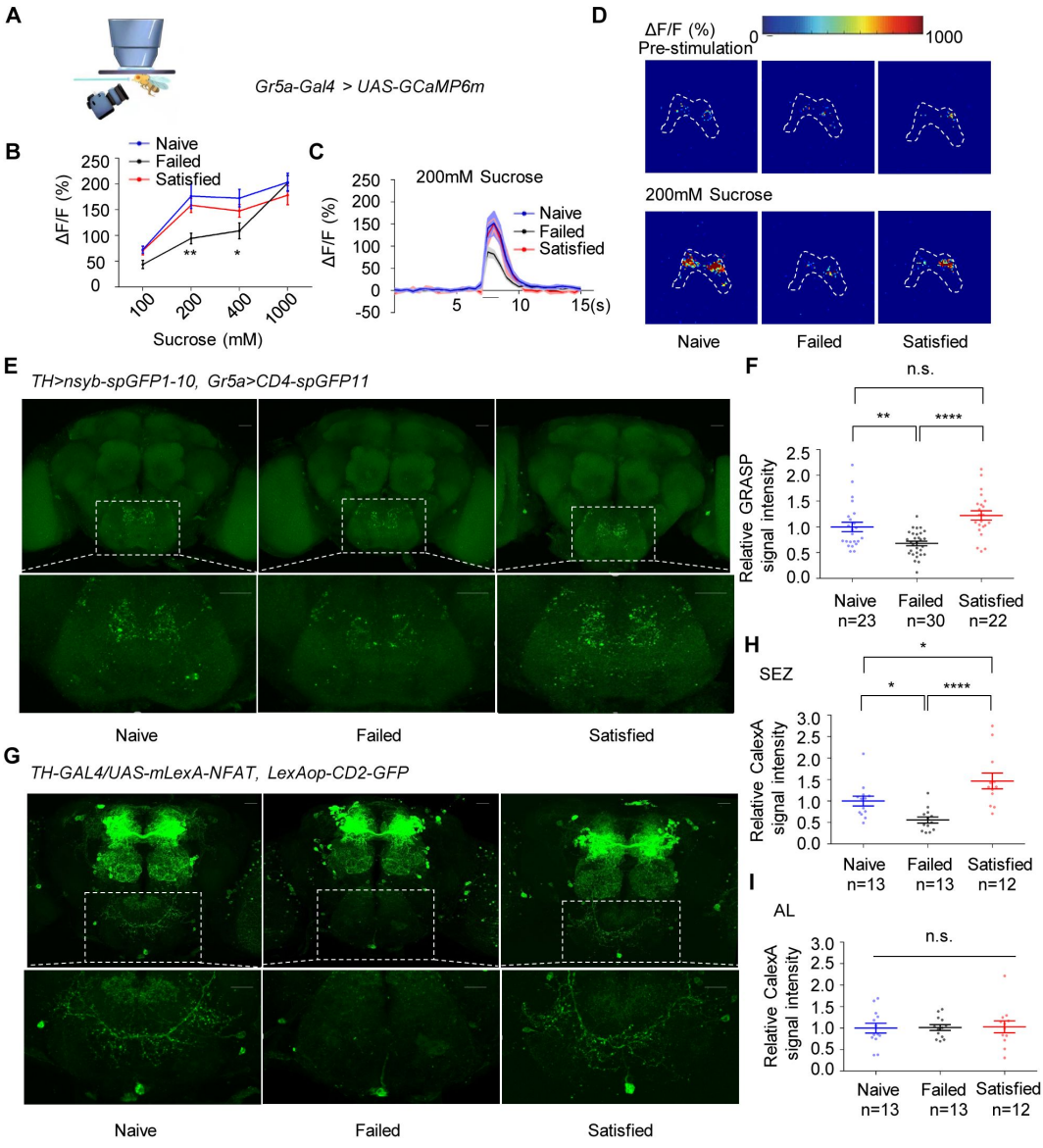

Figure 3

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 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 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. 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 shown on the 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). Lines represent mean ± SEM, and dots indicate values for individual flies. Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

mutations in *Dop1R2* or *DopEcR* did not prevent the decline in sweet sensitivity following sexual failure (**Figure 4A-F**). 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). We found that consecutive activating of $Dop1R1^+$ or $Dop2R^+$ neurons could prevent the decrease in sweet sensitivity upon mating failure (**Figure 5A-B**). Meanwhile, activating $Dop1R2^+$ or $DopEcR^+$ neurons did not reverse this 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). When these neurons were silenced, male flies that experienced mating failure no longer exhibited reduced sweet sensitivity compared to Naive or Satisfied males (**Figure 5C-D**). These findings indicate that sexual failure suppresses sweet sensitivity by regulating the activity of Dop1R1 and Dop2R neurons.

$Gr5a^+$ 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 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 suppression was reversed when Dop1R1 or Dop2R expression was knocked down in $Gr5a^+$ neurons (**Figures 6E-F**), consistent with the above behavioral observations. 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.

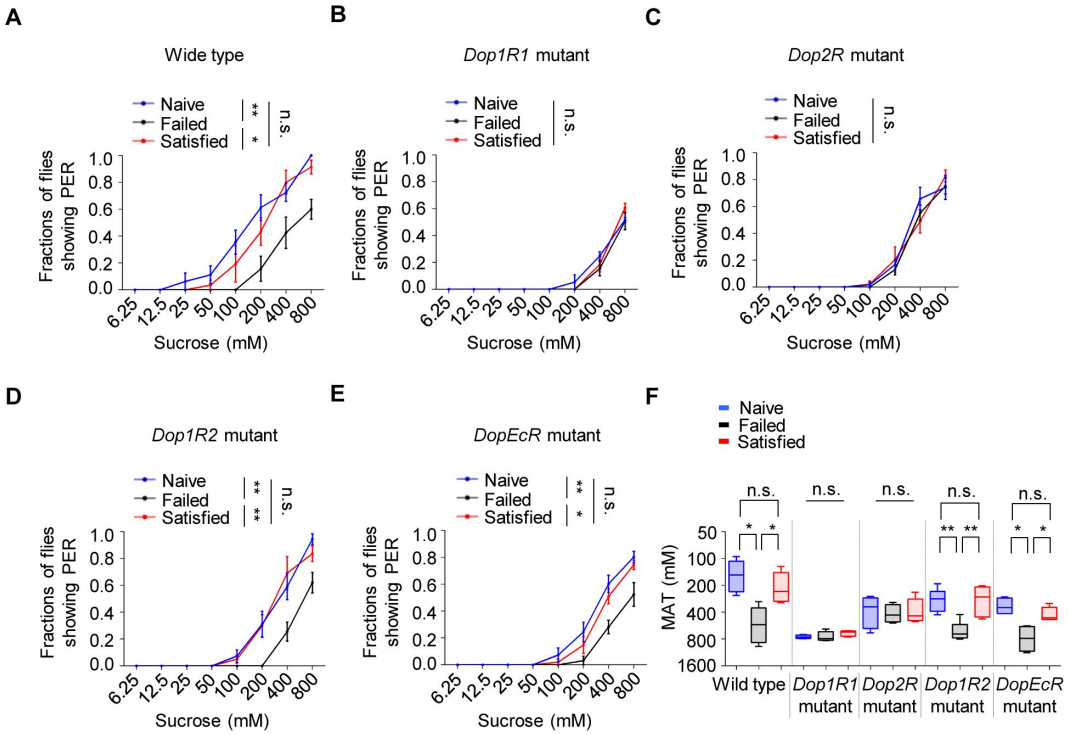

Figure 4

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., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

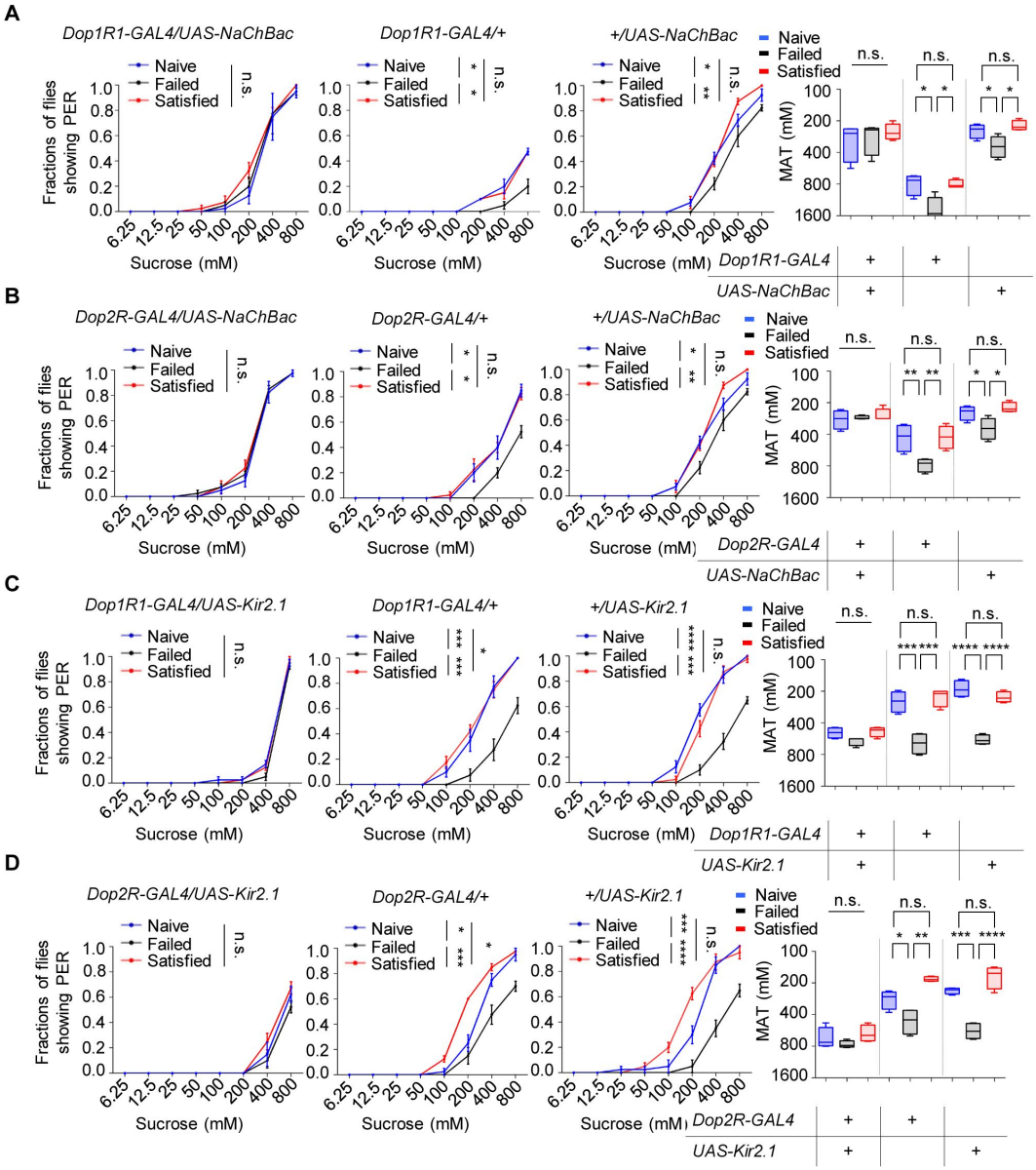

Figure 5

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., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

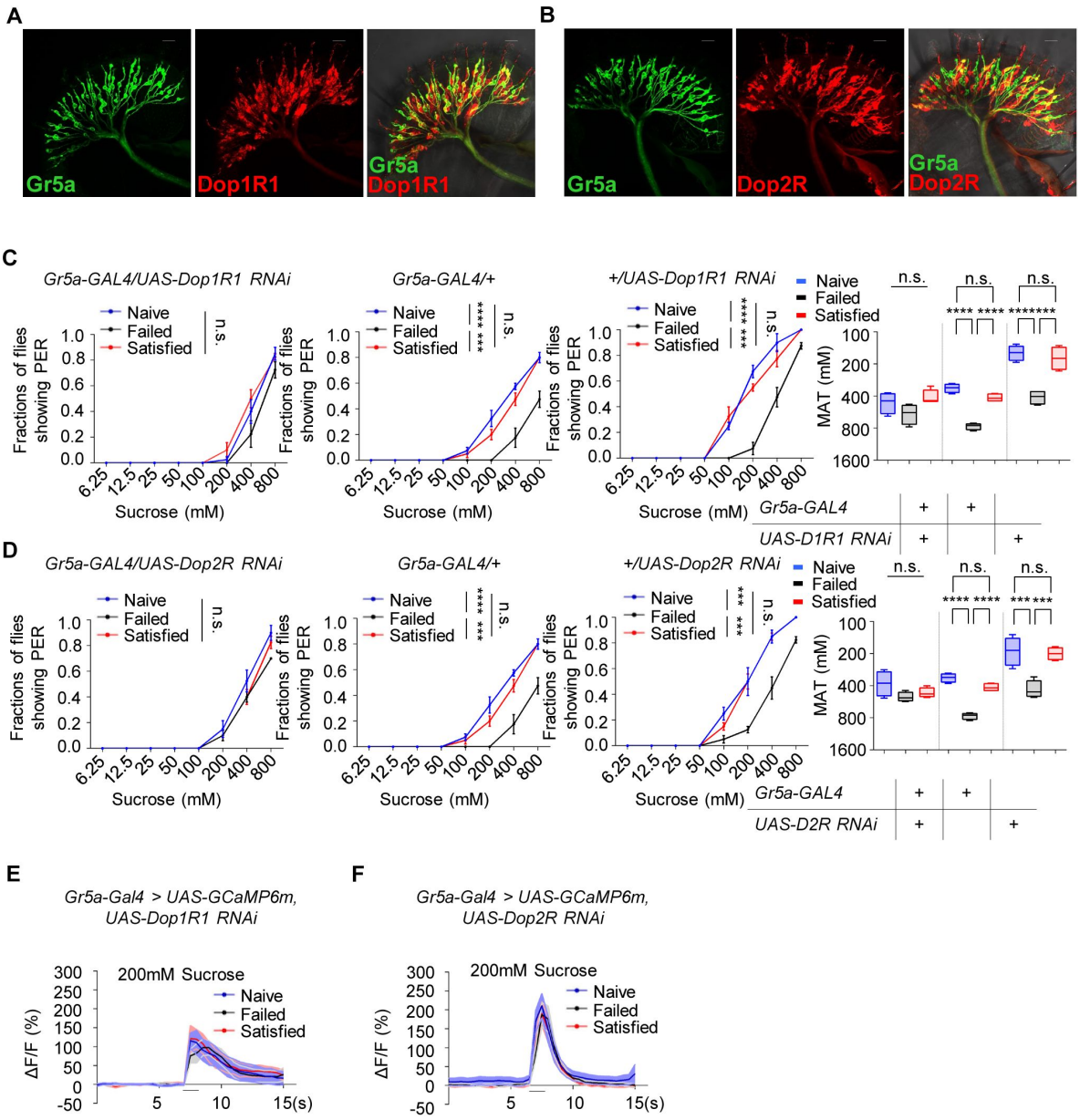

Figure 6

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 receptors 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-F) Averaged traces of the calcium responses to 200mM sucrose in male flies with indicated genotypes (n=7-10). The horizontal black bars represent sucrose application. The solid lines represent average trace, and shaded regions represent ±SEM. Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

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 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?

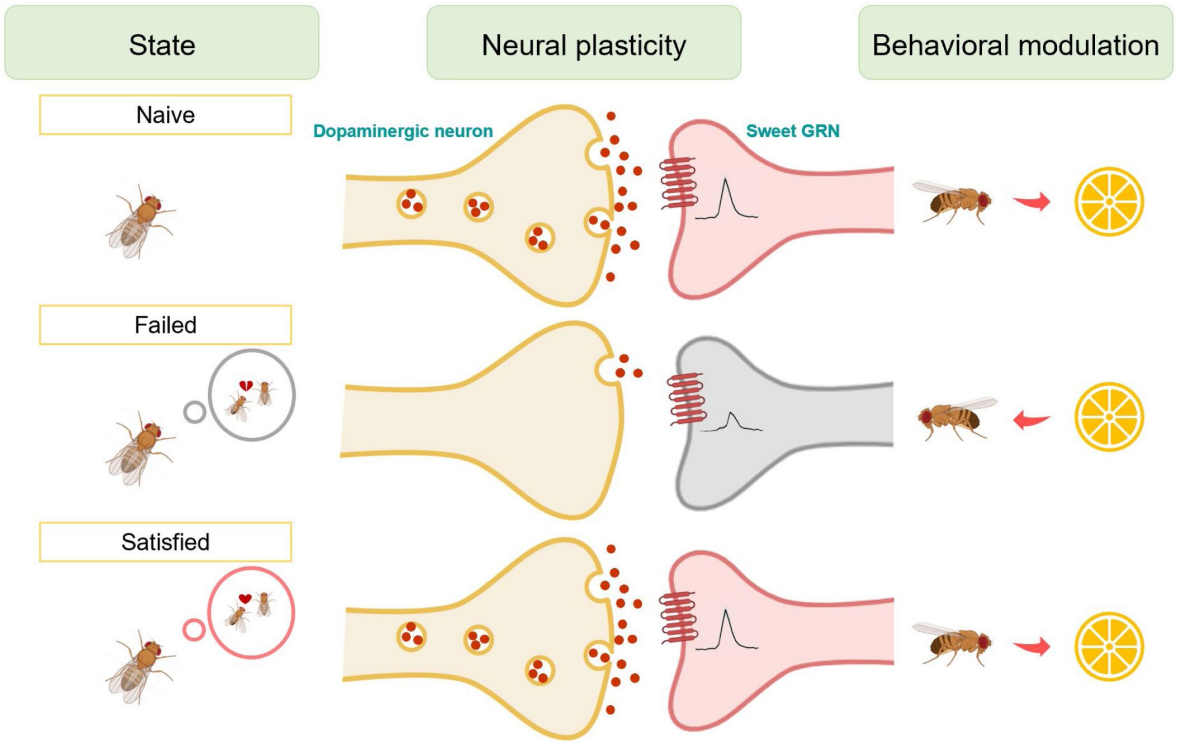

Figure 7

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.

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.

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 regulations 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 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

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-CalexA* (#66542), *UAS-NaChBac* (#9469), *UAS-Kir2.1*(#6595), *Gr5a-GAL4* (#57592), *Gr5a-LexA* (#93014) 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, 10 mg/ml), DL-p-chlorophenylalanine (pCPA) (Sigma, 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 were allowed to mate with virgin females in a food vial 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.

Immunohistochemistry

Fly brains or labella were dissected in PBS on ice and fixed in 4% paraformaldehyde at room temperature for 60 min. 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 4℃ 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, Life Technologies), mouse anti-GFP (1: 1000, Abcam), rabbit anti-DsRed (1:1000, ClonTech), Alexa Fluor 488 goat anti-rabbit (1:1000, Life Technologies), Alexa Fluor 488 goat anti-mouse (1:1000, Life Technologies), Alexa Fluor 546 goat anti-rabbit (1:1000, Life Technologies).

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 MgCl2, 26 mM NaHCO3, 1 mM NaH2PO4, 5 mM KCl, 2 mM CaCl2, 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 R2500) 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.

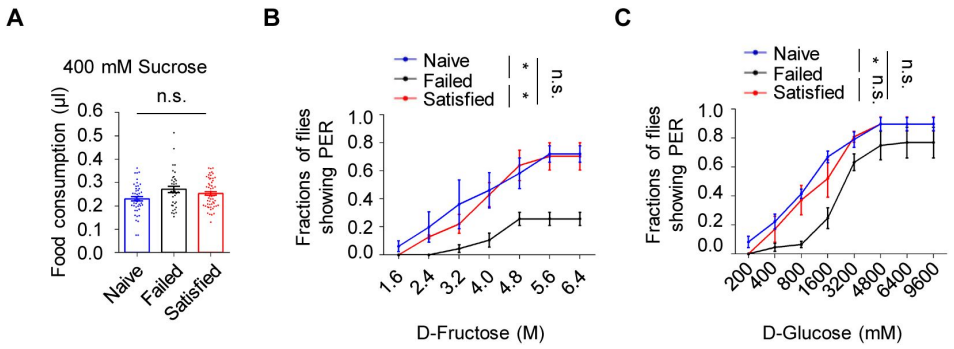

Figure 1-supplement 1

Sexual failure decreased sweet sensitivity.

(A) Volume of 400mM sucrose consumed by males fed *ad libitum* (n = 35-60). (B) Fraction of Naïve, Failed and Satisfied male flies showing PER responses to different concentrations of D-Fructose (n=48-60). (C) Fraction of male flies showing PER responses to different concentrations of D-Glucose (n=48-60). Data are shown as means ± SEM. Error bars represent SEM. n.s., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

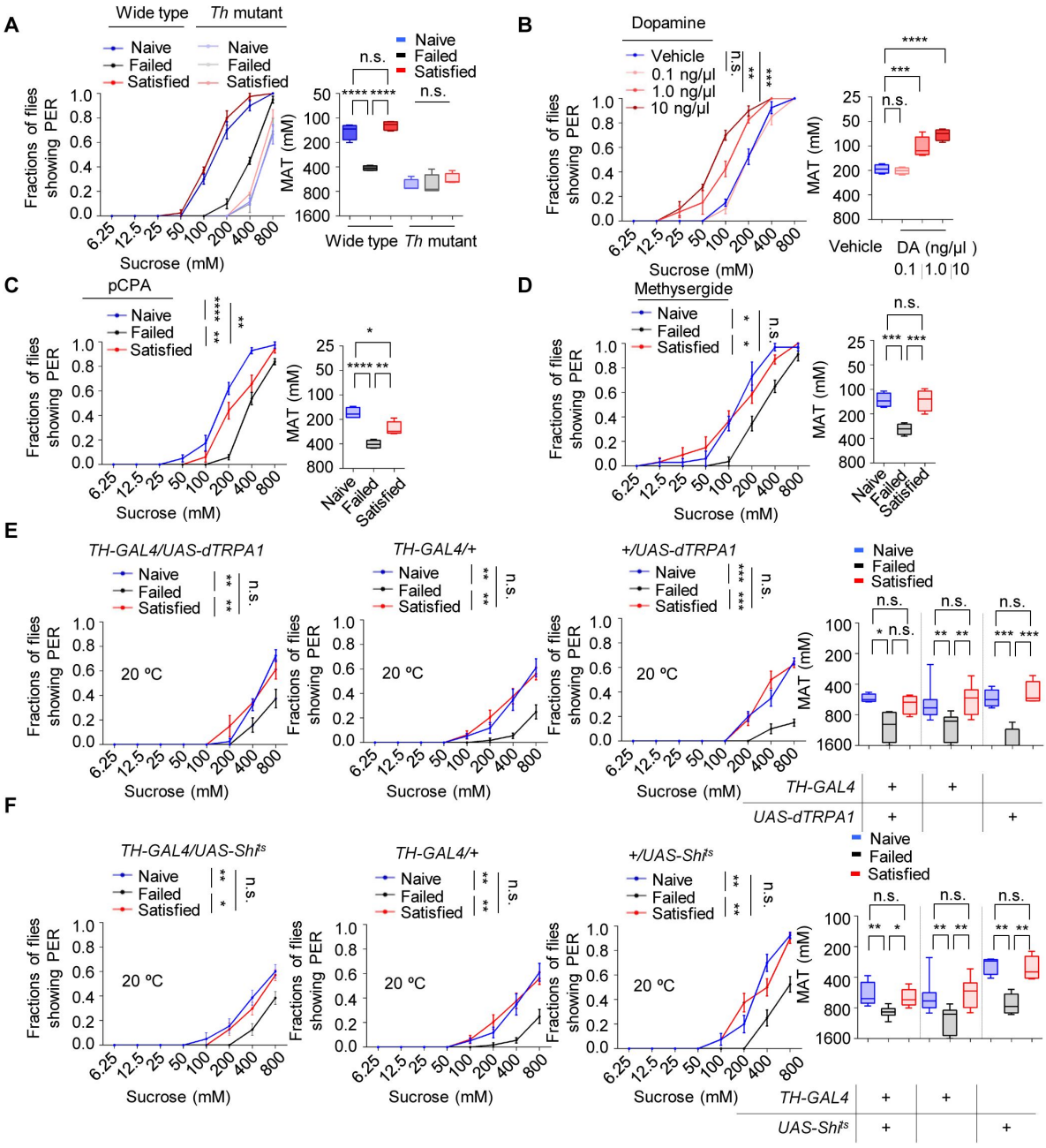

Figure 2-supplement 1

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., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

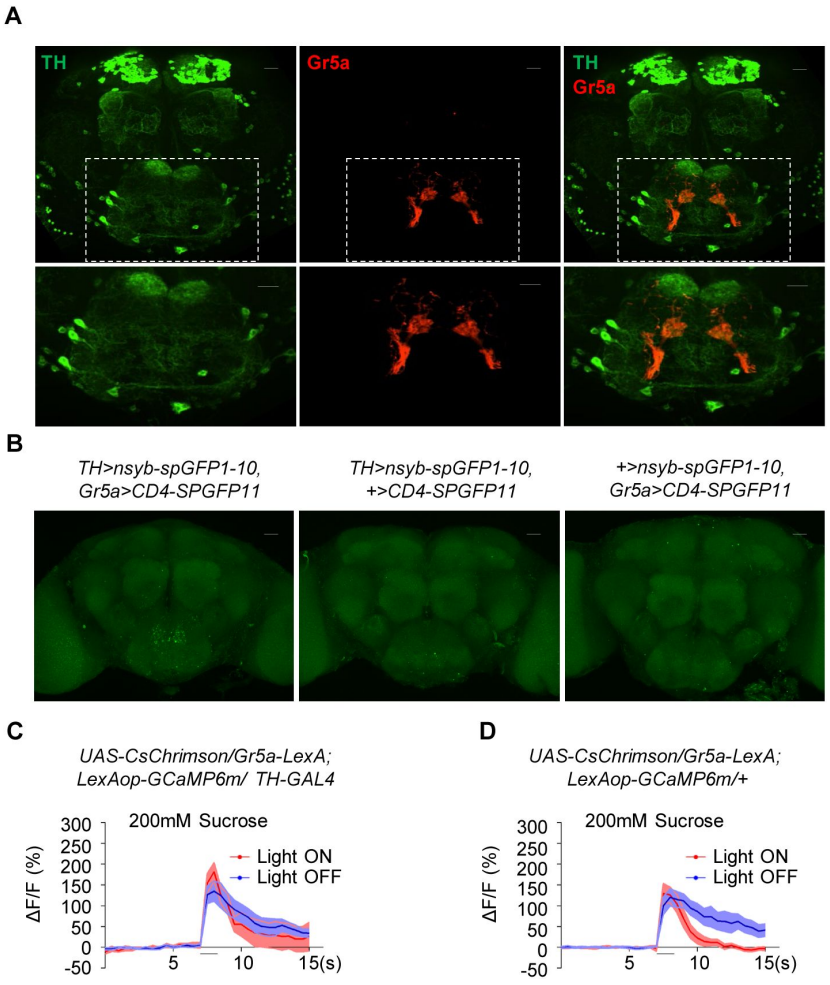

Figure 3-supplement 1

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 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 (n=6-9). The horizontal black bars represent sucrose application. The solid lines represent average trace, and shaded regions represent ±SEM.

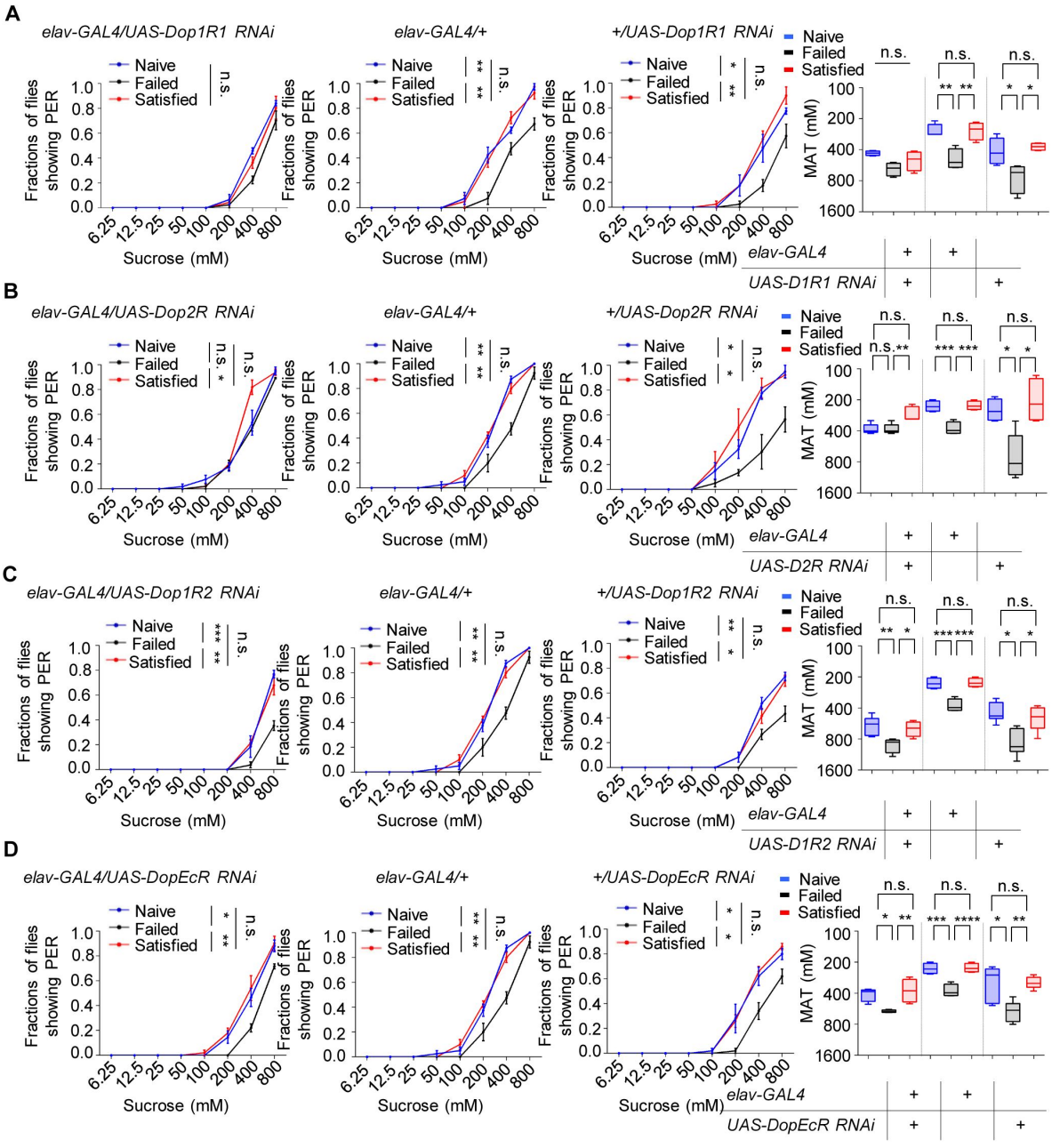

Figure 4-supplement 1

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., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

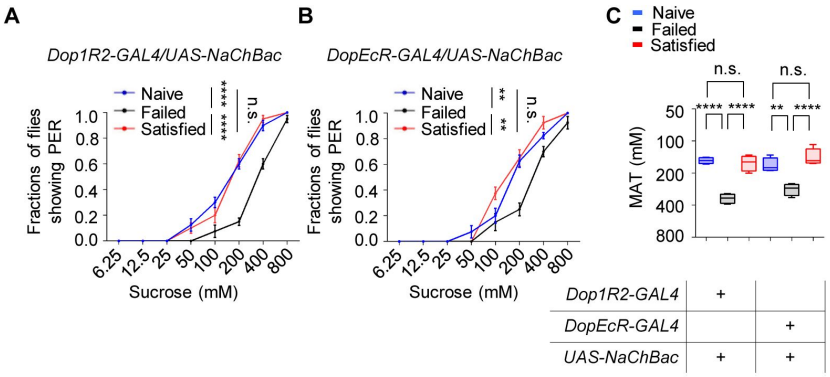

Figure 5-supplement 1

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., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

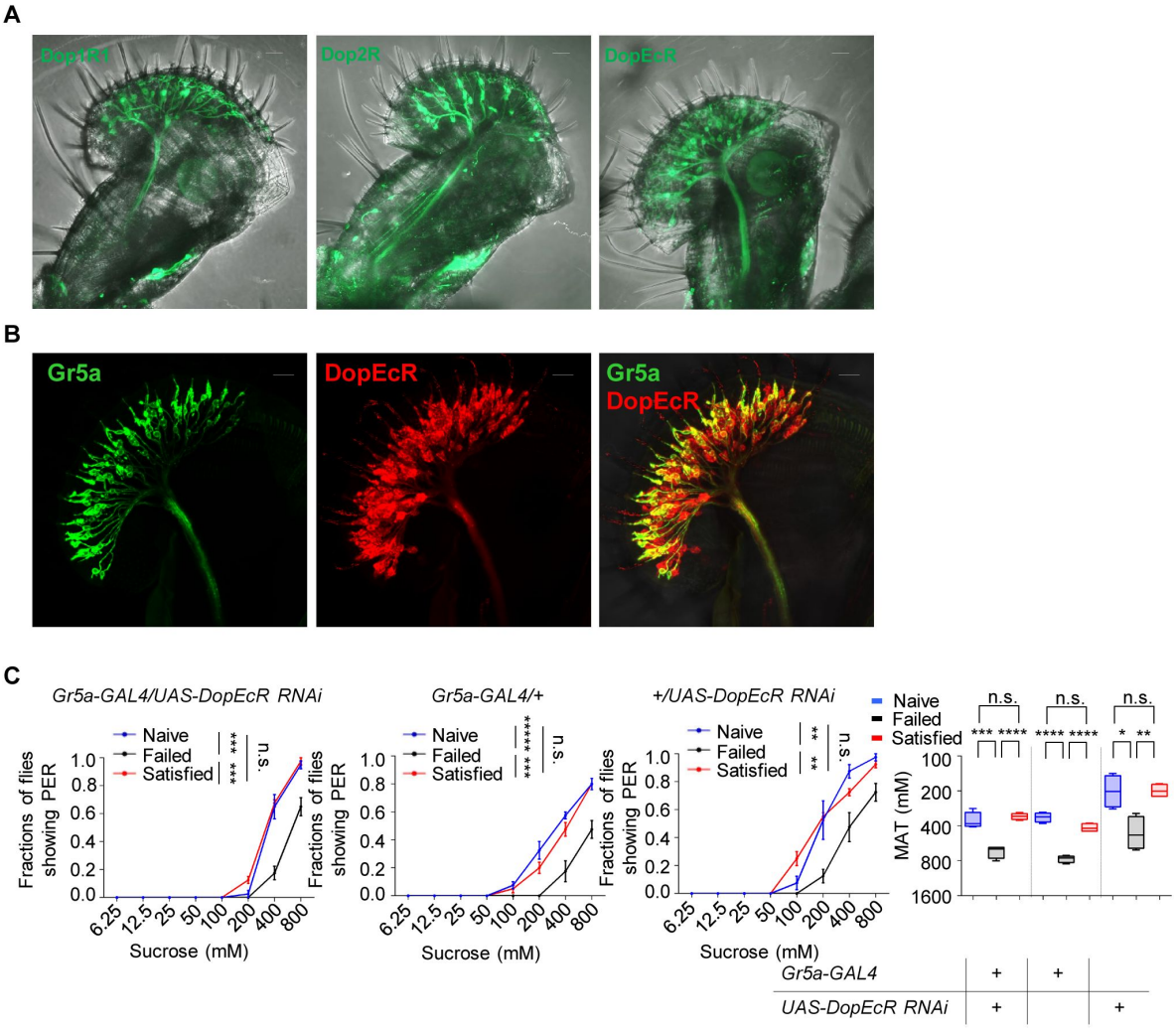

Figure 6-supplement 1

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

(A) The expression of membrane-bound GFP (mCD8GFP) in dopamine receptors 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., $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

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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 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 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. 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.

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.

(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.

(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.

(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.

(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.

(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.

(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.

https://doi.org/10.7554/eLife.105094.1.sa3

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.

https://doi.org/10.7554/eLife.105094.1.sa2

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.

https://doi.org/10.7554/eLife.105094.1.sa1

Author response:

> ***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, DopR1, 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 DopR1 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 different internal states (courtship failure vs. starvation) modulate peripheral sensory system via different signaling pathways (e.g. different subsets of dopaminergic neurons; different dopamine release mechanisms; and different dopamine receptors). We will further discuss 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.*

We think this is a valid concern. We will conduct courtship conditioning in the absence of food and test if courtship failure can still suppress sugar sensitivity in the revised manuscript.

> *(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.*

Here is a brief clarification of our experimental design and we will further clarify the details in the revised manuscript:

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 response curve).

On the other hand, we sought to quantify food consumption of individual flies by using the MAFE assay (Qi et al 2005). 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 (Figure 1B). For these flies, we could quantify the consumed volumes and found there was no change (Figure 1, S1A). We should also note the consistency of these two experiments, e.g. in Figure 1C, only 50-60% of Failed males responded to 400 mM stimulation.

These two experiments in combination suggest that sexual failure suppressed sweet sensitivity of the Failed males. Meanwhile, as long as they still 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.

In addition, to further clarify the potential misunderstanding, we plan to examine food consumption by using 800 mM sucrose in the revised manuscript. As shown in Figure 1C, 800 mM sucrose was adequate to induce feeding in ~100% of the flies.

> *(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 think this is also a valid suggestion. We will directly examine whether activating TH^+ neurons in sexually conditioned males would enhance sugar responses of $Gr5a^+$ neurons in sexually failed males. We will also add in statistical analysis.

Nevertheless, we would still argue our current experiments using Naive males (Figure 3, S1C-D) are adequate to show a functional link between TH^+ neurons and $Gr5a^+$ neurons. Combining with the results that these neurons form active synapses (Figure 3, S1B) and that the activity of TH^+ neurons was dampened in sexually failed males (Figure 3G-I), our current data support the notion that sexual failure suppresses sweet sensitivity via TH-Gr5a circuity.

> *(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 will add 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. In fact, 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. However, our current data are not adequate for the clarification given the experiments shown in Figure 6E-F and the apparent control (Figure 3C) were not conducted under identical settings at the same (that's why we did not directly compare these results). One way to address the issues is to conduct these calcium imaging experiments again with a head-to-head comparison with the control group (Gr5a-GCaMP, +/- Dop1R1 and Dop2R RNAi). We will conduct the experiments and present the data in the revised manuscript.

> *(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 will change our expressions in the revised manuscript. In brief, we think that these manipulations (suppressing $Dop1R1^+$ and $Dop2R^+$ neurons) have two consequences: suppressing the overall sweet sensitivity and eliminating the effect of sexual failure.

> ***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.*

We agree with the reviewer that in the current study, we did not examine 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, S1B-C) upon sexual 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 will further discuss 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.*

Here is a brief clarification of our experimental design and we will further clarify the details in the revised manuscript:

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 response curve).

On the other hand, we sought to quantify food consumption of individual flies by using the MAFE assay (Qi et al 2005). 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 (Figure 1B). For these flies, we could quantify the consumed volumes and found there was no change (Figure 1, S1A). We should also note the consistency of these two experiments, e.g. in Figure 1C, only 50-60% of Failed males responded to 400 mM stimulation.

These two experiments in combination suggest that sexual failure suppressed sweet sensitivity of the Failed males. Meanwhile, as long as they still 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.

In addition, to further clarify the potential misunderstanding, we plan to examine food consumption by using 800 mM sucrose instead. As shown in Figure 1C, 800 mM sucrose was adequate to induce feeding in ~100% of the flies.

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