Salt exposure modulates salt sensing in *Drosophila melanogaster*

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in *Drosophila melanogaster*

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마지막으로 어떤 일이 있어도 항상 저를 지지해주고 든든한 버팀목이 되어 주신 사랑하는 부모님께 감사드립니다. 도움을 주신 모든 분들께 진심으로 감사드리고 항상 건강하고 행복하시길 바랍니다.

2025 년 6 월

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ABSTRACT

Salt exposure modulates salt sensing in *Drosophila melanogaster*

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(Directed by Professor Moon, Seok Jun)

Salt is a vital nutrient with a key role in fluid balance and cellular function. In animals, gustatory sensation and internal physiological state are critical for the regulation of salt-related feeding behavior. Activation of salt-sensing cells in response to low salt concentrations is typically perceived as appetitive, whereas higher salt concentrations stimulate high-salt-sensing cells, eliciting an aversive response. Disturbances in the sodium balance, often resulting from dietary imbalances, are typically corrected through neural circuits and hormonally regulated feeding behaviors that maintain the sodium intake.

While much is known about the taste-independent mechanisms of sodium intake, the effects of chronic dietary salt on taste perception remain underexplored. Questions persist regarding whether chronic salt exposure influences peripheral taste responses, including

those to salt and potential sweetness. It remains unclear whether these alterations are driven by direct effects on taste cells or are contingent on changes in the internal physiological state. Furthermore, the molecular systems that govern taste sensitivity remain poorly understood.

To investigate how dietary salt influences salt perception, I used *Drosophila melanogaster*, a model organism with a well-characterized peripheral taste system. My findings revealed that a chronic salt diet diminishes salt preference without altering the internal state. This effect is associated with loss of electrophysiological responses in low-salt-sensing neurons, likely through internalization in turn impacting both perception and feeding behavior. Notably, the desensitization mechanism exhibits sexual dimorphism: while both sexes induce desensitization through clathrin-mediated endocytosis, macropinocytosis modulates salt perception only in females. Importantly, this female-specific induction of macropinocytosis is critical in the salt diet-induced desensitization of sweet taste perception. Furthermore, Ca2+/calmodulin-dependent protein kinase II mediates salt desensitization, potentially by regulating clathrin-mediated endocytosis.

These findings shed light on the regulatory mechanisms of salt perception, revealing how dietary factors shape sensory responses and potentially informing strategies to manage salt intake and mitigate related health risks.

Key word: Salt, diet, *Drosophila,* desensitization, endocytosis, sexual dimorphism.

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Introduction

1.1 The importance of salt

Taste plays a critical role in nutrient selection and consumption in humans, rats, and flies. Sodium chloride (NaCl), commonly referred to as salt, constitutes one of the five primary taste modalities, along with sweet, umami, bitter, and sour. Unlike other tastes, perceived by activation of specific taste-sensing cells that result in either attraction or aversion, salt preference largely depends on its concentration. Salt is considered attractive at concentrations below 100 mM, while higher concentrations, such as 500 mM NaCl, are typically avoided [1]. Salt sensation and consumption are integral to maintaining bodily homeostasis and regulating essential physiological processes [2]. Sodium, a key electrolyte, acts with others like potassium to regulate critical functions, particularly those related to

the heart, muscles, and nervous system [3]. For instance, sodium is integral to muscle function by facilitating the generation of action potentials that enable muscle contraction and relaxation, while also supporting nerve activity by establishing the electrical gradients essential for nerve impulse transmission. Due to its essential role in cellular and organ function, appropriate salt intake is vital for maintaining overall health.

1.2 Disruption of salt homeostasis causes adverse consequences

While salt is essential, excessive consumption can lead to serious health issues in mammals. Mice are typically fed a standard laboratory diet containing 0.2-0.3% NaCl (34-51 mM), while excessive salt intake usually results from a high-salt diet containing an additional 4-8% NaCl [4][5]. Elevated circulating sodium levels lead to water retention, increased blood volume, and consequently sustained high blood pressure (hypertension), which over time may damage blood vessels and the heart [6][7][8]. Chronic hypertension is associated with an elevated risk of cardiovascular complications, including heart failure, coronary artery disease, myocardial infarction, and stroke [9]. Additionally, excessive sodium intake places strain on the kidneys, which are responsible for regulating the sodium balance. Excess sodium overwhelms the kidneys decreasing its excretion potentially resulting in kidney stones and other renal complications [10]. In addition, a high-salt diet may contribute to gastrointestinal issues, including stomach ulcers, gastritis, and, in severe cases, gastric cancer [11]. Moreover, accumulating evidence suggests that a high-salt diet may adversely impact brain health, contributing to an increased risk of cognitive decline,

memory impairment, and neurodegenerative disorders such as Alzheimer's disease, particularly among older adults [12]. Therefore, managing salt consumption is critical for maintaining both short and long-term health.

In *Drosophila melanogaster*, laboratory diets typically contain salt in the millimolar range. High salt intake experiments are often conducted using food with 1% NaCl or more. A high-salt diet can accelerate muscle aging, manifesting as an age-related decline in climbing ability and increased oxidative stress [13]. In addition, such diets induce premature heart aging, characterized by an increased systolic interval, longer heart periods, and arrhythmias. In young flies, a high-salt diet is associated with poor sleep quality, leading to fragmented sleep resembling that of naturally aging individuals; an effect dependent on the circadian rhythm and dopaminergic system [14]. Furthermore, a high-salt diet induces systematic changes in the composition and structure of bacterial and yeast populations within the microbiome [15]. Bacterial symbionts play a crucial role in motor function and the efficient utilization of food resources. Yeasts, another essential component of the microbiome in *D. melanogaster*, are integral to the organism's life cycle. Variations in yeast populations can have sex-specific effects on key life history traits, such as longevity, fecundity, and mating frequency, influencing both female and male flies.

However, even modest increases in salt levels can lead to excessive salt consumption, although this effect may take longer to manifest. This gradual process seems to align more closely with actual human eating habits and may involve distinct regulatory mechanisms for maintaining salt homeostasis, different from those triggered by a high-salt diet. Despite

the potential significance of these mechanisms, research on this phenomenon remains scarce. I hypothesize that, with prolonged exposure to a salt-enriched diet, the sensory system, particularly taste, as the first line of defense in food intake, would be the first to respond, attempting to modulate salt consumption before homeostasis is disrupted. Therefore, identifying strategies to modulate salt taste perception prior to the onset of homeostatic imbalance may offer promising avenues for preventing excessive salt consumption.

1.3 Salt sensing and regulation in mammals

Salt is considered one of the most complex and intriguing among the basic tastes. Both gustatory sensation and the internal physiological state are critical for regulating salt intake [16][17][18]. In mammals, salt sensation is detected primarily through taste receptors on the tongue. Salt detection begins in the taste buds, which innervate to the chorda tympani nerve. This nerve innervates the fungiform papillae located on the anterior portion of the tongue, a key site for NaCl detection [19]. Each taste bud is composed of specialized taste receptor cells that interface with the oral cavity via taste pores. Salt perception is mediated by two distinct sensory pathways: the amiloride-sensitive pathway, which responds specifically to sodium ions and is associated with salt attraction, and the amiloride-insensitive pathway, involved in high-salt aversion and activated by higher concentrations of salts [20][21]. These pathways work together to create a biphasic response to salt, from attraction at low concentrations to avoidance at high concentrations.

Moreover, the epithelial sodium channel, which consists of α, β, and γ subunits, serves as a crucial low-salt receptor [22]. However, the specific receptors, cell types, and transduction mechanisms responsible for high-salt detection remain incompletely understood and are the subject of ongoing investigation.

Beyond gustatory perception, animals regulate sodium intake based on their physiological needs through central neural circuits and hormonal mechanisms. The subfornical organ (SFO) and the nucleus of the solitary tract (NTS) in the brain detect changes in sodium levels in the cerebrospinal fluid and initiate behaviors to regulate sodium intake [23][24]. An alternative neural mechanism involves serotonin 2C receptor-expressing neurons in the lateral parabrachial nucleus (LPBN), which suppress sodium appetite via projections to the central amygdala. In conditions of disrupted sodium homeostasis, hormones such as angiotensin II (ANG II) activate ANG II type 1 receptor-expressing neurons in the SFO, which in turn project to the dorsal bed nucleus of the stria terminalis (BNST) to promote salt intake. Additionally, ANG II binds to receptors in the zona glomerulosa of the adrenal cortex, stimulating the release of aldosterone. Aldosterone subsequently activates aldosterone-sensitive neurons expressing 11β-hydroxysteroid dehydrogenase type 2 in the NTS, which is sufficient to drive the consumption of sodium-containing solutions in mice. In addition, the gastrointestinal peptide secretin, derived from the colon and involved in the gut-brain axis, is involved in regulating salt intake [25]. These well-established, taste-independent mechanisms are crucial for maintaining the sodium balance and regulating intake behavior.

1.4 Salt sensing and regulation in *Drosophila melanogaster*

In contrast to mammals, the functional characterization of peripheral taste neurons in *Drosophila melanogaster* is well understood, as it exhibits similar patterns of salt attraction at low concentrations and aversion at high concentrations. This research has provided valuable insights into how the gustatory system regulates feeding behavior, laying a strong foundation for further research into the underlying mechanisms.

In *Drosophila,* gustatory receptor neurons (GRNs) in the peripheral nervous system are essential for direct taste detection. These neurons are housed within specialized structures known as gustatory sensilla, which are distributed across multiple body regions, including the labellum, legs, pharynx, wings, and ovipositor (Fig. 1A) [26]. The labellum, located at the tip of the proboscis, serves as the primary taste organ and contains 31 sensilla on each side [27][28]. Each sensillum houses several chemosensory neurons, a mechanosensory neuron, and supporting cells such as thecogen, tormogen, and trichogen [29]. These sensilla are innervated by multiple GRNs, each tuned to detect specific taste modalities. Sensilla on the labellum are categorized into three morphological types: long (L), short (S), and intermediate (I), each housing different numbers of GRNs (Fig. 1B). L- and S-type sensilla each house four GRNs, whereas I-type sensilla contain only two. GRNs classes are classified based on distinct electrophysiological properties, calcium imaging responses, receptor expression profiles, and behavioral assays. Recent research suggests that five molecularly defined GRN classes account for the majority of GRNs in the labellum. These include sweet *Gr64f*-positive GRNs, which respond to sugars and low salt are present

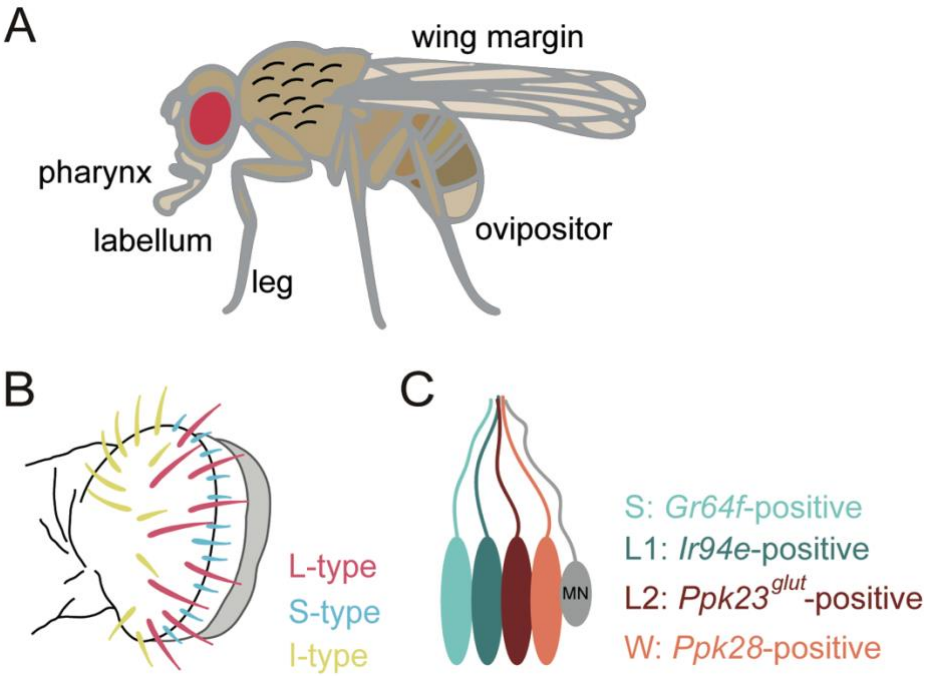

Figure 1. Gustatory organs in *Drosophila melanogaster*

(A) Flies have a broad distribution of gustatory receptor neurons (GRNs) across their bodies, including on peripheral organs such as the labellum, pharynx, legs, wings, and female ovipositor.

(B) Based on their length, labellum sensilla are classified into long (L-type), intermediate (I-type), and short (S-type) types.

(C) Representative single L-type sensillum containing four kinds of chemosensory GRNs: the S cell, responsive to sugars and low salt; the L1 cell, exhibiting moderate sensitivity to low salt; the L2 cell, which detects high salt and bitter compounds; and the W cell, responsive to water, as well as a mechanosensory neuron (MN).

across all sensillum types [30][31]; bitter *Gr66a*-positive GRNs, activated by bitter compounds and located in S- and I-type sensilla [32][33]; water GRNs, which express the epithelial sodium channel homolog pickpocket28 (*Ppk28*) and are sensitive to low osmolality, found in L- and S-type sensilla [34]; pickpocket23 (*Ppk23*) glutamate receptor-expressing GRNs, which respond to high salt concentrations and are located in L- and S-type sensilla [35]; and ionotropic receptor 94e (*Ir94e*) GRNs, expressing *IR94e* and involved in low-salt detection, feeding aversion, and oviposition behaviors, primarily found in L-type sensilla (Fig. 1C) [36].

While these GRN classes are primarily characterized by their sensitivity to specific taste modalities, all five exhibit some degree of salt sensitivity. Sweet GRNs display notable responses to low concentrations of sodium, whereas *Ir94e* GRNs are mildly activated by low salt [37]. Bitter and *Ppk23*-glutamate receptor GRNs are activated by high concentrations of both monovalent and divalent salts, and water GRNs are inhibited by salt and other osmolytes. Notably, salt deprivation in *Drosophila* reduces their avoidance of high concentrations of NaCl, suggesting that this environmental change may either enhance the attractiveness of sodium or suppress high-salt aversion. In addition, although bitter GRNs are essential for salt avoidance in both salt-deprived and salt-fed flies, *Ppk23*-glutamate receptor GRNs specifically contribute to salt avoidance in salt-fed flies. These findings indicate that, in contrast to most other taste modalities, salt perception in *Drosophila* is encoded through a complex combinatorial code.

In addition to the labellum, the tarsal segments of the legs and pharyngeal taste neurons

were also well-studied [38]. The tarsal taste hairs located on the forelegs display sexual dimorphism, with males exhibiting a greater number of these sensilla compared to females. These male-specific sensilla are integral to the detection of pheromones involved in courtship behavior. The pharyngeal sensory organ in *Drosophila* plays a critical role in feeding behavior by linking external taste detection with internal nutrient-sensing mechanisms [39][40]. It comprises three anatomically and functionally distinct pharyngeal sensory structures: the labral sense organ, the ventral cibarial sense organ, and the dorsal cibarial sense organ. Both the legs and pharyngeal taste organs in *Drosophila* contain salt-sensitive taste cells. These organs detect tastants, which activate chemosensory neurons that send signals to the subesophageal zone of the brain. This neural processing ultimately influences the decision to continue or stop feeding.

In addition, taste receptors specifically salt receptors, which have not been identified in mammals are well understood in *Drosophila*. The ionotropic receptor (IR) family plays a central role in salt perception in the labellum [41][42]. This receptor family comprises a small set of conserved, broadly expressed coreceptors and more diverse group of tuning subunits that confer specific ligand sensitivities. IR channels are proposed to operate as heteromeric complexes formed by the assembly of coreceptors with tuning subunits. For instance, *Ir25a* and *Ir76b* act as co-receptors for salt detection, *Ir56b* contributes to low-salt sensitivity, and *Ir7c* specifically mediates high-salt responses [43][44][45][46]. Furthermore, three tarsal sensilla: f4s, f5s and f5b respond to low concentrations of NaCl in sweet GRNs, with *Ir56b* also playing a critical role in the response of tarsal sensilla to

NaCl. Additionally, internal taste organs, particularly in the pharynx, are involved in salt sensing, with *Ir60b* GRNs being essential for detecting high sodium levels and facilitating salt rejection [47]. Alongside *Ir76b*, the gustatory receptors *Gr2a* and *Gr23a*, expressed in the pharyngeal GRNs of the labral sense organ, also contribute to salt perception.

The gustatory response to salt displays sexual dimorphism and is modulated by mating status, with mated females exhibiting enhanced proboscis extension reflexes upon stimulation of either the labellum or tarsi compared to virgin females and males [48]. It is dependent in a sex peptide-dependent manner, indicating a link between reproductive status and salt sensitivity. Salt sensitivity is also heightened under salt deprivation, and a taste-independent internal sodium-sensing system involving anterior enteric neurons has a role in regulating salt perception [49]. These findings illustrate the complexity of salt sensing in *Drosophila*, highlighting the intricate interaction between peripheral taste receptors and internal systems in modulating salt preference and sensitivity.

1.5 Dietary habits influence taste preferences

Taste preferences are dynamic and can be influenced by environmental factors such as dietary habits [50]. For instance, while most organisms show aversion to bitter-tasting substances, prolonged exposure to bitter compounds can lead to their increased acceptance, a phenomenon known as taste adaptation [51]. In *Drosophila*, this adaptive response is exemplified by the downregulation of the transient receptor potential-like (TRPL) cation channel in GRNs after chronic exposure to camphor, a substance that initially elicits an

aversive response. The reduction in TRPL protein levels diminishes camphor rejection, thereby increasing the fruit fly's tolerance to its taste over time.

Similarly, exposure to a high-sugar diet can impair the perception of sweetness in *Drosophila*, reducing the responsiveness of sweet-sensing neurons to sugar. High sugar intake causes a cell-autonomous effect in sweet-sensing neurons through the action of O-linked N-acetylglucosamine transferase, a sugar sensor [52]. This leads to decreased responsiveness to sweet stimuli, contributing to increased food consumption and potential obesity. A high-sugar diet also leads to a global increase in H3K27me3 levels in progeny, and behavioral changes can be transmitted to offspring through the maternal germline [53]. Similarly, rats fed a high sucrose diet significantly reduced chorda tympani responses to sucrose, alongside a reduction in the number of PLCβ2+ taste bud cells in fungiform papillae [54]. This form of sensory adaptation highlights the plasticity of the gustatory system in response to changing dietary conditions, which enables quicker adaptation to potential threats or nutrient availability.

Replacing sucrose with sorbitol, a tasteless sugar with similar caloric content, induces sweet taste sensitization in *Drosophila* [55]. An effect mediated by a dopamine/cAMP/ cAMP response element-binding protein signaling pathway in sweet taste neurons, which, while not directly involved in acute taste transduction, regulates taste sensitivity in response to nutrient and sensory conditions. The pathway's activation converges on PGC1α, a critical regulator of metabolic health and lifespan, necessary and sufficient to enhance sucrose taste sensitivity in response to an unsweetened diet. These findings demonstrate that continuous

exposure to foods lacking a specific taste can increase sensitivity to that taste, indicating the dynamic and adaptive nature of the gustatory system.

However, the dietary conditions used in these studies lead to both continuous exposure of taste cells to stimuli and physiological changes in the body. Consequently, it remains unclear whether changes in taste perception are primarily driven by prolonged exposure of taste-sensing cells to dietary factors or by physiological alterations due to these factors. Further, how the elements regulate the observed changes is unclear. Therefore, clarifying the relative contributions of these two factors would be needed.

1.6 Possibility of a salt diet inducing changes in peripheral taste responses

There is growing evidence that changes in a need-based sodium appetite and other state-dependent factors affect salt behavior. For example, studies in mice have shown that prolonged sodium deprivation leads to an increase in the amiloride-sensitive Na^+ current in the taste cells of the fungiform papillae [56]. Sodium depletion enhances both appetite and tolerance to high concentrations of salt [57]. In addition, human studies have examined the effects of increased dietary salt on taste sensitivity. One group added crystalline salt to their food daily for four weeks, another took salt tablets without altering taste perception, and the third was a control group with a placebo. The group adding salt to their food developed a preference for higher concentrations of salt, while the tablet group showed no change compared to the control group [58]. This suggests that continuous salt taste stimulation from dietary salt supplementation alters salt preference, with an increase in salt taste

stimulation likely playing a crucial role in this adaptation. Furthermore, a separate study found that keeping gustatory stimulation from a few seconds to minutes produced reversible reductions in the chorda tympani response to a subsequent stimulus [59]. These responses exhibited a gradual recovery following a distilled water rinse. The underlying adaptive mechanism likely involves modifications at the level of the taste receptor cell, potentially through alterations in specific receptor sites or changes in the excitability of the receptor cell membrane. Such adaptation may be mediated by conformational changes in the receptor proteins, resulting in diminished responsiveness despite sustained stimulus presence.

Nonetheless, the precise regulatory mechanisms underlying these effects remain largely unexplored. For instance, it is still unclear how a salt-rich diet alters peripheral taste responses to salt. Furthermore, whether these changes result from direct effects on taste cells or changes in the internal physiological state is poorly understood. There is also limited understanding of how salt intake affects the responses of peripheral GRNs to other tastes, such as sweetness. Additionally, the mechanisms underlying alterations in taste perception due to dietary salt intake remain poorly understood. These gaps in knowledge underscore the need for further research on the effects of dietary salt on taste perception and neuronal plasticity.

Building on existing literature, I hypothesize that a continuous salt diet can induce salt taste adaptation in *Drosophila*, mediated by salt-responsive taste cells, without disrupting homeostasis. This adaptation likely causes a shift in salt preference. In this study, I aim to

investigate the effects of chronic exposure to a sodium-enriched diet on taste perception and feeding behavior in *Drosophila melanogaster*. In addition, I will explore the mechanisms that contribute to these behavioral changes, addressing the question of how dietary salt influences gustatory function.

1.7 Various mechanisms of desensitization

Desensitization, or adaptation, occurs when prolonged exposure to a stimulus leads to a reduced response from taste receptor cells. Desensitization of taste receptors may involve conformational alterations of the receptor protein, leading to a diminished response despite continuous exposure to a constant stimulus concentration. Such desensitization to signaling molecules can occur through multiple molecular mechanisms.

One common desensitization mechanism involves the inactivation of the receptors themselves [60]. For example, when signaling molecules bind to cell-surface receptors, it can trigger receptor endocytosis, leading to the sequestration of receptors into endosomes. In some cases, this endocytosis may progress to receptor degradation in lysosomes, a process known as receptor downregulation. Alternatively, receptors may undergo post-translational modifications, such as phosphorylation or methylation, shortly after activation, leading to their desensitization. Another mechanism of desensitization occurs at intracellular signaling sites, where modifications to signaling proteins involved in the transduction process or the production of inhibitory proteins can block further signal propagation.

Considering these mechanisms, this study used electrophysiological recordings and feeding behavior assays to clarify whether a salt diet induces taste desensitization in *Drosophila* [61][62]. Furthermore, I aimed to investigate the underlying regulatory mechanisms in greater detail, providing new insights into how chronic dietary exposure to salt may affect gustatory responses.

2. MATERIALS AND METHODS

2.1 Fly stocks

All *Drosophila melanogaster* strains were maintained at 25 °C with 60% relative humidity under a 12-hour light/12-hour dark cycle. The following transgenic lines were acquired from the Bloomington Drosophila Stock Center: *UAS-Shibire^{ts}* (BL#44222), *UAS-CtBP^{RNAi}* (BL#32889), *UAS-TraF^{RNAi}* (BL#44109), *UAS-TraF* (BL#4590), *UAS-Kir2.1* (BL#6595), *Tub-GAL80^{ts}* (BL#7108), and *Ir94e-GAL4* (BL#81246). RNA interference (RNAi) lines were sourced from the Vienna Drosophila Resource Center (VDRC), including *UAS-Shibire^{RNAi}* (VDRC#105971, VDRC#3799), *UAS-Chc^{RNAi}* (VDRC#103383, VDRC#23666), *UAS-Rho1^{RNAi}* (VDRC#109420, VDRC#12734), *UAS-Flotillin1^{RNAi}* (VDRC#103938, VDRC#42130), *UAS-Graf1^{RNAi}* (VDRC#110812, VDRC#42165), *UAS-Arf6^{RNAi}* (VDRC#24224, VDRC#100726), *UAS-CAM^{RNAi}* (VDRC#100265) and *UAS-CAMKII^{RNAi}* (VDRC#100265). *UAS-Dicer2* (VDRC#60009) was co-expressed to enhance RNAi efficiency. Drosophila Genetic Resource Center provided *MN11+12-GAL4* (NP1363). Additional GAL4 driver lines were kindly provided by other laboratories: *Ppk23-GAL4* and *Ppk28-GAL4* from Kristin Scott, *Gr64f-GAL4* from John Carlson, and *INSO-GAL4 (R10H08AD; R59H02DBD)* from Greg S. B. Suh. *Ir56b^{1}* mutant line from Jae Young Kwon and *Gr5a^{LEXA}* mutant line from Hubert Amrein. The *Gr64af* allele was previously characterized (Kim et al., 2018). To reduce variability due to genetic background, all mutant lines were outcrossed to *w^{1118}* for five successive generations.

2.2 Dietary manipulations

The standard cornmeal-based medium (referred to as the regular diet) consisted of 7% cornmeal (Seunglim Food, Seoul, Korea), 10% dextrose (3020-4400, Daejung, Seoul, Korea), 5% yeast (SAF Instant Yeast), 1% agar (DFA-30301, Hansol Tech, Seoul, Korea), 0.6% propionic acid (P1386, Sigma-Aldrich, St. Louis, MO, USA), and 0.2% methyl 4-hydroxybenzoate (H5501, Sigma-Aldrich).

To generate the salt diet, 10 mM NaCl (7647-14-5, Duchefa Biochemie, Haarlem, Netherlands) was added to the regular diet. The high-sugar diet was prepared by supplementing the regular diet with 15% sucrose (S9378, Sigma-Aldrich).

2.3 Measuring sodium concentrations

A 1 g sample of fly food was weighed and placed into a Teflon digestion vessel compatible with microwave-assisted digestion. The sample was mixed with 8 mL concentrated nitric acid and 2 mL electronic-grade hydrogen peroxide (Dongwoo FineChem, Korea), then sealed and digested using microwave irradiation. After cooling, the digest was diluted to 50 g with distilled water, homogenized, and filtered to prepare the test solution. Sodium concentrations were quantified using inductively coupled plasma atomic emission spectrometry (ICP-AES) on an Avio 500 spectrometer (PerkinElmer, Springfield, IL, USA) under the following parameters: radiofrequency power of 1500 W, argon plasma, peristaltic pump speed of 2.50 rpm, nebulizer flow rate of 0.7 L/min, and detection wavelength set at 589.592 nm for sodium. Sodium quantification analyses were

performed by the Korea Food Research Institute.

2.4 Two-way choice behavior test

Flies were subjected to a two-choice feeding assay for 90 minutes at room temperature in complete darkness, with simultaneous access to two distinct food sources. To differentiate ingestion, each food was supplemented with a non-toxic dye: either 0.125 mg/mL Brilliant Blue FCF (027-12842, Wako Pure Chemical Industry, Ltd., Osaka, Japan) or 0.2 mg/mL sulforhodamine B (S9012, Sigma-Aldrich). Following the feeding period, flies were immediately frozen, and abdominal coloration was evaluated under a stereomicroscope. Feeding preference was quantified using a preference index (PI), calculated as follows:

PI = (Number of red or blue abdomens + ½ × Number of purple abdomens) / Total number of fed flies.

Temporal neuronal inhibition was achieved using *Tub-GAL80^{ts}*; *UAS-Kir2.1* and *UAS-Shibire^{ts}* fly strains. Flies expressing *Tub-GAL80^{ts}* and *UAS-Kir2.1*, an inward rectifying potassium channel, were reared and maintained at 21 °C for development and recovery, followed by a 24-hour incubation at 31 °C to inactivate GAL80 and permit Kir2.1-mediated neuronal silencing. For temperature-sensitive *UAS-Shibire^{ts}* experiments, flies were similarly reared at 21 °C and subsequently exposed to either 21 °C or 31 °C for 6 hours while presented with either a regular or salt diet. Following this exposure, a two-choice feeding assay was conducted at 21 °C for 90 minutes. Control groups for both *Tub-GAL80^{ts}*;

UAS-Kir2.1 and *UAS-Shibire^{ts}* strains were maintained at 21 °C throughout, and choice assays were likewise performed at this temperature.

2.5 Measurement of feeding amount

To assess food intake, 10 female and 10 male flies expressing Shibire^{ts} in motor neuron (MN)11 and 12, or genetic controls, were aged for 2 days and then housed by sex. After a 24-hour acclimation, each group received two 10 μL capillaries filled with 100 mM sucrose. Feeding was measured over 6 hours at either 21 °C or 31 °C by calculating the volume change. To test reversibility, the same flies were returned to 21 °C for 1.5 hours and re-tested under identical conditions.

2.6 Tip recording

Flies were briefly anesthetized on ice prior to electrophysiological recordings and transferred to fresh vials. A glass capillary filled with Ringer's solution was inserted through the thorax and positioned at the base of the labellum to function as a reference electrode, grounded to the recording system. Tastant solutions were prepared in 30 mM tricholine citrate (T0252, Sigma-Aldrich) to enhance conductivity and were delivered via a recording electrode with a tip diameter of 10–20 μm. The recording electrode was connected to the TastePROBE system (Syntech, Hilversum, The Netherlands), and extracellular signals were acquired using a Syntech acquisition controller interfaced with a computer. Signals were amplified 10-fold, band-pass filtered between 100 and 3000 Hz,

and digitized at a sampling rate of 12 kHz. Spike detection and analysis of neuronal firing rates were conducted using Autospike 3.1 software (Syntech).

For localized stimulation of taste sensilla, flies prepared for tip recordings were first stimulated with 30 mM NaCl to establish baseline action potential responses in the L4 sensilla. The same sensilla were then intermittently stimulated with either distilled water or 10 mM NaCl at 2–5-minute intervals over a 3-hour period. Following the repeated stimulation protocol, neuronal responses to 30 mM NaCl were reassessed to evaluate changes in sensory activity.

2.7 Statistical analyses

All data are presented as mean ± standard error of the mean (SEM). Error bars represent SEM. Data distribution was evaluated using the Shapiro–Wilk test to assess normality. For comparisons between two groups, unpaired or paired Student's t-tests were performed when data followed a normal distribution; otherwise, the Mann–Whitney U test was used. For multiple group comparisons, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was conducted for normally distributed data. When normality was not met, Dunn's multiple comparison test was applied. All statistical analyses were performed using GraphPad Prism software (version 10; GraphPad Software, San Diego, CA, USA). Statistical significance is denoted by asterisks ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$.

Table 1. Complete genotypes of flies in the indicated figures

Figure #	Genotype
Figure 2	*w*^{1118}
Figure 3	*w*^{1118}
Figure 4A	*UAS-Kir2.1/+*
	Gr64f-Gal4/UAS-Kir2.1
	Ppk23-Gal4/UAS-Kir2.1
	Ppk28-Gal4/UAS-Kir2.1
	Ir94e-Gal4/UAS-Kir2.1
Figure 4B	*w*^{1118}
	Ir56b^{1}
	Gr5a^{LEXA};;*Gr64af*
Figure 5	*w*^{1118}
Figure 6A-B	*Gr64f-Gal4/+* ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Shibire^{RNAi}(#105971) ; *UAS-Dcr2/+*
	Gr64f-Gal4/+ ; *UAS-Dcr2/ UAS-Shibire*^{RNAi}(#3799)
	Gr64f-Gal4/UAS-Chc^{RNAi}(#103383) ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Chc^{RNAi}(#23666) ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Rho1^{RNAi}(#109420) ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Rho1^{RNAi}(#12734) ; *UAS-Dcr2/+*

	Gr64f-Gal4/UAS-Flotillin1^{RNAi}(#103938) ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Flotillin1^{RNAi}(#42130) ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Graf1^{RNAi}(#110812) ; *UAS-Dcr2/+*
	Gr64f-Gal4/+ ; *UAS-Dcr2/UAS-Graf1^{RNAi}*(#42165*)*
	Gr64f-Gal4/UAS-Arf6^{RNAi}(#24224) ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Arf6^{RNAi}(#100726) ; *UAS-Dcr2/+*
	Gr64f-Gal4/+ ; *UAS-CtBP^{RNAi}/+*
Figure 6C-D	*Gr64f-Gal4/+* ; *UAS-Dcr2/+*
	UAS-Chc^{RNAi}(#103383)*/+*
	Gr64f-Gal4/UAS-Chc^{RNAi}(#103383) ; *UAS-Dcr2/+*
	Gr64f-Gal4/+
	UAS-CtBP^{RNAi}/+
	Gr64f-Gal4/+ ; *UAS-CtBP^{RNAi}/+*
	UAS-Chc^{RNAi}(#103383)*/+* ; *UAS-CtBP^{RNAi}/+*
	Gr64f-Gal4/UAS-Chc^{RNAi}(#103383) ; *UAS-Dcr2/ UAS-CtBP^{RNAi}*
Figure 7A-B	*Gr64f-Gal4/+* ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Shibire^{RNAi}(#105971) ; *UAS-Dcr2/+*
	Gr64f-Gal4/+ ; *UAS-Dcr2/ Shi^{RNAi}*(#3799)
	Gr64f-Gal4/UAS-Chc^{RNAi}(#103383) ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Chc^{RNAi}(#23666) ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Rho1^{RNAi}(#109420) ; *UAS-Dcr2/+*

	Gr64f-Gal4/UAS-Rho1^{RNAi}(#12734) ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Flotillin1^{RNAi}(#103938) ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Flotillin1^{RNAi}(#42130) ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Graf1^{RNAi}(#110812) ; *UAS-Dcr2/+*
	Gr64f-Gal4/+ ; *UAS-Dcr2/UAS-Graf1^{RNAi}*(#42165*)*
	Gr64f-Gal4/UAS-Arf6^{RNAi}(#24224) ; *UAS-Dcr2/+*
	Gr64f-Gal4/UAS-Arf6^{RNAi}(#100726) ; *UAS-Dcr2/+*
	Gr64f-Gal4/+ ; *UAS-CtBP^{RNAi}/+*
Figure 7C-D	*Gr64f-Gal4/+* ; *UAS-Dcr2/+*
	UAS-Chc^{RNAi}(#103383)/+
	Gr64f-Gal4/UAS-Chc^{RNAi}(#103383) ; *UAS-Dcr2/+*
	Gr64f-Gal4/+
	UAS-CtBP^{RNAi}/+
	Gr64f-Gal4/+ ; *UAS-CtBP^{RNAi}/+*
Figure 8B-C	*Gr64f-Gal4/+*
	Gr64f-Gal4/+ ; *UAS-CtBP^{RNAi}/+*
	UAS-traF^{RNAi}/+
	UAS-traF^{RNAi} /+ ; *Gr64f-Gal4/+*
	UAS-traF^{RNAi} /+ ; *Gr64f-Gal4/UAS-CtBP^{RNAi}*
Figure 8D	*Gr64f-Gal4/+*
	Gr64f-Gal4/+ ; *UAS-CtBP^{RNAi}/+*

	UAS-traF^{RNAi}/+
	UAS-traF^{RNAi} /+ ; Gr64f-Gal4/+
	UAS-traF^{RNAi} /+ ; Gr64f-Gal4/UAS-CtBP^{RNAi}
	UAS-traF^{RNAi} /UAS-Chc^{RNAi}(#103383) ; *Gr64f-Gal4/UAS-Dcr2*
Figure 9A-B	*Gr64f-Gal4/+*
	Gr64f-Gal4/+ ; UAS-CtBP^{RNAi}/+
	UAS-traF/+
	UAS-traF/+ ; Gr64f-Gal4/+
	UAS-traF/+ ; Gr64f-Gal4/UAS-CtBP^{RNAi}
Figure 9C	*Gr64f-Gal4/+*
	Gr64f-Gal4/+ ; UAS-CtBP^{RNAi}/+
	UAS-traF/+
	UAS-traF/+ ; Gr64f-Gal4/+
	UAS-traF/+ ; Gr64f-Gal4/UAS-CtBP^{RNAi}
	UAS-traF /UAS-Chc^{RNAi}(#103383) ; *Gr64f-Gal4/UAS-Dcr2*
Figure 10A-D	*Gr64f-Gal4/+ ; UAS-Dcr2/+*
	UAS-CAMKII^{RNAi} /+
	Gr64f-Gal4/UAS-CAMKII^{RNAi} ; UAS-Dcr2/+
	UAS-CAM^{RNAi}/+
	Gr64f-Gal4/UAS-CAM^{RNAi} ; UAS-Dcr2/+
	Gr64f-Gal4/+ ; UAS-Dcr2/+

Figure 10E-F	*UAS-CAMKII^{RNAi}/+*
	Gr64f-Gal4/UAS-CAMKII^{RNAi} ; UAS-Dcr2/+
	UAS-CAM^{RNAi}/+
	Gr64f-Gal4/UAS-CAM^{RNAi} ; UAS-Dcr2/+
	UAS-CAMKII^{RNAi} /+ ; UAS-CtBP^{RNAi}/+
	Gr64f-Gal4/ UAS-CAMKII^{RNAi} ; UAS-Dcr2/ UAS-CtBP^{RNAi}
	UAS-CAM^{RNAi} /+ ; UAS-CtBP^{RNAi}/+
	Gr64f-Gal4/ UAS-CAM^{RNAi} ; UAS-Dcr2/ UAS-CtBP^{RNAi}
Figure 11A-D	*w^{1118}*
Figure 11E-F	*Gr64f-Gal4/+*
	UAS-CtBP^{RNAi}/+
	Gr64f-Gal4/+ ; UAS-CtBP^{RNAi}/+
Figure 12	*w^{1118}*
Figure 13	*w^{1118}*
Figure 14A	*R10H08-AD/+ ; R59H02-DBD/+*
	Tub-Gal80^{ts}/+ ; UAS-Kir2.1/+
	R10H08-AD/ Tub-Gal80^{ts} ; R59H02-DBD/UAS- Kir2.1
Figure 14B	*R10H08-AD/+ ; R59H02-DBD/+*
	UAS-Shibire^{ts}/+
	R10H08-AD/+ ; R59H02-DBD/UAS-Shibire^{ts}
	MN11+12 – Gal4/+

Figure 15	*UAS-Shibire^{ts}/+*
	MN11+12-Gal4/ UAS-Shibire^{ts}
Figure 16	*w^{1118}*

3. Results

3.1 Dietary salt reduces low-salt attraction

To assess whether daily salt intake alters salt preference, I first measured the sodium (Na^+) concentration in the standard *Drosophila* laboratory diet (hereafter referred to as the "regular diet"), which was approximately 3 mM NaCl. Flies maintained on regular diet showed a clear preference for low NaCl concentrations (≤100 mM), and aversion to higher concentrations (>200 mM). To examine the effects of increased dietary salt, I prepared a salt-enriched diet (hereafter referred to as the "salt diet") by adding 10 mM NaCl to the regular medium, resulting in a total NaCl concentration of 13 mM. After 6 hours of exposure to the salt diet, flies showed a significant reduction in their preference for low salt concentrations and enhanced aversion to high salt, compared to flies maintained on the regular diet (Fig. 2A). Notably, flies fed the regular diet exhibited peak attraction at 50 mM NaCl. This salt preference was sensitive to both exposure duration and dietary salt concentration, with a marked decrease in attraction evident after 6 hours on the salt diet in both sexes (Fig. 2B). When salt exposure time was fixed and dietary sodium levels further increased, salt preference was further suppressed (Fig. 2C).

To rule out potential interactions between NaCl and other dietary components, I repeated the behavioral assay using a defined agarose-based diet consisting of 5% sucrose and either 3 mM or 13 mM NaCl in 1% agarose. Flies fed the 13 mM NaCl agarose diet for 6 hours showed significantly reduced attraction to 50 mM NaCl compared to those fed the 3 mM NaCl agarose diet (Fig. 2D). These results indicate that the observed behavioral shift is

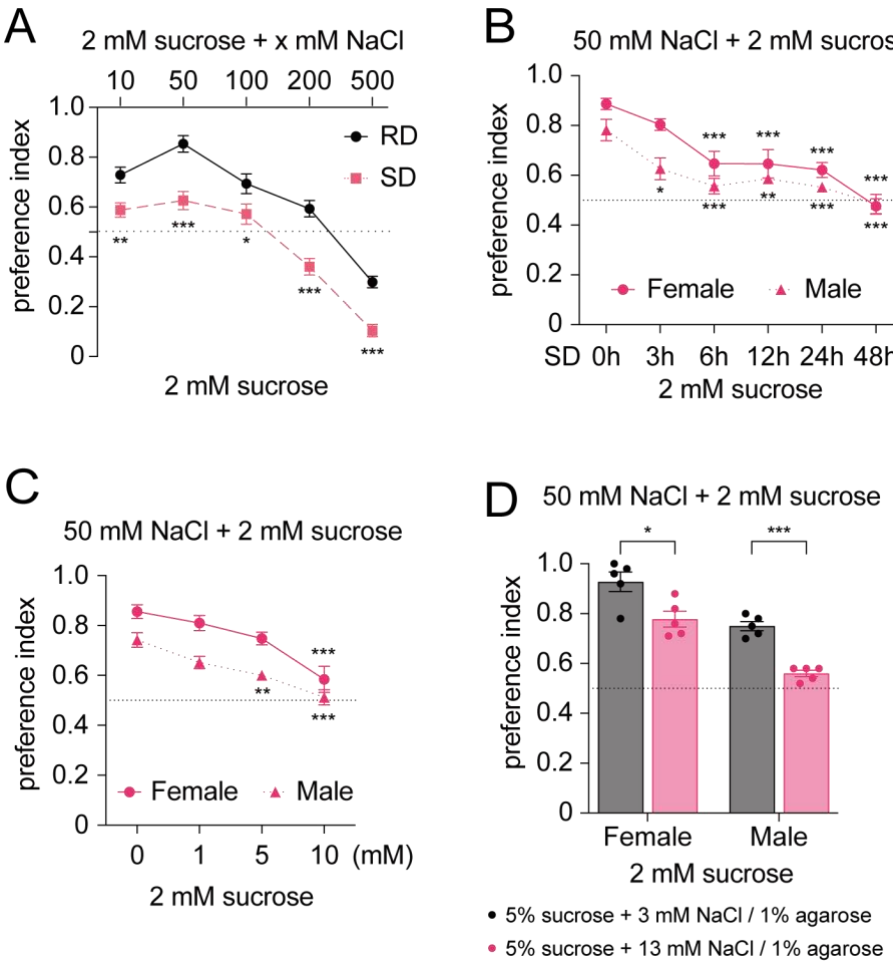

Figure 2. Dietary salt exposure reduces attraction to low salt concentrations

(A) Behavioral salt preference was assessed using a binary choice between 2 mM sucrose in 1% agarose alone or with increasing concentrations of NaCl (10, 50, 100, 200, or 500 mM), after 6 hours on a regular diet (RD) or salt diet (SD).

(B) Behavioral salt preference in female and male flies after exposure to RD or SD for 3, 6, 12, 24, or 48 hours. Flies were tested using a binary choice between 2 mM sucrose alone and 2 mM sucrose with 50 mM NaCl in 1% agarose.

(C) Behavioral salt preference following 6-hour exposure to RD or SD supplemented with different NaCl levels (1, 5, or 10 mM), using a choice between 2 mM sucrose and 2 mM sucrose with 50 mM NaCl.

(D) Behavioral salt preference was assessed after 6-hour feeding on agarose-based RD (3 mM NaCl) or SD (13 mM NaCl), using the same choice assay as above.

specifically caused by elevated dietary sodium.

3.2 Dietary salt decreases electrophysiological responses to low salt in sweet taste neurons

The reduction in salt preference following salt diet exposure may result from changes in peripheral GRNs or central processing mechanisms. To evaluate the contribution of peripheral taste adaptation, I conducted tip recordings from L-type sensilla to measure electrophysiological responses to low NaCl concentrations after salt diet exposure (Fig. 3A). In flies maintained on the regular diet, 30 mM NaCl evoked robust action potentials in both sexes (Fig. 3B–C). However, flies exposed to the salt diet exhibited a time-dependent reduction in spike frequency, paralleling the behavioral decline in salt preference (Fig. 3B–C). All NaCl-responsive L-type sensilla showed significantly reduced activity after salt exposure in both females and males (Fig. 3D–E), supporting a role for peripheral desensitization in modulating salt attraction.

To identify the neurons responsible for this response, I used the inward-rectifying potassium channel Kir2.1 to selectively silence GRN subtypes within L-type sensilla. Silencing sweet GRNs using *Gr64f-GAL4* abolished the 30 mM NaCl-evoked response. In contrast, silencing water-sensing (*Ppk28-GAL4*), pheromone-sensitive (*Ppk23-GAL4*), or low-salt (*Ir94e-GAL4*) neurons had no effect on salt responses or salt-induced desensitization (Fig. 4A). Furthermore, in $Ir56b^{1}$ mutants, NaCl-evoked spiking was abolished, whereas sugar receptor mutants ($Gr5a^{LEXA}$; ; *Gr64af*) retained normal NaCl

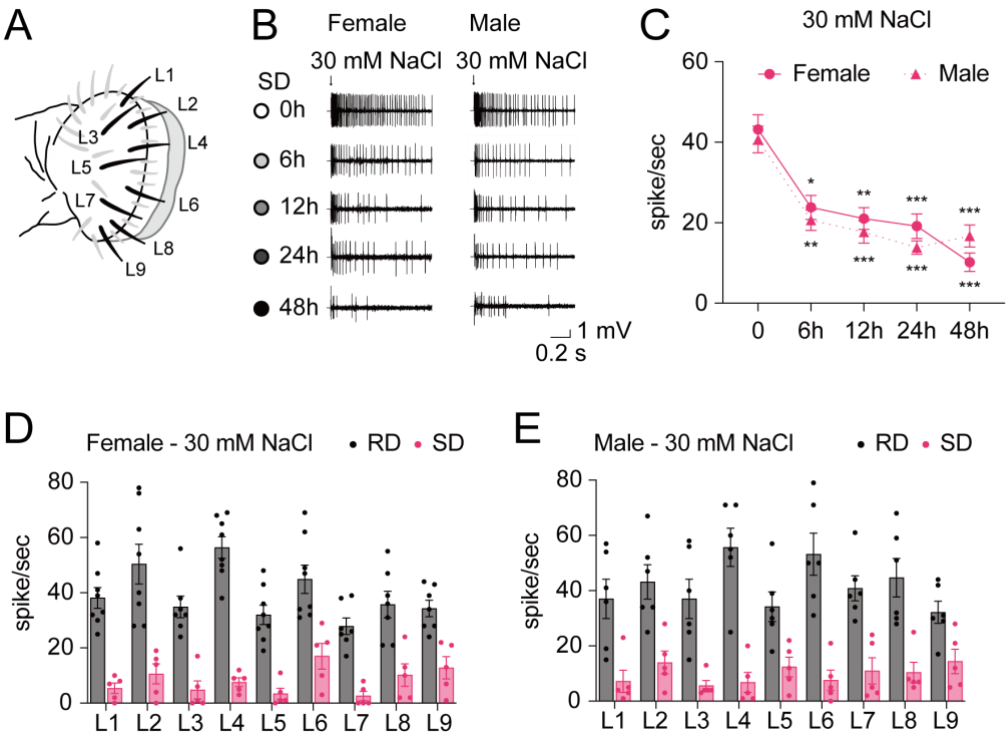

Figure 3. Dietary salt suppresses NaCl-evoked responses in sweet-sensing GRNs

(A) Schematic representation of L-type sensilla in the labellum that detect low concentrations of NaCl.

(B) Representative electrophysiological traces recorded from L-type sensilla in female and male flies after 6, 12, 24, or 48 hours on regular diet (RD) or salt diet (SD), using 30 mM NaCl as stimulus.

(C) Quantification of spike frequency from L-type sensilla in female and male flies after 6, 12, 24, or 48 hours on RD or SD, using 30 mM NaCl as stimulus.

(D) Quantification of NaCl-evoked spike frequency in female flies after 6-hour exposure to RD or SD.

(E) Quantification of NaCl-evoked spike frequency in male flies after 6-hour exposure to RD or SD.

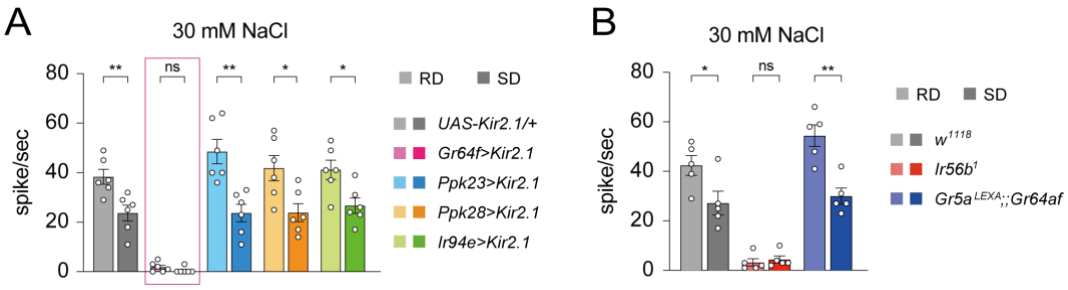

Figure 4. Sweet-sensing GRNs mediate diet-induced salt desensitization through a cell-autonomous mechanism

(A) Quantification of spike frequency in response to 30 mM NaCl recorded from L-type sensilla after silencing sweet GRNs using *UAS-Kir2.1* driven by *Gr64f-GAL4*, *Ppk23-GAL4*, *Ppk28-GAL4*, or *Ir94e-GAL4*, following 6-hour regular diet (RD) or salt diet (SD). (B) Quantification of NaCl-evoked responses from L-type sensilla in w^{1118} control, $Ir56b^{1}$ mutant, and $Gr5a^{LEXA}$;;*Gr64af* mutant flies after 6-hour RD or SD.

responses and still exhibited desensitization following salt exposure (Fig. 4B). These results indicate that salt sensitivity and desensitization occur in a cell-autonomous manner within sweet GRNs.

3.3 Dietary salt causes a reversible reduction in low-salt perception

To determine whether salt-induced desensitization is reversible, flies were transferred from the salt diet back to the regular diet for 3, 6, or 12 hours. After 12 hours of recovery, flies fully regained their original salt preference (Fig. 5A). This behavioral recovery was accompanied by restoration of NaCl-induced neural responses in L-type sensilla (Fig. 5B–C), demonstrating that salt desensitization is a reversible, experience-dependent process involving GRN activity modulation.

3.4 Dietary salt–induced desensitization to low salt depends on receptor-mediated endocytosis

The attenuation of cellular signaling is typically achieved through ligand-induced receptor internalization. These processes are commonly interpreted as forms of receptor adaptation, where sustained ligand exposure induces a refractory state, thus preventing excessive cellular responses to prolonged stimuli. Eukaryotic cells utilize various endocytic pathways for the internalization of cargo, such as nutrients, with the plasma membrane's cytoplasmic regions containing signals that are recognized by specific adaptor proteins [63]. This interaction facilitates the concentration of cargo at the sites of budding for

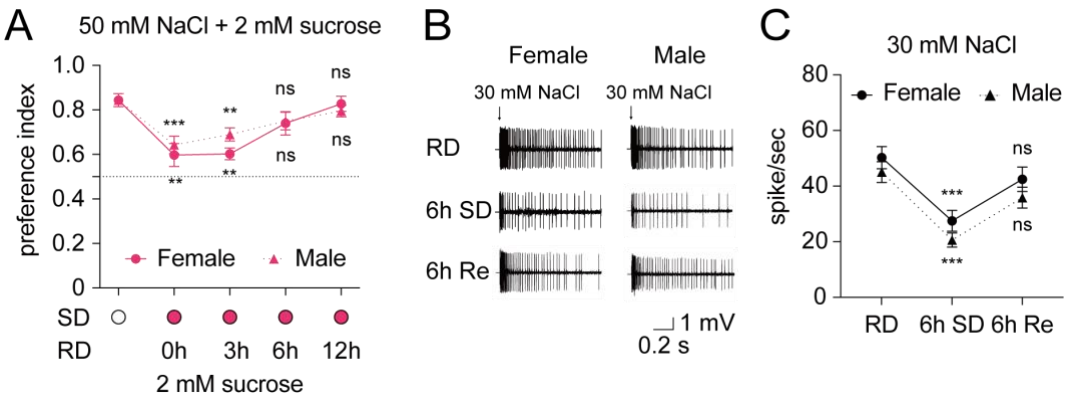

Figure 5. Dietary salt causes a reversible reduction in low-salt perception

(A) Assessment of salt taste recovery in female and male flies after 6 hours on a salt diet (SD), followed by a return to a regular diet (RD) for 3, 6, or 12 hours. Flies were tested using a binary choice between 2 mM sucrose alone and 2 mM sucrose with 50 mM NaCl in 1% agarose.

(B) Representative electrophysiological traces recorded from L-type sensilla in female and male flies maintained on RD, SD, or SD followed by 6-hour RD (Re, recovery), stimulated with 30 mM NaCl.

(C) Quantification of spike frequency from L-type sensilla in female and male flies maintained on RD, SD, or SD followed by 6-hour RD (Re, recovery), stimulated with 30 mM NaCl.

internalization via vesicles. Among various endocytic pathways, clathrin-mediated endocytosis predominates in internalizing many protein cargoes. In addition, dynamin-dependent pathways are associated with small-scale invaginations of the plasma membrane, such as those involved in clathrin, caveolar, or RhoA-dependent processes. In contrast, dynamin-independent pathways exhibit greater diversity, ranging from small-scale to larger membrane invaginations, including flotillin, cdc42, and Afr6-dependent mechanisms. Macropinocytosis, a large-scale endocytic pathway, further expands the spectrum of cellular internalization routes [64]. Notably, *Drosophila* lacks caveolin genes, which precludes caveolar-mediated endocytosis in this organism [65].

To investigate the possibility of taste desensitization after prolonged exposure to a salt diet through receptor internalization, I tested whether internalization of the salt receptor contributes to this process. The inhibition of clathrin-mediated, small-scale receptor-mediated endocytosis pathways, as well as the macropinocytosis-mediated large-scale endocytosis pathway, in salt-sensing neurons of female flies partially restored the action potential frequency after a salt diet (Fig. 6A-B). These electrophysiological results were further supported by behavioral assays, where blocking either clathrin-mediated endocytosis or macropinocytosis partially restored the flies' salt preference. In addition, simultaneous inhibition of both internalization pathways fully restored salt preference in female flies (Fig. 6C-D).

Surprisingly, in male flies, inhibition of clathrin-mediated endocytosis was the only intervention capable of restoring salt neuron activity (Fig. 7A-B), with macropinocytosis

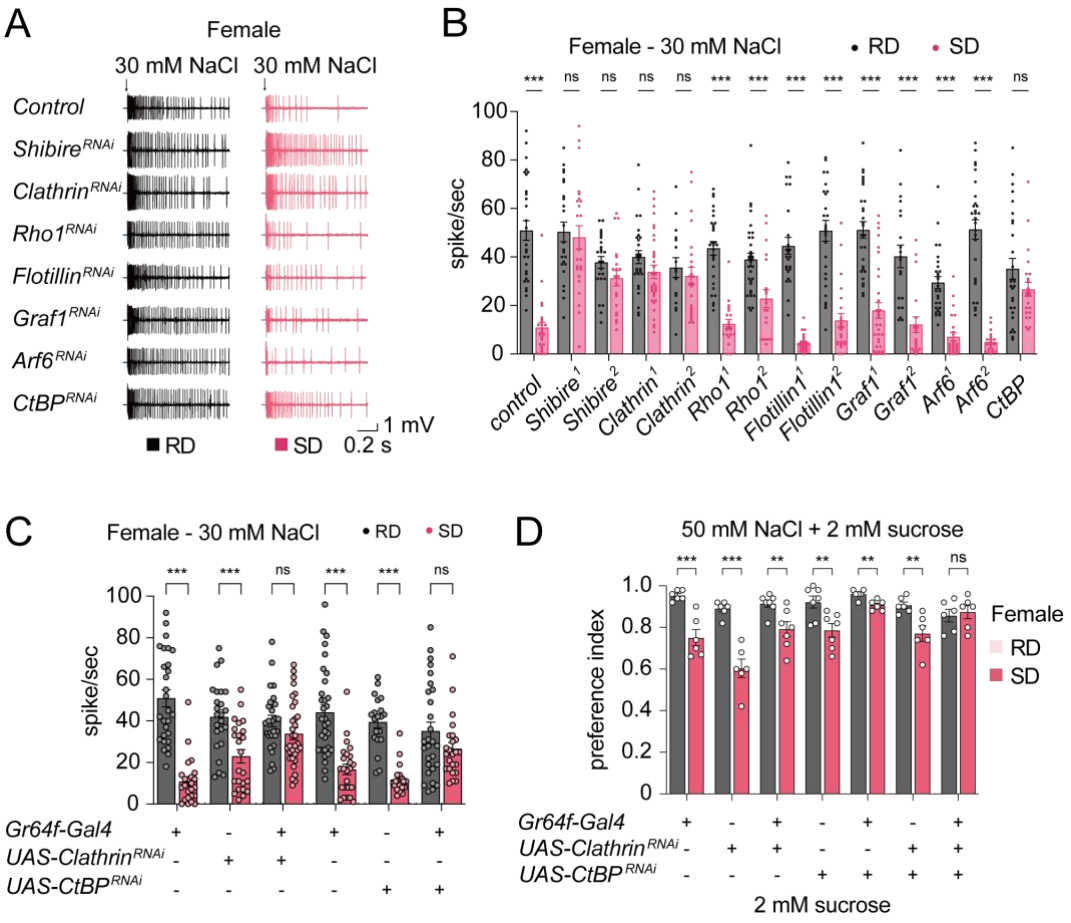

Figure 6. Salt desensitization in female flies depends on clathrin-mediated endocytosis and macropinocytosis

(A) Representative electrophysiological traces recorded from L-type sensilla stimulated with 30 mM NaCl in female flies with RNAi knockdown of endocytosis-related genes in sweet GRNs, after 2-day regular diet (RD) or salt diet (SD).

(B) Quantification of spike frequency from L-type sensilla stimulated with 30 mM NaCl in female flies with RNAi knockdown of endocytosis-related genes in sweet GRNs, after 2-day RD or SD.

(C) Quantification of spike frequency from L-type sensilla stimulated with 30 mM NaCl in female flies with sweet GRN-specific knockdown of clathrin and CtBP, after 2-day RD or SD.

(D) Behavioral salt preference was assessed in females with sweet GRN-specific knockdown of clathrin and CtBP, after 6-hour RD or SD. Flies were tested using a binary choice between 2 mM sucrose alone and 2 mM sucrose with 50 mM NaCl in 1% agarose.

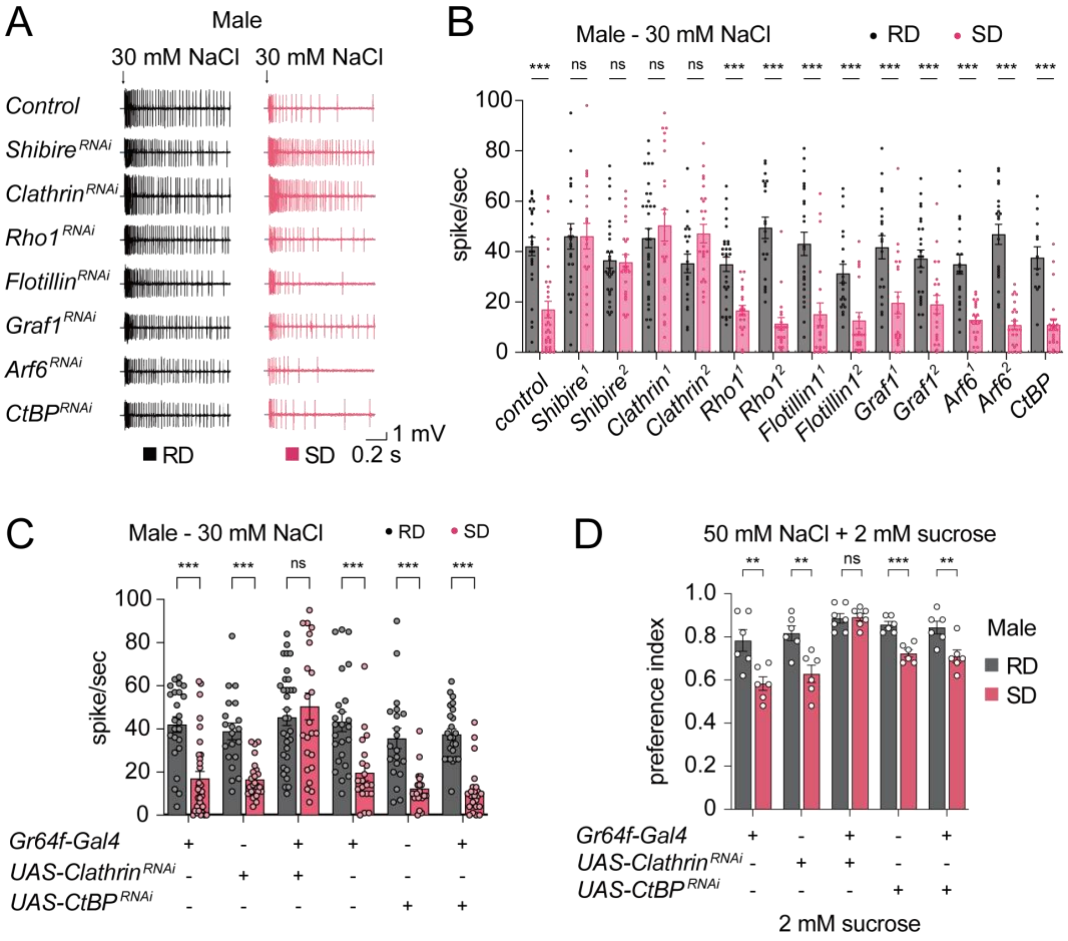

Figure 7. Clathrin-dependent endocytosis regulates salt desensitization in male flies

(A) Representative electrophysiological traces recorded from L-type sensilla stimulated with 30 mM NaCl in pseudomale flies (females with TraF knockdown), after 2-day regular diet (RD) or salt diet (SD).

(B) Quantification of spike frequency from L-type sensilla stimulated with 30 mM NaCl in male flies with RNAi knockdown of endocytosis-related genes in sweet GRNs, after 2-day RD or SD.

(C) Quantification of spike frequency from L-type sensilla stimulated with 30 mM NaCl in male flies with sweet GRN-specific knockdown of clathrin and CtBP, after 2-day RD or SD.

(D) Behavioral salt preference was assessed in males with sweet GRN-specific knockdown of clathrin and CtBP, after 6-hour RD or SD. Flies were tested using a binary choice between 2 mM sucrose alone and 2 mM sucrose with 50 mM NaCl in 1% agarose.

contributing little to the desensitization process. Behavioral assays corroborated these findings, showing that inhibition of clathrin-mediated endocytosis alone was sufficient to fully restore salt preference in male flies (Fig. 7C-D). These results suggest that dietary salt-induced desensitization of salt-sensing neurons is mediated by internalization mechanisms that exhibit sexual dimorphism.

3.5 Dietary salt–induced desensitization exhibits sexual dimorphism

The process of sex determination in organisms is governed by the balance of X chromosome and autosome factors, with different sex chromosome combinations (XX or XY) triggering distinct patterns of gene expression that regulate sexual development [66]. In females, the presence of two X chromosomes activates the expression of the sex-lethal (sxl) gene, which promotes female-specific splicing of the transformer (tra) pre-mRNA, resulting in the production of the functional Tra protein. This protein, in turn, interacts with Transformer-2 (Tra2) to splice doublesex (dsx) pre-mRNA, generating the female-specific DsxF protein that drives the development of female anatomical traits. Conversely, in males, the single X chromosome leads to insufficient sxl expression, resulting in the production of a nonfunctional TraM protein and the default splicing of dsx and fruitless (fru) pre-mRNAs, which produce the male-specific DsxM and FruM proteins, essential for male anatomical features and behavior (Fig. 8A).

To explore the sex-specific nature of the macropinocytosis-dependent internalization in response to a salt diet, two distinct perturbations of the sex determination hierarchy master

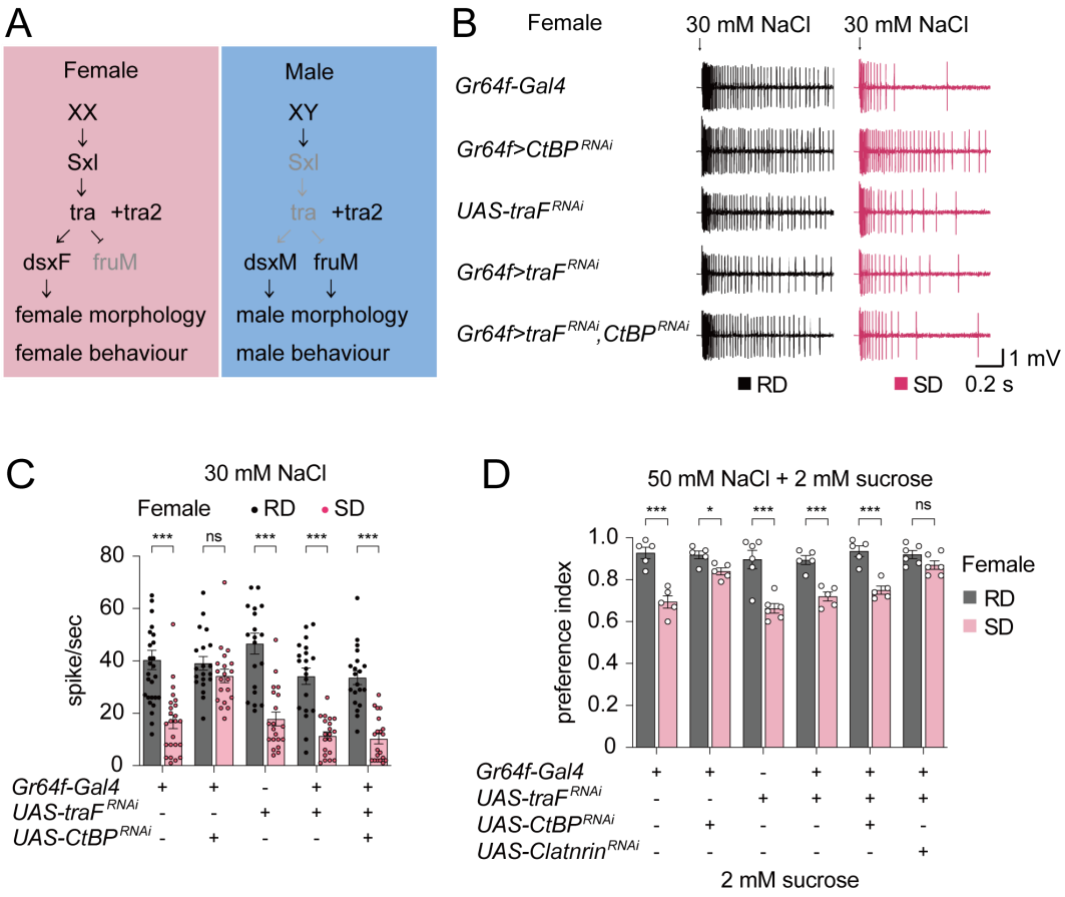

Figure 8. Macropinocytosis-driven desensitization requires female neuronal identity

(A) Schematic of the *Drosophila* sex determination pathway highlighting tra-mediated sexual identity.

(B) Representative electrophysiological traces recorded from L-type sensilla stimulated with 30 mM NaCl in pseudomale flies (females with TraF knockdown) with sweet GRN-specific knockdown of clathrin and CtBP, after 2-day regular diet (RD) or salt diet (SD).

(C) Quantification of spike frequency from L-type sensilla stimulated with 30 mM NaCl in pseudomale flies with sweet GRN-specific knockdown of clathrin and CtBP, after 2-day RD or SD.

(D) Behavioral salt preference was assessed in pseudomale flies with sweet GRN-specific knockdown of clathrin and CtBP, after 6-hour RD or SD. Flies were tested using a binary choice between 2 mM sucrose alone and 2 mM sucrose with 50 mM NaCl in 1% agarose.

regulator, transformer (tra), were examined: pseudomales (TraF knockdown females) and pseudofemales (tra overexpression in males). In pseudomale flies on a low-salt diet, the female flies' knockdown of TraF in salt-sensing neurons disrupted macropinocytosis-dependent salt desensitization (Fig. 8B-C). Behavioral assays demonstrated that, similar to normal males, inhibiting clathrin-mediated endocytosis in pseudomales restored salt preference and macropinocytosis-independent desensitization after a salt diet (Fig. 8D). In contrast, pseudofemale flies, male flies ectopically expressing TraF in salt-sensing neurons, exhibited macropinocytosis-dependent desensitization, resulting in female-like electrophysiological responses (Fig. 9A-B). Behavioral data showed that, in pseudofemales, the inhibition of clathrin-mediated endocytosis did not fully restore the salt preference (Fig. 9C). These findings suggest that salt diet-induced desensitization is sexually dimorphic, with macropinocytosis-related desensitization occurring specifically in female flies.

3.6 Ca^{2+}/calmodulin signaling is essential for salt desensitization in sweet GRNs

Next, I investigated the molecular mechanisms underlying salt diet-induced desensitization. Typically, upon ligand binding, receptor activation triggers downstream signaling pathways that result in receptor phosphorylation [67][68]. This modification enables the receptor to interact with adaptor proteins that link to the clathrin endocytic machinery. To assess the role of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), a key protein in excitatory synapses with plays a central role in synaptic plasticity a key protein in excitatory synapses, plays a central role in synaptic plasticity [69]. CaMKII

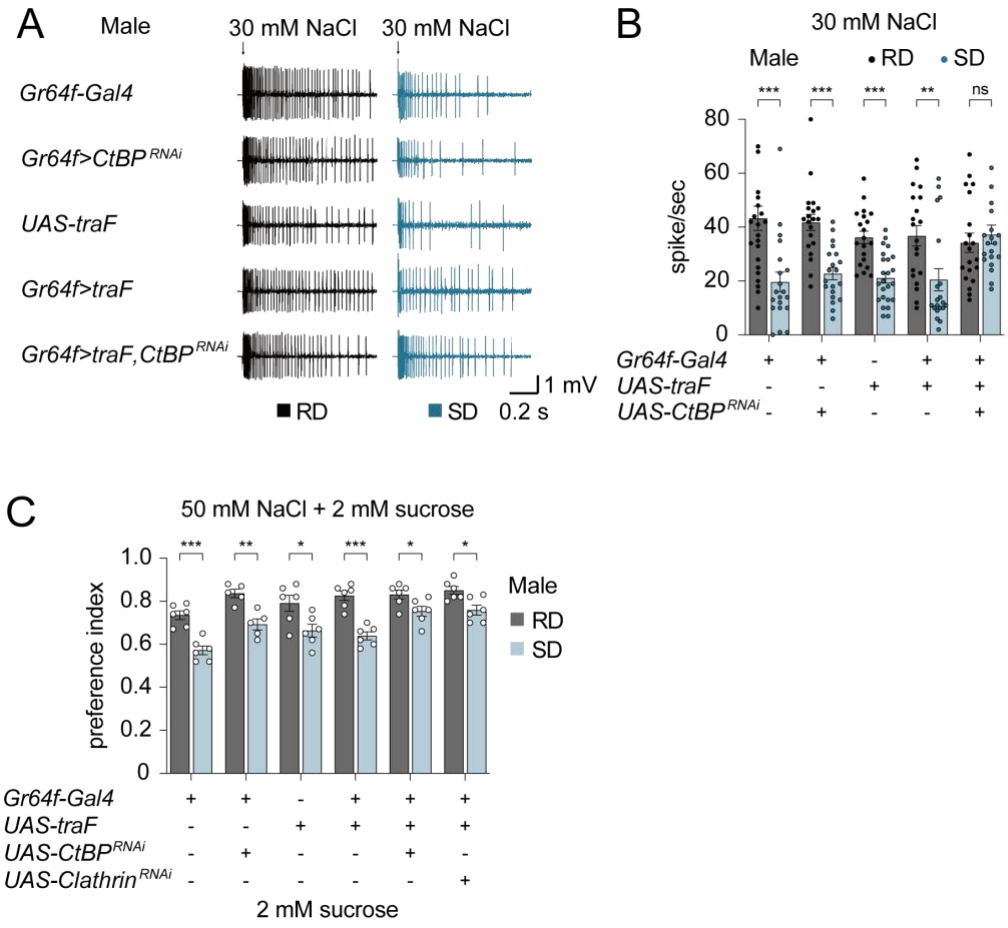

Figure 9. Feminization of male GRNs permits macropinocytosis-dependent salt desensitization

(A) Representative electrophysiological traces recorded from L-type sensilla stimulated with 30 mM NaCl in pseudofemale flies (males expressing TraF) with sweet GRN-specific knockdown of clathrin and CtBP, after 2-day regular diet (RD) or salt diet (SD).

(B) Quantification of spike frequency from L-type sensilla stimulated with 30 mM NaCl in pseudofemale flies with sweet GRN-specific knockdown of clathrin and CtBP, after 2-day RD or SD.

(C) Behavioral salt preference was assessed in pseudofemale flies with sweet GRN-specific knockdown of clathrin and CtBP, after 6-hour RD or SD. Flies were tested using a binary choice between 2 mM sucrose alone and 2 mM sucrose with 50 mM NaCl in 1% agarose.

phosphorylates several synaptic proteins, regulating their structure and function, which in turn governs essential molecular events such as receptor trafficking, localization, and activity.

To assess the role of CaMKII in salt diet-induced desensitization, CaMKII knockdown experiments were conducted. Electrophysiological recordings showed that CaMKII knockdown in low-salt neurons restored salt-induced action potentials in both female and male flies (Fig. 10A-D). Given that CaMKII activation is dependent on Ca^{2+}/calmodulin binding, calmodulin knockdown was also tested. Similarly, neuron-specific calmodulin knockdown in low-salt conditions restored the electrophysiological responses to salt in both female and male flies (Fig. 10A-D). As expected, CaMKII knockdown and clathrin endocytic knockdown flies exhibited similar behaviors; only male flies showed full restoration of salt preference with CaMKII inhibition, whereas female flies did not recover their salt preference (Fig. 10E-F). These results suggest that CaMKII and calmodulin are critical for salt-induced desensitization, with a sexually dimorphic response.

3.7 Dietary salt induces sugar desensitization in female flies

Considering the overlap between low-salt salt and sugar sensing neurons, I investigated whether a salt diet influences sugar responsiveness. Interestingly, female flies previously exposed to a salt diet exhibited a marked reduction in sucrose-evoked neural activity. This reduction in the frequency of action potentials was not observed in males, suggesting a sex-specific physiological response (Fig. 11A-B). Complementary behavioral assays revealed

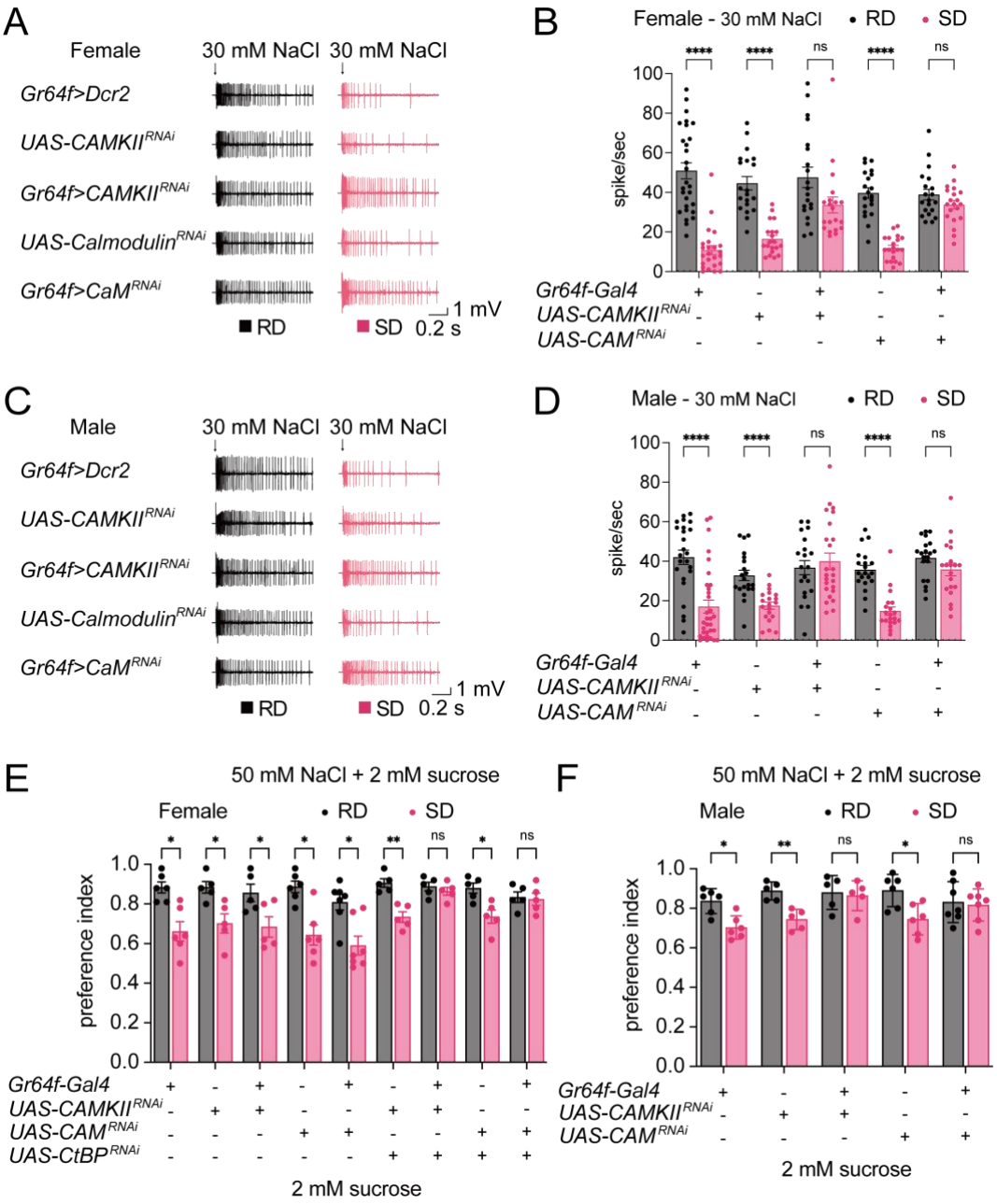

Figure 10. Ca^{2+}/calmodulin signaling is essential for salt desensitization in sweet GRNs

(A) Representative electrophysiological traces recorded from L-type sensilla in female flies with CAMKII or calmodulin knockdown in sweet GRNs, after 2-day regular diet (RD) or salt diet (SD).

(B) Quantification of spike frequency from L-type sensilla in female flies with CAMKII or calmodulin knockdown in sweet GRNs, after 2-day RD or SD.

(C) Representative electrophysiological traces recorded from L-type sensilla in male flies with CAMKII or calmodulin knockdown in sweet GRNs, after 2-day RD or SD.

(D) Quantification of spike frequency from L-type sensilla in male flies with CAMKII or calmodulin knockdown in sweet GRNs, after 2-day RD or SD.

(E) Behavioral salt preference was assessed in female flies with sweet GRN-specific knockdown of CtBP and CAMKII or calmodulin, after 6-hour RD or SD. Flies were tested using a binary choice between 2 mM sucrose alone and 2 mM sucrose with 50 mM NaCl in 1% agarose.

(F) Behavioral salt preference was assessed in male flies with sweet GRN-specific knockdown of CtBP and CAMKII or calmodulin, after 6-hour RD or SD. Flies were tested using a binary choice between 2 mM sucrose alone and 2 mM sucrose with 50 mM NaCl in 1% agarose.

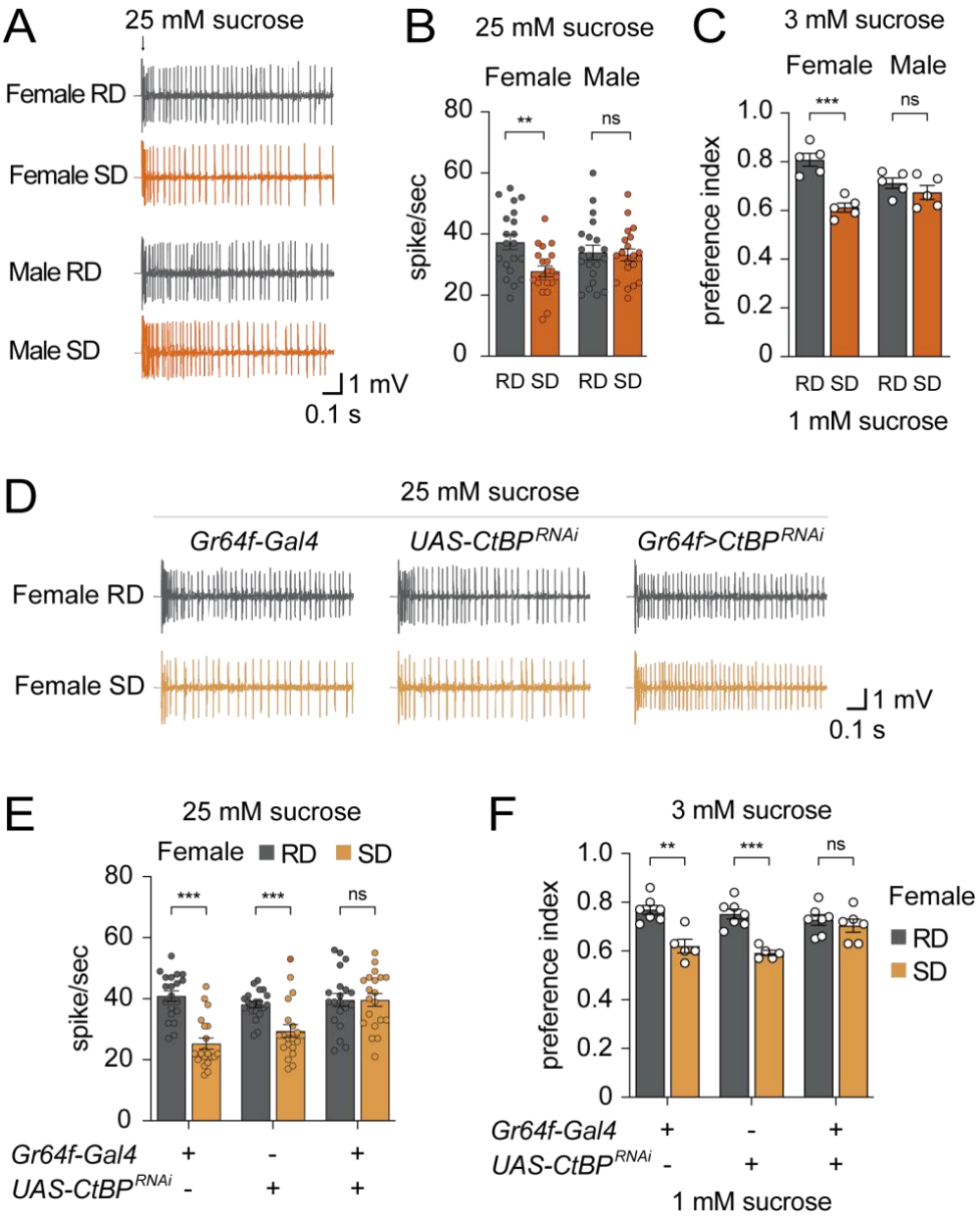

Figure 11. Salt diet reduces sugar sensitivity in female flies

(A) Representative electrophysiological traces recorded from L-type sensilla in female and male flies after 6-hour regular diet (RD) or salt diet (SD), stimulated with 25 mM sucrose.

(B) Quantification of spike frequency from L-type sensilla in female and male flies after 6-hour RD or SD, stimulated with 25 mM sucrose.

(C) Behavioral sucrose preference was assessed in female and male flies after 6-hour RD or SD. Flies were tested using a binary choice between 1mM sucrose alone and 3 mM sucrose in 1% agarose.

(D) Representative electrophysiological traces recorded from L-type sensilla in female flies with sweet GRN-specific knockdown of macropinocytosis pathway components, after 6-hour RD or SD, stimulated with 25 mM sucrose.

(E) Quantification of spike frequency from L-type sensilla in female flies with sweet GRN-specific knockdown of macropinocytosis pathway components, after 6-hour RD or SD, stimulated with 25 mM sucrose.

(F) Behavioral sucrose preference was assessed in female flies with sweet GRN-specific knockdown of macropinocytosis pathway components after either RD or SD for 6 hours. Flies were tested using a binary choice between 1mM sucrose alone and 3 mM sucrose in 1% agarose.

a corresponding decline in sucrose preference among females. In contrast, male feeding behavior remained unchanged (Fig. 11C).

Given the previous implication of macropinocytosis in sensory plasticity, I hypothesized that this cellular process may underlie the reduced sweet sensitivity observed in females. To test this, I check female flies with inhibited macropinocytosis selectively in sweet GRNs. As expected, flies with blocked macropinocytosis return to normal levels of physiological response and behavioral result after two days on a salt diet. In contrast, control groups still showed a decrease in sugar perception (Fig. 11D-F). These findings highlight a critical role for macropinocytosis in mediating salt-induced sweet taste desensitization, in a female-specific manner.

3.8 Sugar in the diet does not contribute to salt diet–induced salt desensitization

To rule out the potential contribution of sugar present in the diet to desensitization of salt responses, I used electrophysiological recordings, and flies were reared on agarose diets with or without 5% sucrose, supplemented with either 3 mM or 13 mM NaCl. Notably, a six-hour exposure to the 13 mM NaCl diet significantly reduced action potential frequency in response to 30 mM NaCl, independent of the presence of sucrose (Fig. 12A-B). These results suggest that sugar does not influence the salt-induced attenuation of salt taste, confirming that salt alone drives this desensitization.

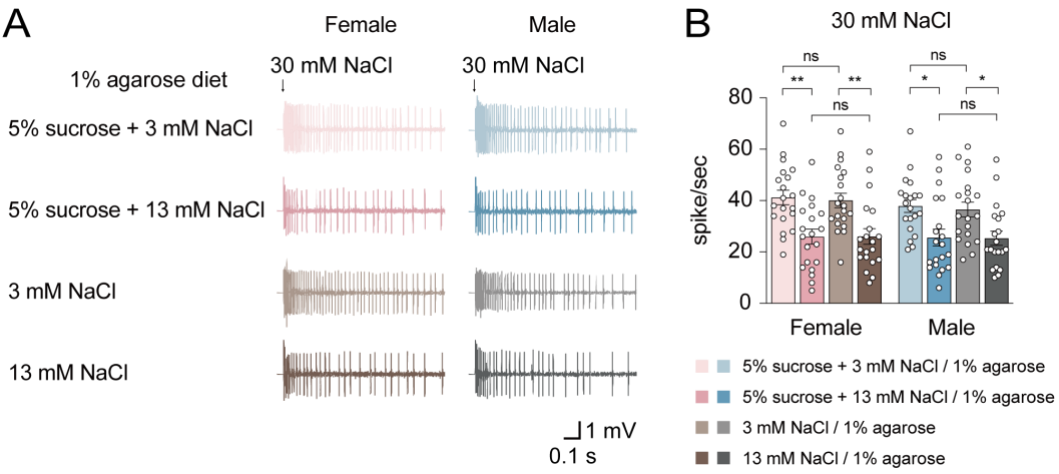

Figure 12. Sugar does not contribute to salt diet-induced salt desensitization

(A) Representative electrophysiological traces recorded from L-type sensilla of female and male flies fed an agarose-based regular diet with or without sucrose, or after 6 hours on an agarose-based salt diet with or without sucrose.

(B) Quantification of spike frequency from L-type sensilla of female and male flies fed an agarose-based regular diet with or without sucrose, or after 6 hours on an agarose-based salt diet with or without sucrose.

3.9 High-sugar diet alters sweet but not salt perception

Next, I tested flies after feeding a high-sugar diet. As previous literature has reported that excessive dietary sugar impairs sweet taste sensitivity, both female and male flies exhibited decreased neural responses and lowered behavioral preference for sucrose after 6 or 48 hours of a high sugar diet (Fig. 13A-C). However, this dietary intervention did not alter salt sensitivity or salt preference across sexes within the same time frame (Fig. 13D-F).

3.10 Dietary salt reduces low-salt attraction independently of internal sodium regulation

Finally, to determine whether internal physiological state contributes to the reduction in salt perception following dietary salt exposure, I investigated the role of internal sodium-sensing (INSO) neurons. The same as previously reported, flies that underwent salt deprivation for three days displayed an increased behavioral attraction to 10 mM NaCl, indicating a compensatory response to internal sodium deficiency. However, when INSO neurons were silenced in these salt-deprived flies, this enhanced preference for low salt was eliminated (Fig. 14A), confirming their role in mediating salt appetite under sodium-deficient conditions.

Interestingly, in flies previously exposed to a salt diet, silencing INSO neurons had no impact on the decreased preference for low salt compared to controls maintained on a regular diet (Fig. 14B). Electrophysiological experiments also support this conclusion (Fig.

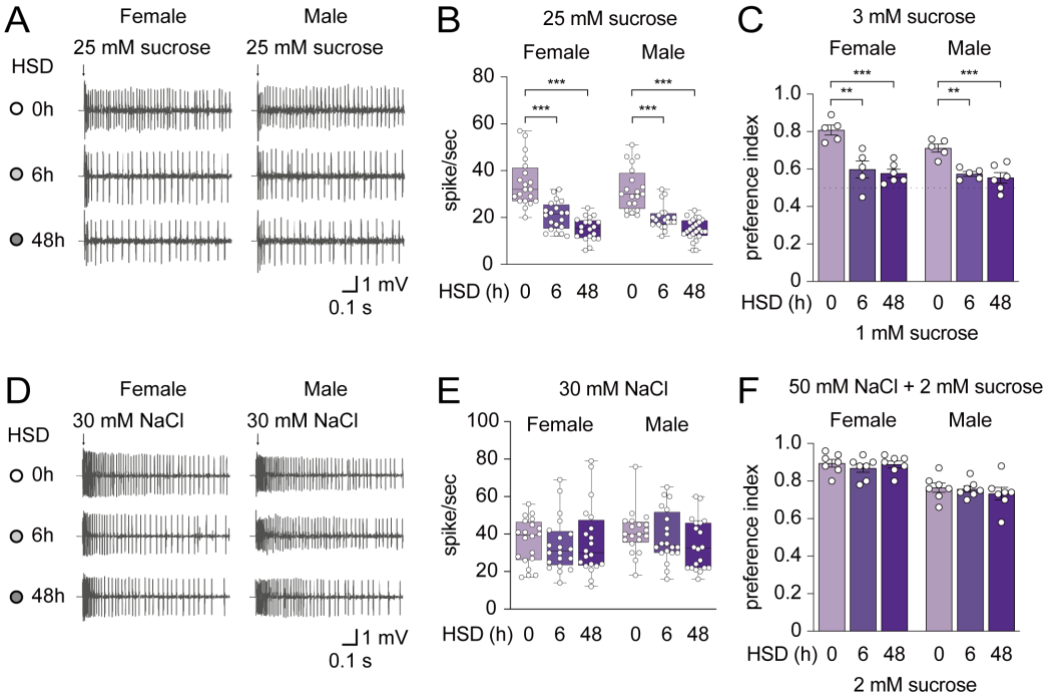

Figure 13. High-sugar diet impairs sugar sensing but does not affect salt response

(A) Representative electrophysiological traces recorded from L-type sensilla in female and male flies fed regular diet (RD) or high-sugar diet (HSD) for 6 or 48 hours, stimulated with 25 mM sucrose.

(B) Quantification of spike frequency from L-type sensilla in female and male flies fed RD or HSD for 6 or 48 hours, stimulated with 25 mM sucrose.

(C) Behavioral sucrose preference was assessed in female and male flies after RD or HSD for 6 or 48 hours. Flies were tested using a binary choice between 1mM sucrose alone and 3 mM sucrose in 1% agarose.

(D) Representative electrophysiological traces recorded from L-type sensilla in female and male flies fed RD or HSD for 6 or 48 hours, stimulated with 30 mM NaCl.

(E) Quantification of spike frequency from L-type sensilla in female and male flies fed RD or HSD for 6 or 48 hours, stimulated with 30 mM NaCl.

(F) Behavioral sucrose preference was assessed in female and male flies after RD or HSD for 6 or 48 hours. Flies were tested using a binary choice between 2 mM sucrose alone and 2 mM sucrose with 50 mM NaCl in 1% agarose.

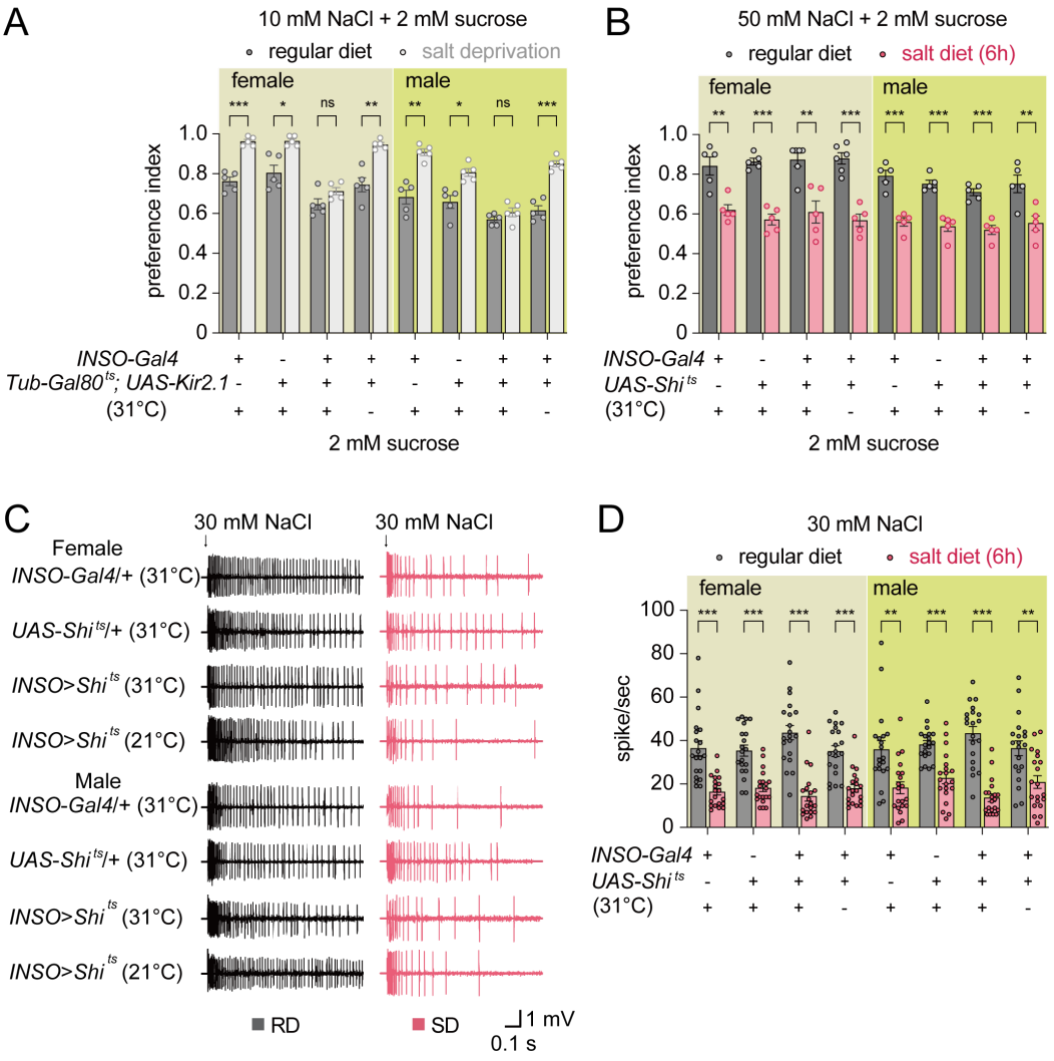

Figure 14. Salt desensitization is independent of internal sodium-sensing neurons

(A) Behavioral salt preference was assessed in female and male flies with indicated genotypes after regular condition or 3 days salt deprivation at 31°C or 21°C. Flies were tested using a binary choice between 2 mM sucrose alone and 2 mM sucrose with 10 mM NaCl in 1% agarose.

(B) Behavioral salt preference was assessed in female and male flies with indicated genotypes after 6-hour regular diet (RD) or salt diet (SD) at 31°C or 21°C. Flies were tested using a binary choice between 2 mM sucrose alone and 2 mM sucrose with 50 mM NaCl in 1% agarose.

(C) Representative electrophysiological traces recorded from L-type sensilla stimulated with 30 mM NaCl in female and male flies with indicated genotypes and temperatures after 6-hour RD or SD.

(D) Quantification of spike frequency from L-type sensilla stimulated with 30 mM NaCl in female and male flies with indicated genotypes and temperatures after 6-hour RD or SD.

14C-D). These results imply that the salt desensitization induced by dietary salt operates through a pathway that is independent of INSO neuron signaling (Fig. 14C-D).

To further isolate peripheral effects of salt exposure from potential changes in internal sodium balance, I developed a method to limit ingestion of salt-containing food while allowing sensory exposure. This was achieved by transiently inhibiting labellar MN11 and 12 using a temperature-sensitive shibire construct (*UAS-Shibire^{ts}*). Silencing these neurons at the restrictive temperature (31°C) significantly reduced food intake over a six-hour window, as confirmed using the CAFÉ assay (Fig. 15A-B). After this period, flies were shifted to the permissive temperature (21°C) and allowed to feed for 1.5 hours, ensuring consistent intake across genotypes for preference testing. Importantly, flies that had motor neurons silenced showed similar salt sensitivity as genetic control groups following salt exposure, suggesting that internal sodium intake did not drive the behavioral changes (Fig. 15C-E).

Additionally, to directly assess whether sensory adaptation contributes to reduced salt responsiveness, I exposed immobilized flies to repetitive salt stimulation for three hours. This localized stimulation resulted in a decreased neural response from L-type sensilla, supporting the hypothesis that salt desensitization is occurring at the level of peripheral sensory neurons (Fig. 16A-B).

Taken together, these findings provide strong evidence that the reduced attraction to low salt following dietary salt intake is not mediated by internal sodium homeostasis. Instead,

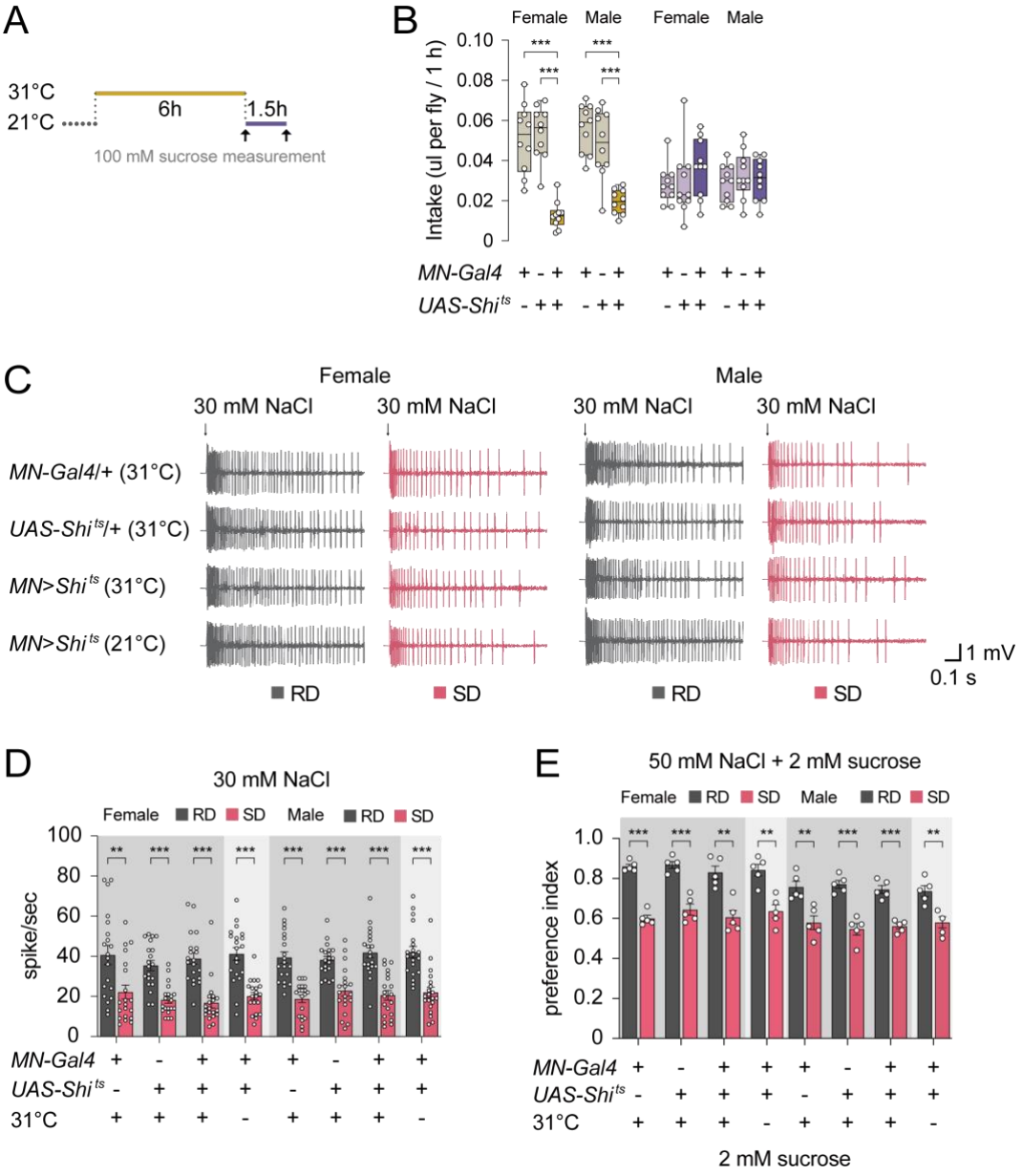

Figure 15. Dietary salt reduces salt attraction irrelevant with internal state

(A) Schematic representation of the CAFE assay used to measure food intake (100 mM sucrose) in female and male flies with silenced MN11 and MN12 motor neurons.

(B) Sucrose intake (100 mM) measured using the CAFE assay in female and male flies of the indicated genotypes. Food consumption was recorded over a 6-hour period at 31 °C, followed by a second measurement after a 1.5-hour recovery period at 21 °C.

(C) Representative electrophysiological traces recorded from L-type sensilla stimulated with 30 mM NaCl in female and male flies with indicated genotypes and temperatures after 6-hour regular diet (RD) or salt diet (SD).

(D) Quantification of spike frequency from L-type sensilla stimulated with 30 mM NaCl in female and male flies with indicated genotypes and temperatures after 6-hour RD or SD.

(E) Behavioral salt preference was assessed in female and male flies with indicated genotypes after 6-hour regular diet (RD) or salt diet (SD) at 31°C or 21°C. Flies were tested using a binary choice between 2 mM sucrose alone and 2 mM sucrose with 50 mM NaCl in 1% agarose.

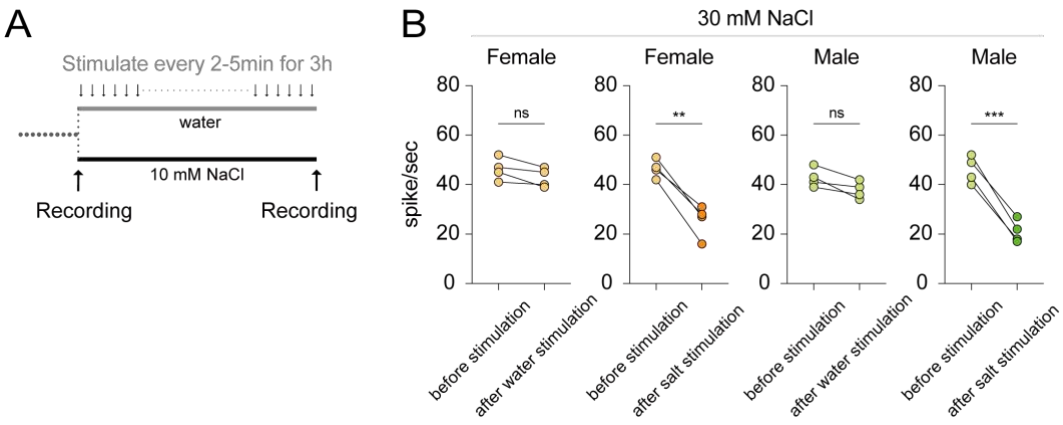

Figure 16. Dietary salt induces peripheral salt desensitization independent of internal Na^+ homeostasis

(A) Diagram of the stimulation setup delivering salt or water to labellum without ingestion.

(B) Quantification of the mean number of electrophysiological response frequencies from L-type sensilla stimulated with 30 mM NaCl in female and male flies before and after continuous water or NaCl stimulation.

the results point to a peripheral desensitization mechanism within the gustatory system as the primary driver of altered salt perception.

4. Discussion

The current findings demonstrate that a salt diet induces significant yet selective modifications in the peripheral taste system of *Drosophila*. First, both female and male flies exhibited reduced electrophysiological responses to salt stimuli following salt exposure. This suggests a general salt desensitization across sexes, likely driven by alterations in receptor-level mechanisms rather than downstream sensory processing or integration.

Second, while endocytosis is implicated in salt-induced desensitization in both sexes, the underlying mechanisms appear to be more complex in females. While male flies utilize clathrin-mediated endocytosis. However, females seem to utilize not only clathrin-mediated endocytosis but also macropinocytosis. This sex-specific difference was further supported by the finding that only females exhibited diminished sugar sensitivity after a salt diet, an effect mediated by a female-specific endocytic pathway. Collectively, these results reveal that the impact of dietary salt on taste perception is sexually dimorphic, engaging distinct cellular and molecular processes in males and females.

Sexual dimorphism in *Drosophila* is well established, particularly regarding feeding behavior, sensory processing, and nutrient preferences—traits closely tied to reproductive roles. Females, for example, require higher protein intake to support oogenesis, whereas males prioritize carbohydrate intake for mating and courtship activities [71][72]. These physiological differences shape sex-specific feeding behaviors and sensory responses.

Recent research has highlighted the role of enteroendocrine cells in the adult female midgut, which detect nutrient status and release neuropeptide F (NPF) to promote protein

consumption in mated females [73]. NPF acts through glucagon-like adipokinetic hormone (AKH) signaling to induce sugar satiety while stimulating increased protein intake and promoting nutrient storage in adipose tissue. Additionally, it has been suggested that a salt diet can enhance egg production in female fruit flies. My findings align with this result, showing that a salt diet specifically reduces sugar sensitivity in females. This suggests that salt intake may influence feeding behavior by inhibiting sugar consumption and, in turn, promoting protein intake to support egg production. Such findings highlight the complex interplay between diet, reproductive needs, and feeding behaviors in *Drosophila* and suggest that salt may serve as a modulator of nutritional preferences that are critical for reproduction.

In the context of cellular internalization processes, clathrin-mediated endocytosis (CME) and macropinocytosis are two distinct, yet crucial mechanisms by which cells internalize extracellular material, differing markedly in their mechanisms, vesicle sizes, and the types of cargo they handle. Clathrin-mediated endocytosis is a highly specific, receptor-mediated process wherein cell surface receptors bind to ligands such as hormones or nutrients, triggering the formation of clathrin-coated pits. These pits, typically 100 to 200 nm in diameter, bud off from the plasma membrane to form small vesicles containing the receptor-ligand complexes [74][75]. After internalization, these vesicles uncoat and fuse with early endosomes for further processing, facilitating the uptake of specific cargo such as low-density lipoproteins and growth factors. Conversely, macropinocytosis is a nonselective process driven by dynamic changes in the actin cytoskeleton, leading to large-

scale membrane ruffling and the formation of macropinosomes. These vesicles, ranging from 0.5 to 5 micrometers, engulf extracellular fluid, solutes, and larger particles. Unlike clathrin-mediated endocytosis, macropinocytosis does not rely on receptor specificity, allowing for a broader and more indiscriminate uptake of extracellular material. These differences underscore the distinct roles of each process in cellular physiology and their implications for material uptake and cellular signaling.

Moreover, the study of epidermal growth factor receptor (EGFR) endocytosis further illustrates the dynamic interplay between clathrin-dependent and clathrin-independent mechanisms [76]. EGFR internalization is regulated by both clathrin-dependent endocytosis and clathrin-independent endocytosis, depending on the ligand concentration. Clathrin-mediated endocytosis plays a critical role in assembling the initial signaling platform necessary for EGFR activation, whereas clathrin-independent endocytosis occurs at higher doses of EGFR [77]. This nuanced mechanism may also be relevant to female flies, who possess higher salt requirements than males and may have evolved a more intricate, salt-sensitive endocytic system—a potential adaptation to reproductive needs. Such insights underscore the importance of internalization mechanisms in cellular adaptation and signaling regulation under different environmental and physiological conditions.

Despite the valuable insights obtained in this study, several limitations must be acknowledged. Although these results suggest that the desensitization of sugar sensitivity induced by a salt diet can be mitigated through the inhibition of receptor endocytosis,

technical constraints prevented us from conclusively verifying that this desensitization is specifically due to the endocytosis of salt receptors. As a result, I cannot entirely exclude the possibility that other salt detection-related signal molecules might be involved in the desensitization process. Future research could explore whether the low-salt receptor complex—comprising Ir25a, Ir56b, and Ir76b—is internalized following salt exposure, and whether this response varies between males and females.

Inhibited CAMKII impaired recovery of salt preference after a salt diet, despite the near-complete recovery of electrophysiological responses. I hypothesize that CAMKII may regulate a previously unknown function in the salt diet response, independent of clathrin-mediated endocytosis. However, inhibition of both clathrin-mediated endocytosis and macropinocytosis may be enough compensation.

While much of the literature focuses on the links between dietary salt and various diseases or metabolic alterations in humans, there is a paucity of data examining its direct effects on the peripheral taste system. My findings offer a crucial starting point for further research into this area, laying the groundwork for more in-depth behavioral and neurobiological investigations. These results contribute to my understanding of the complex neurobiological consequences of salt consumption, shedding light on the intricate interactions between dietary factors and sensory responses. This knowledge could potentially inform strategies aimed at managing salt intake and mitigating the health issues associated with excessive consumption.

5. Conclusion

This study sheds light on how dietary salt exposure modulates gustatory perception in *Drosophila melanogaster*, revealing a plastic and adaptive sensory system shaped by dietary experience.

1. In *Drosophila melanogaster*, chronic exposure to a salt diet induces peripheral salt taste desensitization in sweet GRNs through cell-autonomous and receptor mediated endocytosis.
2. The mechanisms behind salt diet-induced salt desensitization differ between sexes. Both males and females use clathrin-mediated endocytosis, but females also engage a macropinocytosis-dependent pathway to adjust their salt sensitivity.
3. In female flies, salt diet exposure specifically reduces sugar sensitivity through a process that also relies on macropinocytosis.
4. Sugar does not play a role in salt sensitivity.
5. Salt diet-induced desensitization is reversible, do not rely on internal sodium levels.

Thus, by elucidating the molecular mechanisms governing salt perception, this study provides valuable insights into the intricate interactions between diet, sensory systems, and homeostatic regulation. The discovery of sex-specific mechanisms, particularly involving macropinocytosis, suggests new directions for developing strategies to control salt consumption.

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국문 요약 (IN KOREAN)

노랑초파리에서 식이 소금에 의한 짠맛 인지 변화

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소금은 체액 균형과 세포 기능 유지에 중요한 필수 영양소로, 포유류와 곤충에서 소금 선호도 및 섭취는 미각과 체내 생리적 상태에 의해 조절된다. 낮은 소금 농도에서는 나트륨 감지 세포가 활성화되어 섭취를 유도하고, 높은 농도에서는 고농도 소금 감지 세포가 활성화되어 소금에 대한 혐오 반응을 유발한다. 체내 이온 불균형은 신경 회로와 호르몬을 통해 소금 선호도를 변화시켜 적절한 섭취를 유도하는 조절 메커니즘을 작동시킨다.

소금 섭취 행동의 생리적 조절 메커니즘에 대한 연구는 많지만, 환경 변화나 식이 습관 변화가 미각을 통한 소금 섭식에 미치는 영향에 대한 연구는 부족하다. 특히, 짠 음식을 섭취했을 때 미각 변화와 그 메커니즘에 대해서는 아직 명확하지 않다. 예를 들어, 짠 음식을 먹었을 때 소금 맛에 대한 미각이 변화하는지, 다른 맛에 대해서는 어떤 영향을 미치는지에 대해서는 알려진 바가 없다. 또한, 맛 감지에 변화가 생긴다면 그 기전이

무엇인지, 미각 세포의 직접적인 조절을 통한 것인지, 아니면 내부 생리적 상태의 변화에 의존하는지에 대한 연구도 부족하다. 이에 본 연구는 미각에 대해 잘 알려진 노랑초파리(*Drosophila melanogaster*)를 이용하여, 짠 음식에 노출된 초파리의 미각 반응 변화를 통해 환경 변화가 소금 선호도 및 소금 감지에 미치는 영향을 규명하였다.

짠 음식에 노출된 초파리는 소금을 섭취하지 않아도, 체내 생리적 변화 없이 지속적으로 소금에 노출된 것만으로 소금에 대한 선호도가 감소한다는 것을 확인하였다. 이 변화는 나트륨 감지 세포의 전기생리학적 반응 저하에 기인하며, 이는 세포내이입 경로를 통해 조절된다. 특히 성별에 따른 이형성 (sexual dimorphism)을 나타냈는데 수컷과 암컷 모두 클라트린 매개 세포내이입 (Clathrin-mediated endocytosis) 경로를 통해 소금에 대한 탈감작을 일으킬 수 있지만, 암컷은 특이적으로 대음세포작용 (macropinocytosis)을 통해서도 조절된다. 또한, Ca2+/칼모듈린 의존성 단백질 키나제 II (CaMKII)가 탈감작 현상에 중요한 역할을 하며, 이는 클라트린 매개 세포내이입을 조절할 것으로 예상된다. 추가로, 짠 음식에 노출된 암컷 초파리에서만 단맛 선호도가 감소하는데, 이는 대식세포의 유입을 통해 발생하는 현상임을 확인하였다.

본 연구는 짠 음식 습관이 미각에 미치는 영향을 규명하며, 식이 변화가 미각 반응을 어떻게 조절하는지에 대한 이해를 높이는 데 기여한다. 또한,

이러한 결과는 향후 소금 섭취 관리와 관련된 정책 개선에 중요한 기초 자료를 제공할 것으로 기대된다.

핵심되는 말: 소금, 식이 습관, 초파리, 탈감작, 세포내이입, 성별 이형성.