

Dietary salt induces taste desensitization via receptor internalization in *Drosophila* in a sexually dimorphic manner

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<https://doi.org/10.1016/j.mocell.2025.100242>

ABSTRACT

Sodium homeostasis, which is critical for survival, includes mechanisms for regulating salt intake that integrate central neural pathways with the peripheral taste system. Although the central homeostatic mechanisms of salt appetite are well-studied, the mechanisms by which dietary salt modulates peripheral taste responses remain unclear. We found increased dietary salt reduces salt preference in *Drosophila* by desensitizing sweet gustatory receptor neurons independent of internal sodium levels. We observed a reversible suppression of salt-evoked neural responses following salt exposure accomplished via clathrin-mediated endocytosis in males and both clathrin- and C-terminal binding protein-dependent endocytosis in females. We also found reversing gustatory receptor neuron sexual identity switched the desensitization pattern, indicating cell-autonomous control of this sexual dimorphism. Moreover, C-terminal binding protein-mediated macropinocytosis in females also dampened sweet taste responses, revealing a sex- and modality-specific mechanism underlying sensory adaptation. These findings reveal dietary experience can affect feeding behavior by reprogramming peripheral taste responses, clarifying the plasticity of nutrient sensing.

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Keywords: Desensitization, *Drosophila*, Endocytosis, Salt, Sexual dimorphism

INTRODUCTION

Sodium ions are essential for various biological processes, including body fluid homeostasis, muscle contractions, and neural activity (Lowell, 2019). While dietary salt intake is necessary for sodium homeostasis, excessive salt intake can be detrimental. In mammals, chronic consumption of a high-salt diet is associated with hypertension and other health disorders, and in *Drosophila*, a high-salt diet reduces lifespan and disrupts physiological homeostasis (Dey et al., 2023; Farquhar et al., 2015; Muller et al., 2019; Murashov et al., 2021; Xie et al., 2019). Animals have therefore evolved conserved mechanisms for regulating salt intake, driving sodium-seeking behavior when its levels are too low, and sodium avoidance when levels are too high. These regulatory mechanisms involve both peripheral taste systems that detect dietary sodium and central neural pathways that govern salt appetite and aversion (Taruno and Gordon, 2023).

In the periphery, the gustatory system plays a central role in regulating salt intake via its role in detecting dietary sodium and triggering appropriate behavioral responses. In *Drosophila*, salt drives concentration-dependent innate behaviors. Low-salt concentrations (≤ 100 mM NaCl) activate sweet gustatory

receptor neurons (GRNs) (Gr64f-expressing), promoting attraction, whereas high-salt concentrations (≥ 200 mM NaCl) activate bitter (Gr66a-expressing) and pheromone-sensitive (Ppk23-expressing) GRNs, driving strong aversion (Jaeger et al., 2018; Zhang et al., 2013). These innate salt concentration-dependent responses can, however, be modulated by internal physiological cues such as those associated with sodium deficiency or reproductive status (Jaeger et al., 2018; McDowell et al., 2022; Walker et al., 2015). For example, salt-deprived flies exhibit increased attraction to low salt and reduced avoidance of high salt, suggesting dynamic crosstalk between internal sodium homeostasis and peripheral taste sensitivity.

While salt detection and its immediate behavioral consequences in many animal systems are well-characterized (Ki and Jeong, 2024; Taruno and Gordon, 2023), the ways in which previous exposure to dietary salt can modulate current salt palatability remain poorly understood. This has been challenging because dietary salt exposure inevitably alters internal sodium homeostasis, making it difficult to distinguish direct sensory plasticity from homeostatic feedback effects. It is also unclear whether such plasticity arises from central or peripheral mechanisms, or both.

To address these questions, we investigated the influence of dietary salt exposure on salt preference in *Drosophila*. We found that a diet with increased salt reduced salt preference by desensitizing peripheral salt responses independent of internal sodium homeostasis. This behavioral plasticity occurred via endocytic receptor internalization in sweet GRNs, indicating that salt exposure can modify the sensitivity of primary taste cells. Importantly, we also found that the mechanism underlying receptor internalization exhibited sexual dimorphism. While this salt-induced desensitization required clathrin-mediated endocytosis in males, it required both clathrin- and C-terminal binding protein (CtBP)-mediated endocytosis in females. Thus, we have uncovered an experience-dependent tuning of peripheral salt taste that also highlights a contribution of biological sex to homeostatic plasticity. Our study provides new insights into how sensory systems adapt to dietary changes as they regulate the intake of essential nutrients.

MATERIALS AND METHODS

Fly Stocks

All fly stocks were maintained on standard cornmeal-based food at 25°C and 60% humidity, under a 12-hour/12-hour light/dark cycle. The following fly lines were obtained from the Bloomington *Drosophila* Stock Center: *UAS-Sh¹*^{ts} (BL44222), *UAS-CtBP^{RNAi}* (BL32889), *UAS-TraF^{RNAi}* (BL44109), *UAS-Kir2.1* (BL6595), *Ir94e-GAL4* (BL81246), and *UAS-TraF* (BL4590). The following RNAi lines were obtained from the Vienna *Drosophila* Resource Center: *UAS-shibire^{RNAi}* (#105971, #3799), *UAS-Chc^{RNAi}* (#103383, #23666), *UAS-Rho1^{RNAi}* (#109420, #12734), *UAS-Flotillin1^{RNAi}* (#103938, #42130), *UAS-Graf1^{RNAi}* (#110812, #42165), and *UAS-Arf6^{RNAi}* (#24224, #100726). *UAS-Dicer2* (#60009) was also obtained from the Vienna *Drosophila* Resource Center and used to enhance knockdown efficiency. The *MN11+12-GAL4* (NP1363) line was obtained from the *Drosophila* Genetic Resource Center, and the *Gr64f-GAL4* line was a gift from John Carlson. *Ppk23-GAL4* and *Ppk28-GAL4* were gifts from Kristin Scott. *Ir56b¹* was a gift from Jae Young Kwon. *Gr5a^{LEXA}* was a gift from Hubert Amrein, and *Gr64af* was previously described (Kim et al., 2018). The complete genotypes of the flies used in this study appear in [Supplementary Table S1](#).

Dietary Manipulations

Standard cornmeal-based food composed of 5% yeast (SAF Instant Yeast), 7% cornmeal (Seunglim Food), 10% dextrose (3020-4400, Daejung), 1% agar (DFA-30301, Hansol Tech), 0.2% methyl-4-hydroxybenzoate (H5501, Sigma-Aldrich), and 0.6% propionic acid (P1386, Sigma-Aldrich) was used for the regular diet. For the salt diet, 10 mM NaCl (7647-14-5, Duchefa Biochemie) was added to the regular diet for a total of 13 mM NaCl. For the high-sugar diet, 15% sucrose (S9378, Sigma-Aldrich) was added to the regular diet.

Measuring Sodium Concentrations

About 1 g of fly food was accurately weighed and transferred to a Teflon beaker suitable for microwave digestion. Then, 8 ml of nitric acid and 2 ml of hydrogen peroxide (electronic grade,

Dongwoo FineChem Co, Ltd) were added as the certified reference materials. The beaker was sealed, and the reaction was allowed to progress in the microwave. After digestion, the sample was allowed to cool and then adjusted to 50 g with distilled water. The resulting solution was mixed and passed through filter paper to obtain the test solution. Sodium concentrations were measured via inductively coupled plasma atomic emission spectrometry using the Avio 500 (Perkin-Elmer) instrument (1500-W power; argon gas; 2.50-rpm pump speed; 0.7 L/min nebulizer flow; 589.592-nm wavelength for sodium).

Two-Way Choice Behavior Test

Fed flies were allowed to feed for 90 minutes in a dark room at room temperature with a choice between 2 food options. To monitor food preference, each food option included a nontoxic dye, either blue (0.125 mg/ml Brilliant Blue FCF, 027-12842, Wako Pure Chemical Industry, Ltd) or red (0.2 mg/ml sulforhodamine B, S9012, Sigma-Aldrich). After the 90-minute feeding period, the flies were frozen and the colors of their abdomens were observed under a stereomicroscope. A preference index was calculated using the following equation: preference index = (# of red or blue abdomens + ½ the # of purple abdomens)/total # of fed flies.

In [Fig. 1F](#), to prevent actual ingestion of sodium, the flies were raised at 21°C and then transferred to 21 or 31°C, while simultaneously being exposed to either a regular or salt diet for 6 hours. After that, the 2-way choice assay was performed at 21°C for 90 minutes.

Capillary Feeder Assay

To examine the effect of motor neurons (MN)11/MN12 silencing on feeding, 10 females and 10 males expressing temperature-sensitive shibire (*UAS-shibire^{ts}*) in the MN11 and MN12 or their genetic controls were collected and placed into separate vials. After aging the flies for 2 days, they were sorted into new vials, each containing 10 females or 10 males, and allowed to acclimate for 1 day. Each vial also included 2 10-μl capillaries, each containing 100 mM sucrose (Ja et al., 2007). After 6 hours of feeding at 31°C and 60% relative humidity, the levels of sucrose remaining in the capillaries were measured. To assess the recovery of MN function, food intake was measured again after the same flies were transferred to 21°C for 1.5 hours.

Tip Recordings

Tip recordings were performed as previously described (Pradhan et al., 2023). Flies housed in fresh vials were anesthetized by brief exposure to ice. A glass capillary pipette containing Ringer's solution was inserted through the thorax and advanced to the base of the labellum. This pipette was grounded to the recording system so it could serve as the reference electrode. Tastants were dissolved in 30 mM tricholine citrate (T0252, Sigma-Aldrich) to facilitate electrical conductivity and delivered through a 10- to 20-μm-diameter recording electrode. The recording electrode was connected to a TastePROBE (Syntech) and the resulting signals were captured using an acquisition controller (Syntech) connected to a computer. The raw signals were amplified by a factor of 10, band-

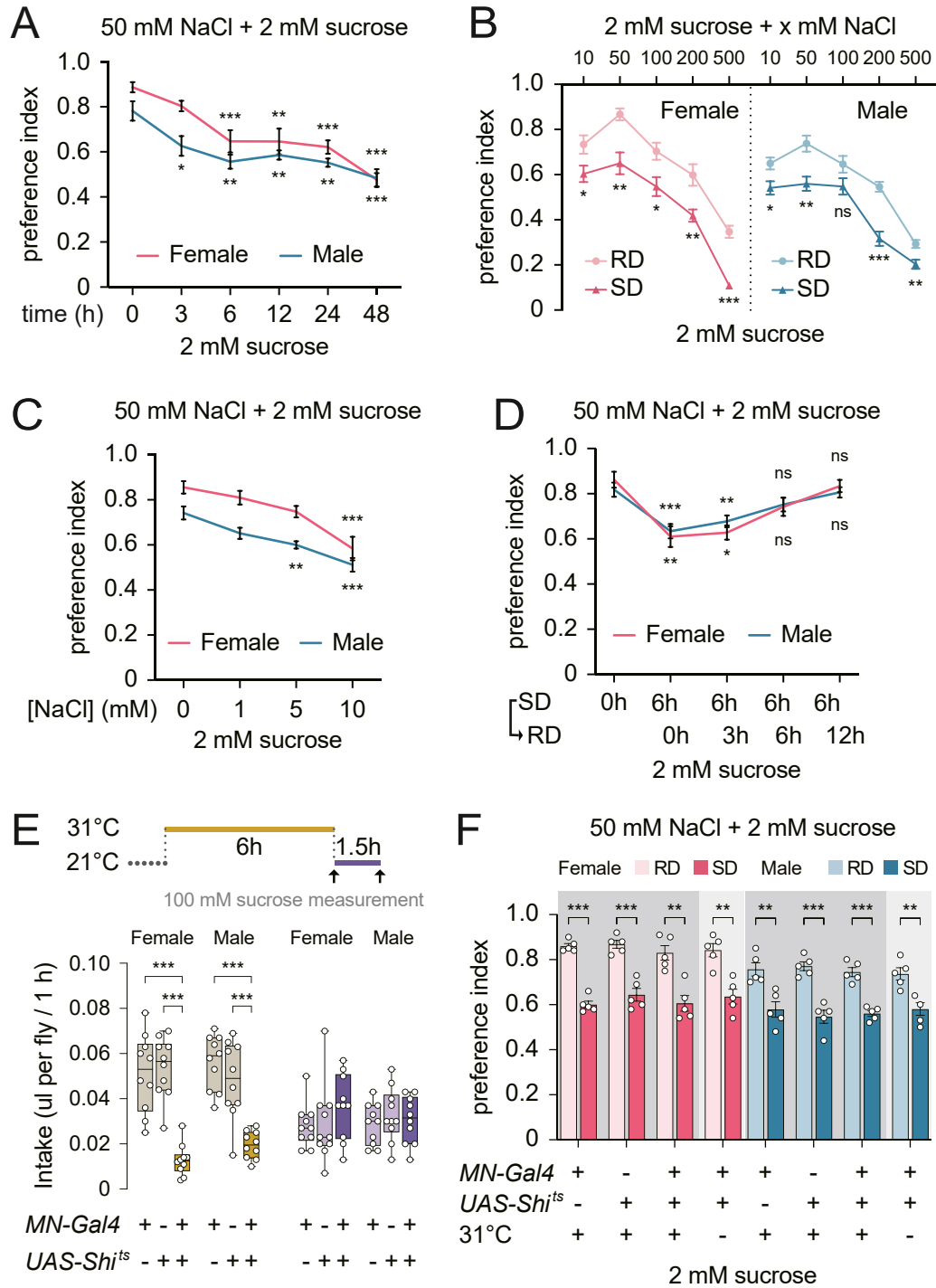


Fig. 1. Dietary salt exposure reduces low-salt preference. (A) Salt preference in both female and male flies fed regular diet (RD) or salt diet (SD) for 3, 6, 12, 24, or 48 hours. Flies were given a choice between 50 mM NaCl plus 2 mM sucrose in 1% agarose or 2 mM sucrose in 1% agarose. $n = 5$ –6, statistical comparisons to RD-fed females or males. (B) Salt preference in both female and male flies fed RD or SD for 6 hours. Flies were given a choice between 2 mM sucrose or 2 mM sucrose with varying NaCl concentrations (10, 50, 100, 200, or 500 mM). $n = 6$, statistical comparisons between RD and SD for each concentration. (C) Salt preference in female and male flies fed RD or SD supplemented with 1, 5, or 10 mM NaCl for 6 hours. Flies were given a choice between 50 mM NaCl plus 2 mM sucrose in 1% agarose or 2 mM sucrose in 1% agarose. $n = 5$, statistical comparisons to RD-fed females or males. (D) Recovery of salt taste desensitization in female and male flies after they were fed SD for 6 hours and transferred to RD for 3, 6, or 12 hours. Flies were given a choice between 50 mM NaCl plus 2 mM sucrose in 1% agarose or 2 mM sucrose in 1% agarose. $n = 5$, statistical comparisons to RD-fed females or males. (E) Sucrose consumption (100 mM) by flies of the indicated genotypes in the CAFÉ assay. Food intake was recorded for 6 hours at 31°C, followed by a second measurement after the flies were transferred to 21°C for 1.5 hours. $n = 10$. (F) Salt preference in flies of the indicated genotypes after 6 hours on either RD or SD at 31 or 21°C. Flies were given a choice between 50 mM NaCl plus 2 mM sucrose in 1% agarose or 2 mM sucrose in 1% agarose. $n = 5$. All data are presented as means $\pm$ SEM, *** $P < .001$, ** $P < .01$, and * $P < .05$ for all panels unless otherwise indicated. CAFÉ, capillary feeder.

pass filtered (100–3000 Hz), and sampled at 12 kHz. Neuronal firing rates were subsequently analyzed using the Autospike 3.1 software (Syntech).

For local sensilla stimulation, after the flies were prepared for tip recordings, action potentials evoked by 30 mM NaCl were first measured in the L4 sensilla. After this initial recording, the L4 sensilla were intermittently stimulated with either distilled water or 10 mM NaCl at 2- to 5-minute intervals over a 3-hour period. After this period of repeated stimulation, neuronal responses to 30 mM NaCl were reassessed.

Statistics

All error bars indicate SEM. The Shapiro-Wilk method was used to test for normality. For comparing 2 groups, unpaired or paired Student's *t*-tests were used when the data followed a normal distribution. For multiple comparisons, 1-way ANOVAs were used with Tukey's tests or Dunn's tests, depending on data normality. Statistical tests were conducted using GraphPad Prism software (version 10). Asterisks indicate *** $P < .001$, ** $P < .01$, and * $P < .05$. More details regarding these statistical analyses appear in [Supplementary Table S2](#).

RESULTS

Dietary Salt Reduces Salt Preference

We aimed to determine whether daily salt intake influences salt preference in *Drosophila*. First, we measured the sodium (Na⁺) content of our standard laboratory fly food (hereinafter referred to as regular diet) and found it to be approximately 3 mM ([Fig. S1A](#)). Flies reared on the regular diet exhibited a strong preference for food containing 50 mM NaCl, the concentration that elicits the highest level of salt attraction ([Fig. 1A](#)). Next, we tested whether exposure to salt affected salt preference. By supplementing the regular diet with an additional 10 mM NaCl, resulting in a total of 13 mM sodium, we prepared what we referred to as salt diet. We then found that flies fed the salt diet displayed a time-dependent decrease in their preference for food containing 50 mM NaCl, which became noticeable at 3 hours and reached its peak at 48 hours in both males and females ([Fig. 1A](#)). To minimize any potential confounding from mechanisms related to internal sodium homeostasis, we selected 6 hours of salt diet feeding as the experimental condition for investigating the effect of dietary salt on salt preference.

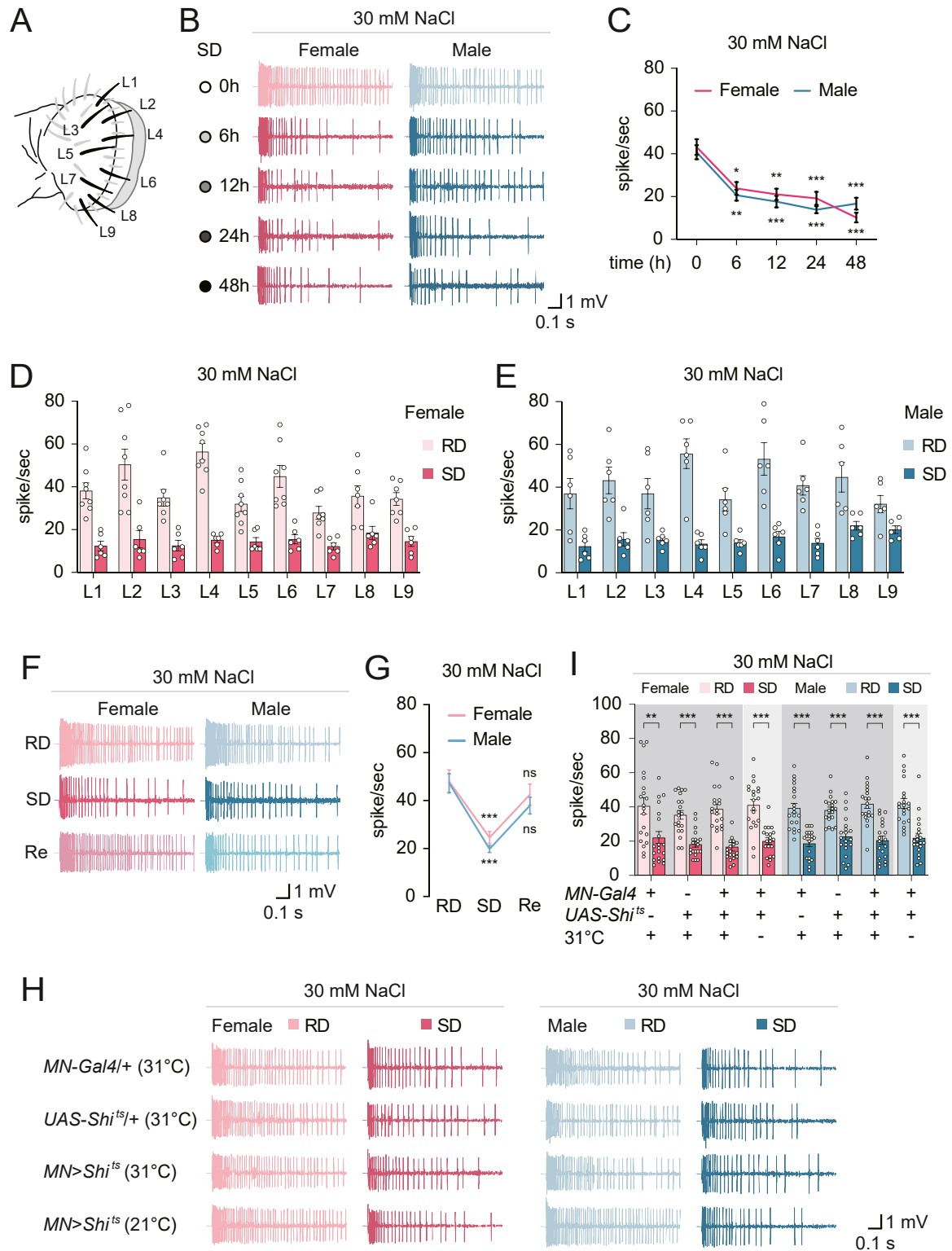
Flies reared on the regular diet exhibited a preference for food containing more salt (≤ 100 mM NaCl). This preference

peaked at 50 mM NaCl and shifted to avoidance at NaCl concentrations above 200 mM ([Fig. 1B](#)). Although flies fed salt diet for 6 hours showed a similar trend, both males and females showed significantly lower preference for food with lower salt levels and stronger avoidance of high-salt food ([Fig. 1B](#)). When we instead kept feeding duration constant but increased dietary sodium content, we observed an even more pronounced suppression of the preference for salt ([Fig. 1C](#)). Notably, this reduction in salt preference following exposure to salt diet was reversible. It began to recover after 6 hours, returning within 12 hours to levels of preference like those of flies fed the regular diet ([Fig. 1D](#)). To rule out potential interactions between the additional NaCl and other dietary nutrients, we performed a salt food preference assay using an agarose-based diet (5% sucrose in 1% agarose) supplemented with either 3 or 13 mM NaCl. Flies fed the 13 mM NaCl diet for 6 hours exhibited reduced preference for food containing 50 mM NaCl compared with those fed the 3 mM NaCl diet. This suggests the effect we observed is attributable specifically to dietary salt and not any other ingredients ([Fig. S1B](#)).

To exclude the possibility that internal sodium homeostasis influenced salt preference during the 6-hour salt diet exposure, we wanted to allow exposure of fly GRNs to salt diet while preventing actual salt ingestion. To achieve this, we silenced labellar MN11 and MN12 ([Manzo et al., 2012](#)) via expression of temperature-sensitive *shibire* (*UAS-shibire^{ts}*) ([Kitamoto, 2001](#)). Using the Capillary Feeder assay, we confirmed that silencing MN11+12 for 6 hours at the restrictive temperature (31°C) significantly reduced food intake ([Fig. 1E](#)). We then immediately shifted the flies to the permissive temperature (21°C) and allowed them to feed for 1.5 hours. The permissive temperature prevents the *Shi^{ts}* silencing effect, allowing flies of all genotypes to consume similar amounts of food and enabling us to assess their food preference. Notably, we found MN11+12 silencing had no effect on salt preference following salt diet exposure, which lends further support to the hypothesis that internal sodium homeostasis is uninvolved in this phenomenon ([Fig. 1F](#)).

Dietary Salt Reduces Salt-Evoked Nerve Firing

While the reduced preference for salt we observed following salt diet exposure could be the result of changes in peripheral GRNs or in higher-order central processing, we prioritized investigating peripheral desensitization because we had already established that internal sodium homeostasis was not involved. Labellar sensilla, which each house 2 to 4 GRNs, are classified



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Fig. 2. Electrophysiological responses to salt in flies exposed to the salt diet. (A) Schematic of the labellum showing the L-type sensilla that respond to low concentrations of salt. (B) Representative action potential traces from L-type sensilla in response to 30 mM NaCl after female and male flies were fed either RD or SD for 6, 12, 24, or 48 hours. (C) Quantification of the mean number of action potentials recorded from L-type sensilla in response to 30 mM NaCl in female and male flies fed either RD or SD for 6, 12, 24, or 48 hours. $n = 20$ –28, statistical comparisons to RD-fed females or males. (D, E) Quantification of the mean number of action potentials recorded from L-type sensilla in response to 30 mM NaCl in female (D) and male (E) flies fed either RD or SD for 6 hours. $n = 6$ –8. (F) Representative action potential traces from L-type sensilla in response to 30 mM NaCl in flies fed RD or SD for 6 hours or SD for 6 hours followed by refeeding with RD for 6 hours (Re). (G) Quantification of the mean number of action potentials recorded from L-type sensilla in response to 30 mM NaCl in flies fed RD or SD for 6 hours or SD for 6 hours followed by RD for 6 hours (Re). $n = 20$ –24, statistical comparisons to RD-fed females or males. (H) Representative action potential traces from L-type sensilla in response to 30 mM NaCl in flies of the indicated genotypes at the indicated temperatures after being fed RD or SD for 6 hours. (I) Quantification of the mean number of action potentials recorded from L-type sensilla in response to 30 mM NaCl in flies of the indicated genotypes at the indicated temperatures fed RD or SD for 6 hours. $n = 20$. All data are presented as means $\pm$ SEM, *** $P < .001$, ** $P < .01$, and * $P < .05$ for all panels unless otherwise indicated.

into 3 distinct types according to their position and length: Long (L-type), Intermediate, and Short sensilla (Fig. 2A) (Hiroi et al., 2002). Attraction to low-salt concentrations depends primarily on sweet GRNs found in all sensillar types (Zhang et al., 2013). We therefore performed tip recordings in L-type sensilla to measure electrophysiological responses to low concentrations of salt following exposure to salt diet. We found salt diet exposure led to a gradual reduction in the number of action potentials evoked by 30 mM NaCl in both male and female L-type sensilla (Fig. 2B–E). Notably, this reduction in action potentials was reversible. After a 6-hour salt diet exposure reduced action potentials evoked by 30 mM NaCl, a subsequent 6-hour return to regular diet restored the number of salt-evoked nerve firings to baseline levels (Fig. 2F, G).

To access the specificity of action potentials evoked by 30 mM NaCl, we used the inward-rectifying potassium channel Kir2.1 to selectively silence 4 types of GRNs in L-type sensilla: sweet GRNs (*Gr64f-GAL4*), water GRNs (*Ppk28-GAL4*), low-salt GRNs (*Ir94e-GAL4*), and pheromone GRNs (*Ppk23-GAL4*) (Jaeger et al., 2018). Only using *Gr64f-GAL4* to drive Kir2.1 expression in sweet GRNs abolished responses to 30 mM NaCl; the silencing of the other GRN types had no effect (Fig. S2A). To further validate the specificity of low-salt responses in sweet GRNs, we analyzed flies carrying mutations in salt and sugar receptor genes. We found mutation of *Ir56b*, which encodes a subunit of a low-salt receptor, abolished salt-evoked nerve firing, but the responses of sugar-blind flies (*Gr5a^{LEXA}::Gr64af*) remained intact (Fig. S2B). Notably, the silencing of the other types of GRNs failed to block salt diet-induced salt desensitization, suggesting that this phenomenon is cell-autonomous to sweet GRNs.

Next, we sought to rule out the potential contribution of sucrose present in the diet, as both sucrose and NaCl could simultaneously activate sweet GRNs. To address this, we performed tip recordings with flies reared on an agarose-based diet containing 5% sucrose with either 3 or 13 mM NaCl, or on similar sucrose-free diets containing only the 3 or 13 mM NaCl. Flies fed the 13 mM NaCl diet for 6 hours—regardless of the presence of sucrose—exhibited fewer action potentials evoked by 30 mM NaCl. These results suggest sugar does not contribute to salt diet-induced salt desensitization (Fig. S3).

We also observed the same reduction in salt-evoked action potentials in flies with silenced labellar MN11 and MN12, regardless of their actual food intake (Fig. 2H, I). In addition, 3 hours of repetitive local salt stimulation in immobilized flies

reduced the salt-evoked responses of L-type sensilla (Fig. S4). These findings confirm that the salt desensitization we observed is not due to internal NaCl homeostasis. Together, these data indicate the reduced salt preference that follows dietary salt exposure is caused by the desensitization of peripheral salt-sensing neurons.

Salt Desensitization Depends on a Sexually Dimorphic Receptor Internalization Mechanism

Receptor endocytosis is a key mechanism underlying sensory desensitization (Dohlman, 2002). We conducted a targeted RNAi screen to determine whether receptor internalization contributes to salt desensitization and to identify the specific pathways involved. To maximize the efficiency of the RNAi screen, we used flies that had been fed a salt diet for 2 days, a condition in which salt diet-induced desensitization reaches its peak (Fig. 2C). We found in female flies that knockdown of genes involved in clathrin-mediated endocytosis (Kaksonen and Roux, 2018), such as *Shibire* and *clathrin*, abolished salt desensitization (Fig. 3A, C), as did knockdown of *CtBP*, a key regulator of macropinocytosis (Kerr and Teasdale, 2009) (Fig. 3A, C). In contrast, for male flies, only clathrin pathway gene knockdown abolished salt desensitization; *CtBP* knockdown had no effect (Fig. 3B, D).

We next inhibited the endocytic pathway and performed a 2-way choice behavioral assay to evaluate the behavioral consequences of impairing salt desensitization. In females, knockdown of *clathrin* or *CtBP* each partially inhibited the salt diet-induced suppression of salt preference, whereas simultaneous knockdown of both genes completely abolished this suppression (Fig. 3E). In males, *clathrin* knockdown alone was sufficient to block salt diet-induced suppression of salt preference, while *CtBP* knockdown had no effect (Fig. 3F). These findings indicate receptor-mediated endocytosis is required for salt desensitization and that the underlying mechanism is regulated in a sexually dimorphic manner.

Sex Determination Genes Regulate Male-Female Differences in Salt Desensitization

To further validate the sexually dimorphic regulation of salt desensitization, we employed a genetic approach to alter the sexual identity of GRNs (Ferveur et al., 1995). *Transformer* (*tra*) is expressed only in females and required in female sexual development (Robinett et al., 2010). To determine whether *CtBP* is specifically required for salt diet-induced

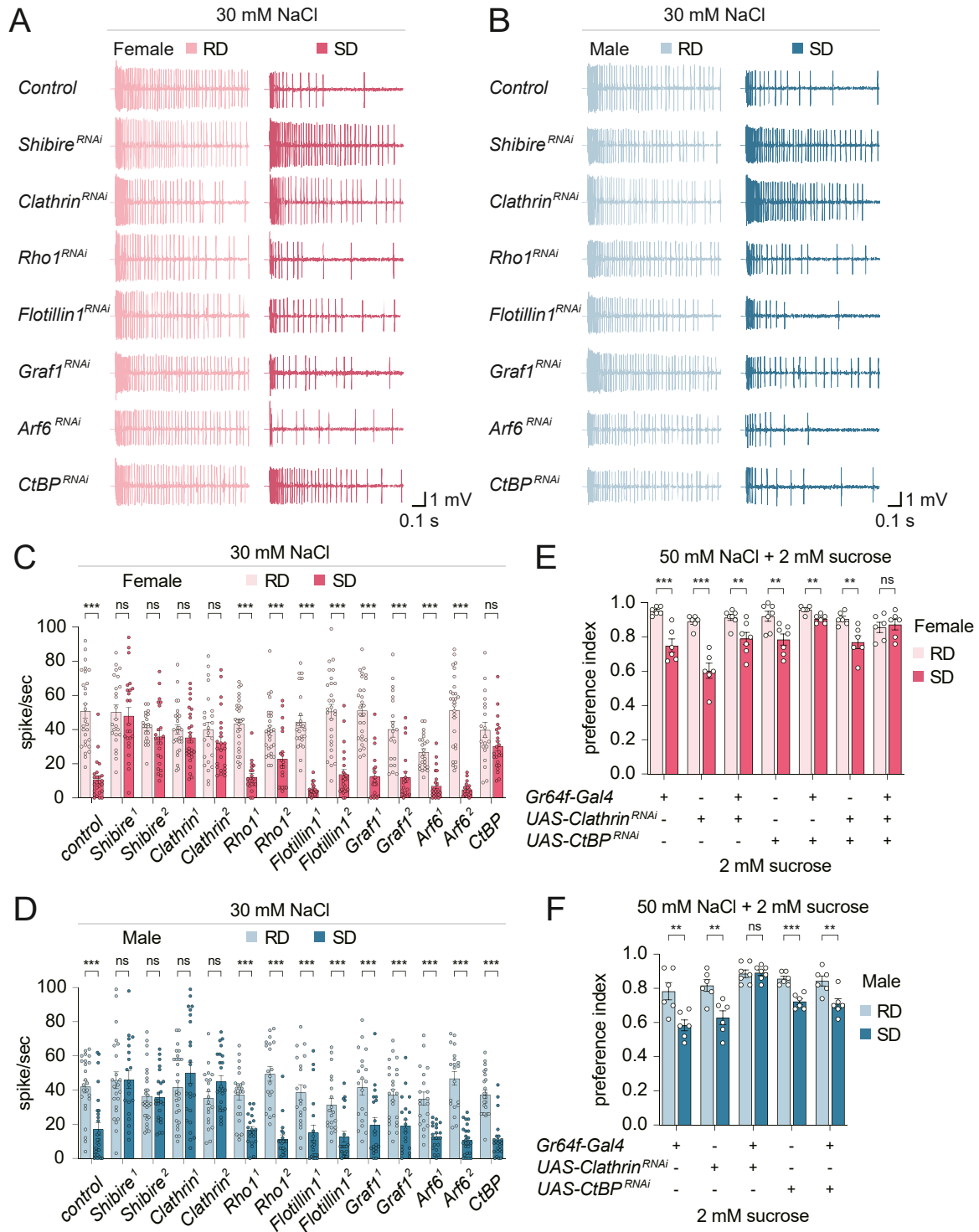


Fig. 3. Salt desensitization requires the endocytic pathway in a sexually dimorphic manner. (A, B) Representative action potential traces recorded from L-type sensilla in female (A) and male (B) flies with sweet GRN-specific knockdown of endocytic pathway components in response to 30 mM NaCl after feeding on RD or SD for 2 days. (C, D) Quantification of the mean number of action potentials recorded from L-type sensilla in female (A) and male (B) flies with sweet GRN-specific knockdown of endocytic pathway components in response to 30 mM NaCl after feeding on RD or SD for 2 days. $n = 20-28$. (E, F) Salt preference in female (E) and male (F) flies with sweet GRN-specific knockdown of clathrin and CtBP after feeding on RD or SD for 6 hours. $n = 6-7$. All data are presented as means $\pm$ SEM, *** $P < .001$, ** $P < .01$, and * $P < .05$ for all panels unless otherwise indicated.

desensitization in female GRNs, we knocked down *tra* in the sweet GRNs of female flies, thereby inducing a loss of female sexual identity in those neurons. In these females with genetically masculinized GRNs, *CtBP* knockdown no longer suppressed salt-evoked neural desensitization following exposure to salt diet. In addition, *clathrin* knockdown alone was sufficient to abolish salt food-induced desensitization, mimicking the male-specific response (Fig. 4A, B). Conversely, ectopic expression of *tra* in the sweet GRNs of male flies, which converted their sexual identity to female, rendered their salt diet-induced desensitization dependent on *CtBP*, mimicking the female-specific regulatory mechanism (Fig. 4C, D). Consistent with these electrophysiological data, we also found in 2-choice feeding assays that salt diet-induced suppression of salt preference showed sex-dependent regulation. Female flies with sweet GRN-specific *tra* knockdown exhibited salt preference suppression in a clathrin-dependent manner, like males. In contrast, the salt diet-induced suppression of salt preference in male flies ectopically expressing *tra* in sweet GRNs was dependent on both *clathrin* and *CtBP*, as observed in females (Fig. 4E, F). These findings indicate that the sexual identity of sweet GRNs dictates the sexually dimorphic regulation of salt diet-induced desensitization.

Salt Diet-Induced Macropinocytosis Desensitizes Sugar Responses in Females

The sexually dimorphic regulation of salt diet-induced desensitization prompted us to investigate whether the macropinocytosis pathway, which is involved exclusively in females, drives the physiological differences observed between male and female flies. Macropinocytosis is a process that engulfs large regions of membrane, potentially leading to receptor internalization (Bryant et al., 2007; Salloum et al., 2023). Since gustatory detection of low concentrations of salt in *Drosophila* is mediated primarily by sweet GRNs, we hypothesized that *CtBP*-mediated macropinocytosis in females might also contribute to sugar response desensitization because the sugar receptors and salt receptors are found in the same portion of the GRN dendritic membranes. Consistent with this hypothesis, we observed that female flies fed the salt diet exhibited reduced sucrose-evoked action potentials compared with those fed the regular diet (Fig. 5A, B). In behavioral assays, these flies also exhibited a diminished preference for food containing 3 mM sucrose over food containing 1 mM sucrose (Fig. 5C). In contrast, we did not observe any desensitization of sweet responses in male flies. To confirm that the salt diet-induced desensitization of sweet responses we observed in female flies depends on *CtBP*-mediated macropinocytosis, we knocked down *CtBP* in the sweet GRNs of female flies. As expected, *CtBP* knockdown abolished salt diet-induced sugar desensitization in female flies and restored their preference for food containing higher sucrose concentrations (Fig. 5D-F).

Flies fed a high-sugar diet exhibit reduced sensitivity to sweet taste (May et al., 2019). Consistent with this, feeding both male and female flies a high-sugar diet for 6 or 48 hours reduced sucrose-evoked action potentials and preference for food with higher concentrations of sucrose (Fig. S5A-C). A high-sugar diet did not, however, reduce salt sensitivity or preference

in either sex at the same time points (Fig. S5D-F). These findings indicate that dietary salt uniquely reduces sweet taste sensation in females, highlighting a sex- and taste modality-specific mechanism for gustatory desensitization.

DISCUSSION

Our study demonstrates that dietary salt exposure rapidly reduces salt taste sensitivity in *Drosophila* peripheral GRNs via receptor internalization. This process occurs independently of internal sodium homeostasis and exhibits clear sexual dimorphism. Previous studies have reported that flies fed a salt-rich diet exhibit stronger avoidance of high-salt food (Jaeger et al., 2018; McDowell et al., 2022), likely due to altered internal sodium levels. Our findings suggest, however, that salt-induced peripheral desensitization of sweet GRNs to low salt may also contribute to this enhanced high-salt avoidance by reducing salt preference. Peripheral desensitization may therefore serve as an additional protective mechanism to prevent excess salt intake and maintain sodium balance.

Peripheral desensitization to salt can be induced without any change in internal sodium levels. Flies in which the MN essential for ingestion were silenced still exhibited salt taste desensitization (Fig. 2H, I). This indicated that peripheral salt desensitization requires direct exposure of sweet GRNs to salt rather salt ingestion. Salt desensitization was also unaffected by silencing the internal salt sensor (data not shown) (Kim et al., 2024).

The precise sodium concentrations required to trigger peripheral desensitization remain unclear. Although the sodium content of standard *Drosophila* food is poorly characterized, Piper et al reported their development of a holidic media containing approximately 20 mM sodium that supported *Drosophila* growth and development (Piper et al., 2014). In our study, the standard laboratory food contained 3 mM sodium, and the salt diet contained 13 mM NaCl. While no defined standards exist for optimal sodium intake in flies, our results suggest that even modest increases in dietary sodium can modulate peripheral taste sensitivity.

One of our most unexpected findings was the sexual dimorphism we observed in salt taste desensitization. In males, desensitization relies solely on clathrin-dependent endocytosis, whereas in females, both clathrin-mediated and *CtBP*-dependent macropinocytosis contribute to receptor internalization (Fig. 3). Genetic manipulation of the sexual identity of sweet GRNs confirmed that this dimorphism is cell-autonomous—neuronal feminization or masculinization each produced the corresponding change in the desensitization response (Fig. 4). This suggests an evolutionary adaptation in which females may have developed greater sensory plasticity to meet sex-specific nutritional demands. Notably, salt-induced macropinocytosis—a key endocytic mechanism in females—also reduces sugar-evoked responses in sweet GRNs via *CtBP*-mediated internalization (Fig. 5). Previous studies using mated females found that increased dietary salt enhances egg production (Walker et al., 2015) and that reduced sugar appetite promotes increased intake of protein-rich foods (Malita et al., 2022). We hope to determine in the future whether salt diet-induced sugar desensitization gives mated females a similar reproductive advantage.

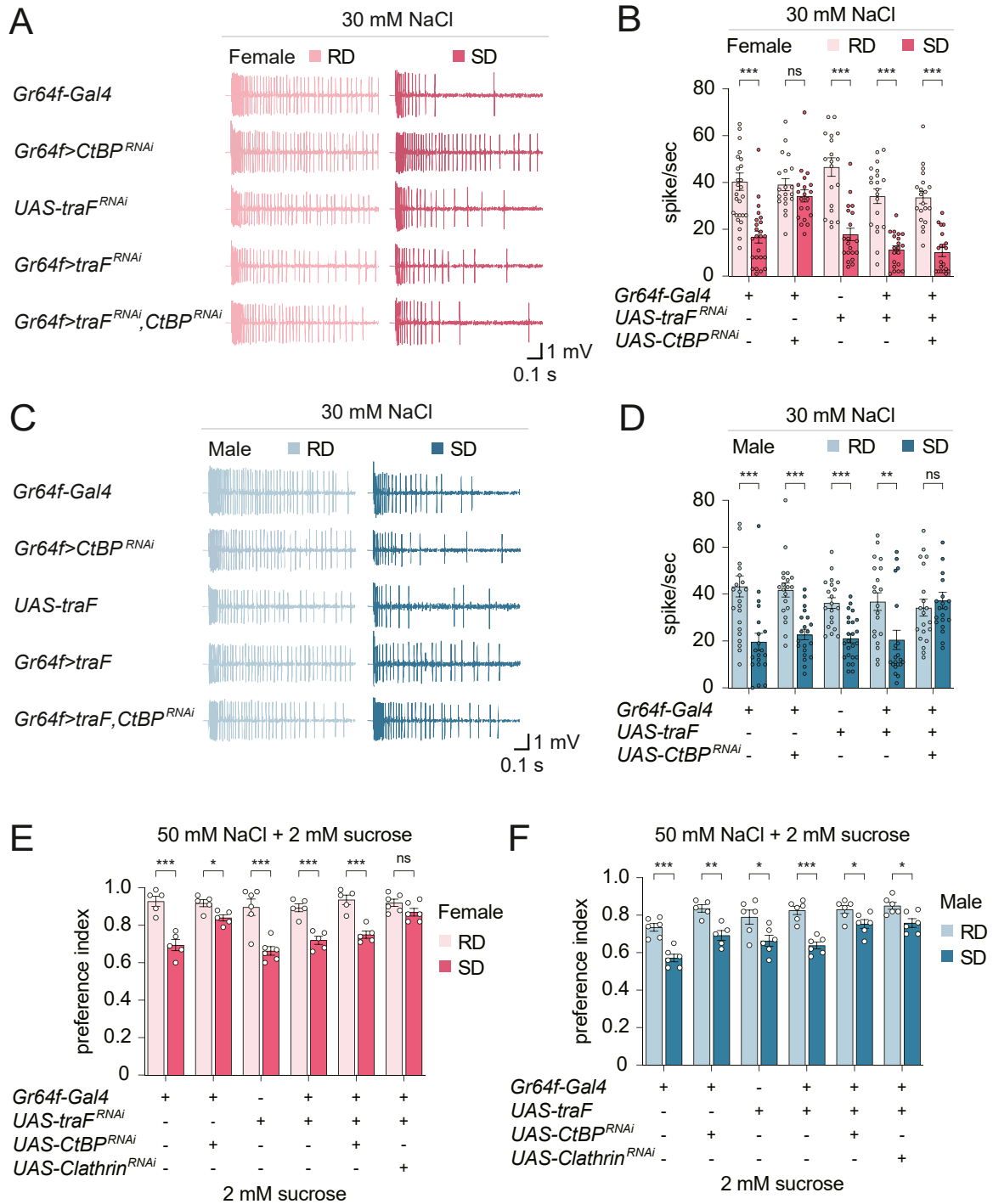


Fig. 4. Sexual identity regulates endocytic pathways in sweet-sensing gustatory neurons. (A) Representative action potential traces recorded from L-type sensilla in females with masculinized sweet GRNs in response to 30 mM NaCl after feeding on RD or SD for 2 days. (B) Quantification of the mean number of action potentials recorded from L-type sensilla in females with masculinized sweet GRNs in response to 30 mM NaCl after feeding on RD or SD for 2 days. $n = 20-28$. (C) Representative action potential traces recorded from L-type sensilla in males with feminized sweet GRNs in response to 30 mM NaCl after feeding on RD or SD for 2 days. (D) Quantification of the mean number of action potentials recorded from L-type sensilla in males with feminized sweet GRNs in response to 30 mM NaCl after feeding on RD or SD for 2 days. $n = 20-24$. (E) Salt preference in females with masculinized sweet GRNs after feeding on RD or SD for 6 hours. $n = 5-6$. (F) Salt preference in males with feminized sweet GRNs after feeding on RD or SD for 6 hours. $n = 5-6$. All data are presented as means $\pm$ SEM, *** $P < .001$, ** $P < .01$, and * $P < .05$ for all panels unless otherwise indicated.

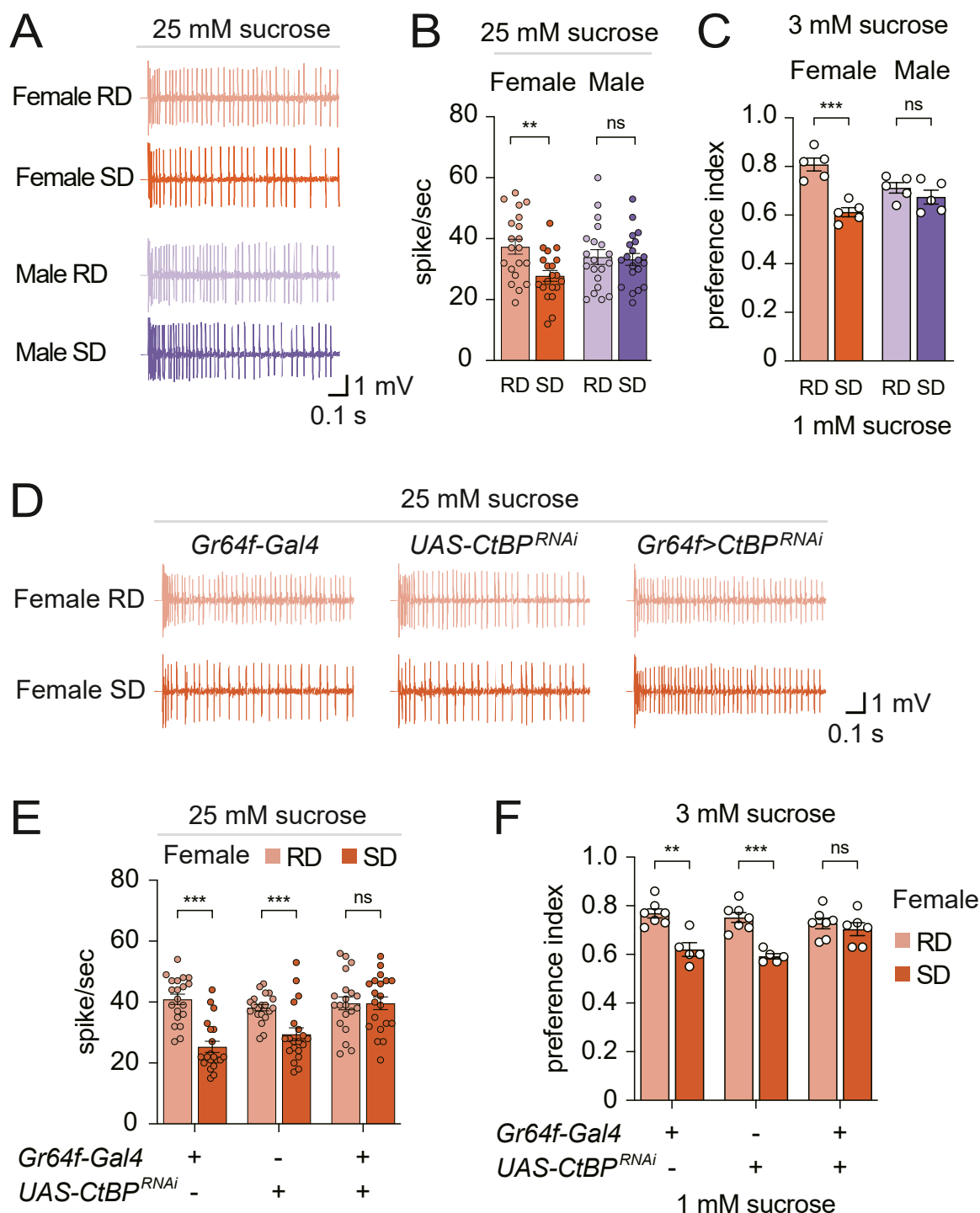


Fig. 5. Sweet desensitization in females after exposure to salt diet. (A) Representative action potential traces recorded from L-type sensilla of female and male flies in response to 25 mM sucrose after feeding on RD or SD for 6 hours. (B) Quantification of the mean number of action potentials recorded from L-type sensilla in response to 25 mM sucrose in female and male flies fed RD or SD for 6 hours. $n = 20$. (C) Sugar preference in female and male flies fed RD or SD for 6 hours. Flies were given a choice between 1 mM sucrose in 1% agarose or 3 mM sucrose in 1% agarose. $n = 5$. (D) Representative action potential traces recorded from L-type sensilla in female flies with sweet GRN-specific knockdown of macropinocytosis pathway components in response to 25 mM sucrose after feeding on RD or SD for 6 hours. (E) Quantification of the mean number of action potentials recorded from L-type sensilla in female flies with sweet GRN-specific knockdown of macropinocytosis pathway components in response to 25 mM sucrose after feeding on RD or SD for 6 hours. $n = 20$. (F) Sugar preference in female flies with sweet GRN-specific knockdown of macropinocytosis pathway components after feeding on RD or SD for 6 hours. Flies were given a choice between 1 mM sucrose in 1% agarose and 3 mM sucrose in 1% agarose. All data are presented as means $\pm$ SEM, *** $P < .001$, ** $P < .01$, and * $P < .05$ for all panels unless otherwise indicated.

Clathrin-mediated endocytosis is a widely conserved mechanism for receptor desensitization. In females, however, salt exposure induces both clathrin-dependent and CtBP-mediated internalization, the latter likely contributing to heterologous internalization of sweet taste receptors. Although the functional significance of this process remains unclear, it is reminiscent of EGFR signaling, where clathrin-mediated endocytosis promotes receptor recycling, while macropinocytosis facilitates degradation (Sigismund et al., 2008). This dual pathway may allow females to fine-tune receptor abundance and responsiveness according to physiological conditions.

Future studies could investigate whether the low-salt receptor complex—composed of Ir25a, Ir56b, and Ir76b (Asefa et al., 2024; Dweck et al., 2022; Zhang et al., 2013)—undergoes internalization in response to salt exposure, and whether this process differs between sexes.

In conclusion, our study reveals a novel form of sensory plasticity in which dietary salt modulates taste sensitivity via receptor internalization in a sex- and taste modality-specific manner. Understanding these adaptive responses provides key insights into how sensory systems dynamically respond to dietary and environmental challenges.

AUTHOR CONTRIBUTIONS

Seok Jun Moon: Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Chul Hoon Kim:** Writing – original draft, Supervision. **Jeong Taeg Seo:** Supervision, Conceptualization. **Linni Jin:** Software, Methodology, Investigation, Data curation.

DECLARATION OF COMPETING INTERESTS

The authors declare the following financial interests/personal relationships that may be considered as potential competing interests: Seok Jun Moon reports financial support was provided by National Research Foundation of Korea. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGMENTS

We thank the Bloomington Stock Center, Vienna *Drosophila* RNAi Center, and *Drosophila* Genetic Resource Center for providing fly stocks. We also thank the Korea Food Research Institute for sodium concentration measurements. This work was supported by National Research Foundation of Korea (NRF) Grants funded by the Korean Government (RS-2024-00406281 and 2022M3E5E8017946 to S.J.M.).

APPENDIX A. SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: [doi:10.1016/j.mocell.2025.100242](https://doi.org/10.1016/j.mocell.2025.100242).

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Received March 26, 2025

Revised May 30, 2025

Accepted June 12, 2025

Available online 18 June 2025.

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