



Figures and figure supplements

Sweet neurons inhibit texture discrimination by signaling TMC-expressing mechanosensitive neurons in *Drosophila*

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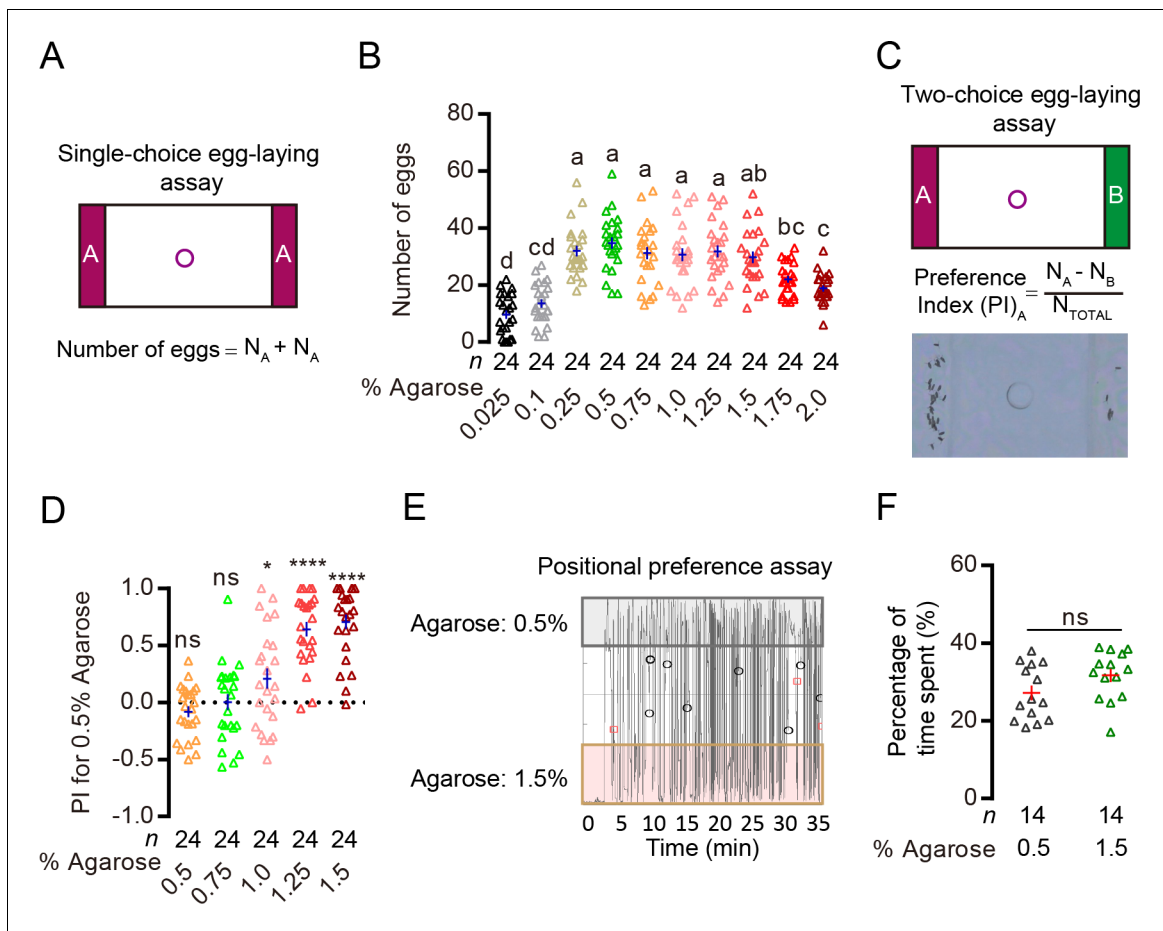


Figure 1. *Drosophila* can discriminate egg-laying substrates of different hardness. (A) Upper image: schematic of our single-choice assay. In this assay, we placed the same agarose (colored strips) on the two sides of the arena and a drop of sucrose in the center hole. The two agarose are separated by acrylic. Each of our apparatus has 30 arenas that can assay egg-laying of 30 individual females. (B) Comparison of acceptance of different concentrations of agarose for egg-laying in single-choice assay. Each data point in a column denotes the number of eggs laid by a single female over 14 hr. The numbers of females examined per group are labeled on the graph ($N = 24$ for each group in this experiment). Note that in this work, when comparisons of multiple groups were needed, we used letters (e.g. **a**, **b**) to describe the statistical relationship between them and used the following rule: groups that share at least one letter (e.g. **ab** vs. **bc**) are statistically indistinguishable, and groups that have different letters (e.g. **a** vs. **b**) are statistically different. One-way ANOVA followed by Tukey's multiple comparisons test. These comparisons may yield different p values (e.g. $p < 0.05$, $p < 0.0001$) at times, in which case, we labeled the highest. Also, throughout this work, the 'cross' labeled in each column denotes sample mean \pm s.e.m. (C) Upper panel: schematic of our two-choice assay. In this assay, we placed two different agarose (colored strips) on the two sides of the arena. Lower panel: formula for calculating egg-laying preference index (PI) and a representative image of eggs laid by a single WT female in a 0.5% vs. 1.5% two-choice assay. (D) PI (for 0.5% agarose) of WT(w^{1118}) females in different two-choice assays where 0.5% agarose was pitted against other concentrations of agarose. ns: not significant, **** $p < 0.0001$, * $p < 0.05$; Wilcoxon signed-rank test ($H_0 = 0$). (E) Representative trajectory of a single WT female as it explored the arena in a 0.5% vs. 1.5% two-choice assay. The x-axis denotes time, and the y-axis denotes the y position of the fly. (F) Quantification of the proportion of time females spent on the 0.5% agarose vs. on the 1.5% agarose in the two-choice arena. ns: not significant; Wilcoxon matched-pairs test.

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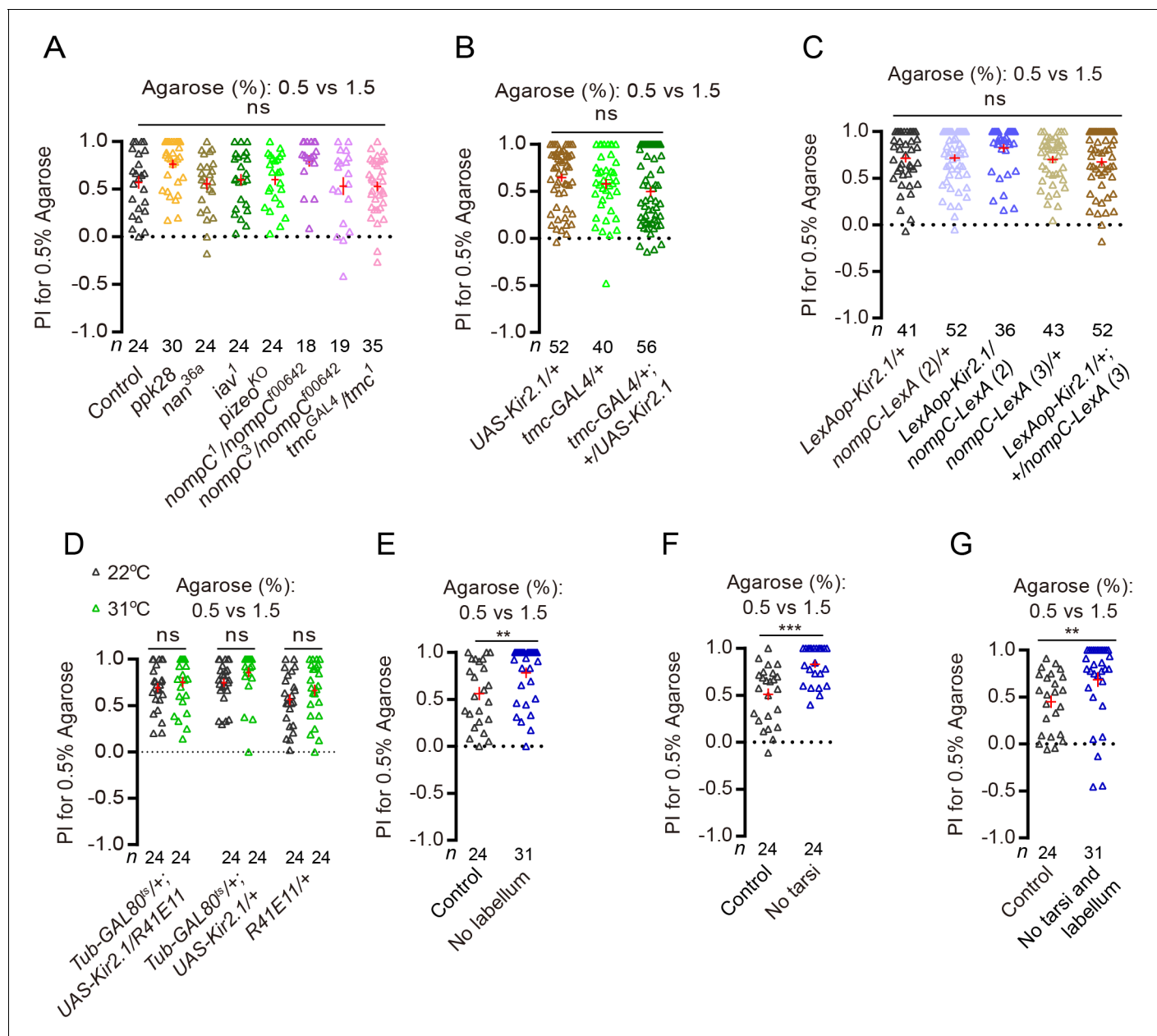


Figure 2. *Drosophila* use different channels and neurons to discriminate substrate hardness during feeding and egg-laying. (A) PI (for 0.5% agarose) of several known channel mutants in a 0.5% vs. 1.5% two-choice assay. ns: not significant; Kruskal–Wallis test followed by Dunn’s multiple comparisons test. (B–D) PI (for 0.5% agarose) of females with TMC-expressing neurons silenced (B) or NompC and Nan-expressing neurons silenced (C–D) in a 0.5% vs. 1.5% two-choice assay. (R41E11-GAL4 is another driver that labels the NompC and Nan-expressing neurons) (Jeong *et al.*, 2016). ns: not significant; Kruskal–Wallis test followed by Dunn’s multiple comparisons test for comparisons in panels (I and J), Mann–Whitney test for comparison in panel K). (E–G) PI (for 0.5% agarose) of WT females with their (E) labellum, (F) tarsi of all six legs, and (G) labellum plus tarsi of all legs removed in a 0.5% vs. 1.5% two-choice assay. *** $p < 0.001$, ** $p < 0.01$; Mann–Whitney test.

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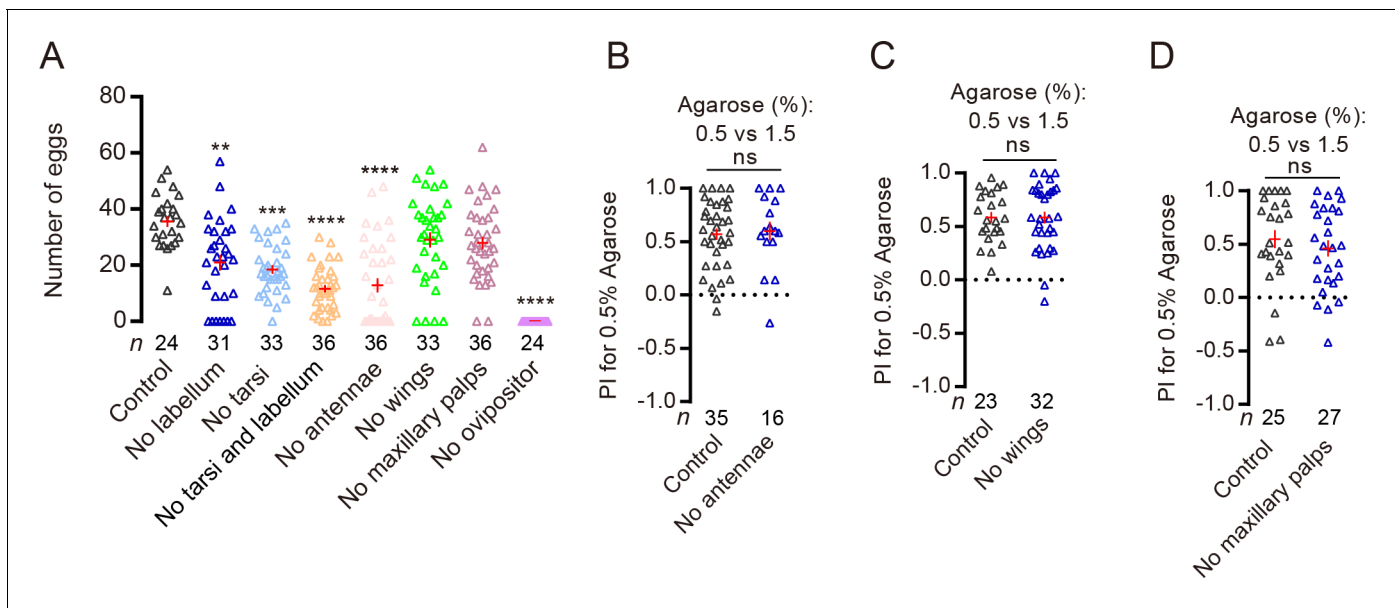


Figure 2—figure supplement 1. Contribution of different appendages on egg-laying rate and discrimination of substrate hardness. **(A)** Number of eggs laid by females with different appendages removed in single-choice assay. ****p<0.0001, ***p<0.001, **p<0.01; Kruskal–Wallis test followed by Dunn’s multiple comparisons test against control. **(B–D)** PI (for 0.5% agarose) of WT females with different appendages removed in a 0.5% vs. 1.5% two-choice assay. ns: not significant; Mann–Whitney test.

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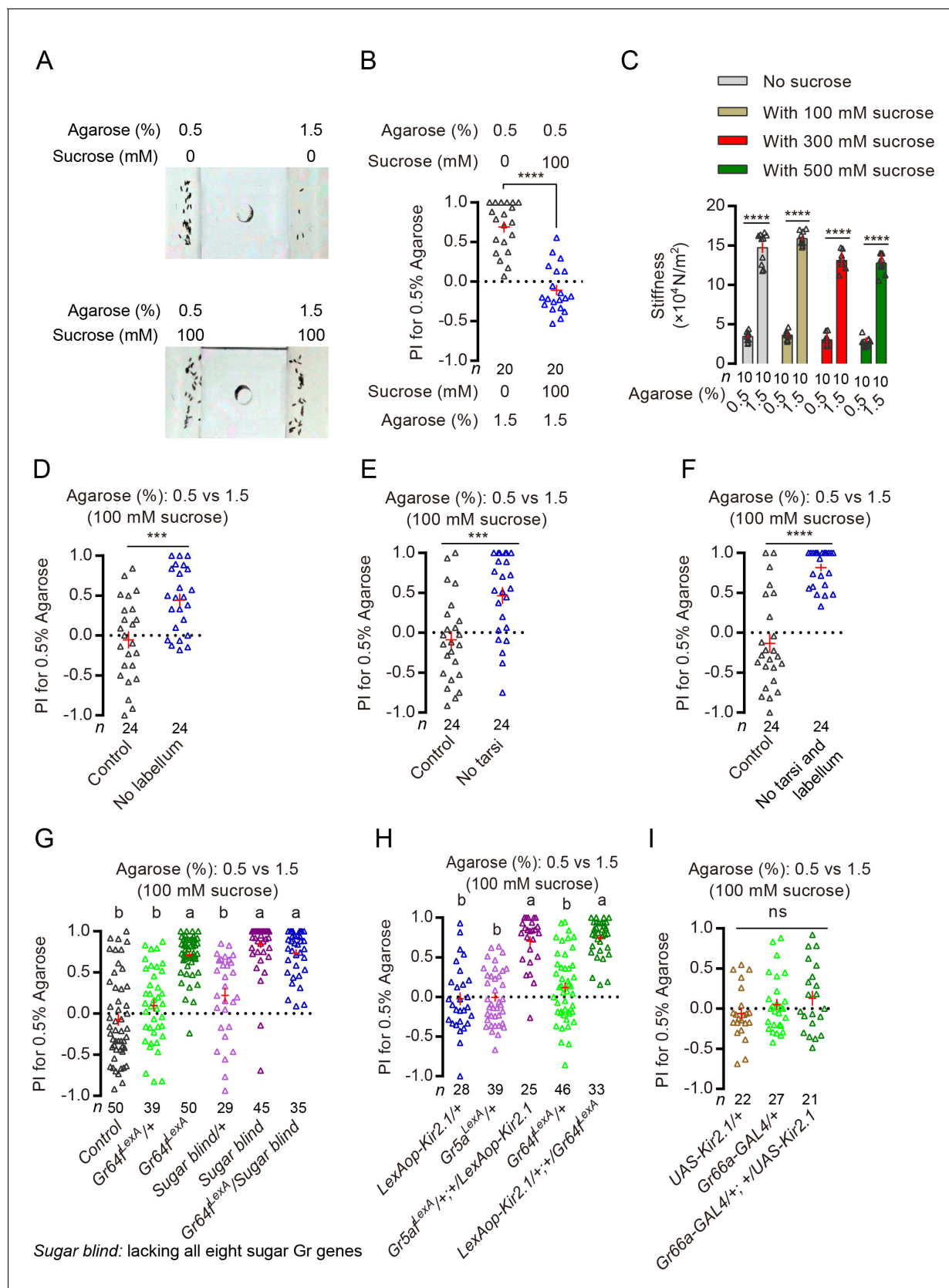


Figure 3. Detection of sucrose on substrates by sweet neurons can inhibit discrimination of substrates of different hardness. (A) Representative images of eggs laid by a single WT female in a 0.5% vs. 1.5% two-choice assay where both substrates were sugar free (top) and where both substrates

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Figure 3 continued

contained 100 mM sucrose (bottom). (B) PI (for 0.5% agarose) of WT females in a 0.5% vs. 1.5% two-choice assay where both substrates were sugar free (black) and where both substrates contained 100 mM sucrose (blue). **** $p < 0.0001$; Mann–Whitney test. The PI for the sucrose-containing group on the right is not significantly different from 0; Wilcoxon signed-rank test ($H_0 = 0$). (C) Stiffness of agarose 0.5% and 1.5% agarose substrates with or without 100 mM, 300 mM, and 500 mM of sucrose in them. **** $p < 0.0001$; Mann–Whitney test. (D–F) PI (for 0.5% agarose) of WT females with different appendages surgically removed in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. **** $p < 0.0001$, *** $p < 0.001$; Mann–Whitney test. (G) PI (for 0.5% agarose) of mutants that lacked either a critical co-receptor Gr64f (*Gr64f^{LexA}*) (Yavuz et al., 2014) for sugar sensing or all eight known sugar receptors ('sugar blind') (Yavuz et al., 2014) in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. Groups that share at least one letter are statistically indistinguishable; Kruskal–Wallis test followed by Dunn's multiple comparisons test with $p < 0.05$. (H) PI (for 0.5% agarose) of females with their *Gr64f^{LexA}* or *Gr5a^{LexA}*-labeled neurons selectively silenced in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. Groups that share at least one letter are statistically indistinguishable; Kruskal–Wallis test followed by Dunn's multiple comparisons test with $p < 0.001$. (I) PI (for 0.5% agarose) of females with their *Gr66a*-GAL4-labeled neurons (aka the bitter-sensing taste neurons) selectively silenced in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. ns: not significant; one-way ANOVA followed by Tukey's multiple comparisons test.

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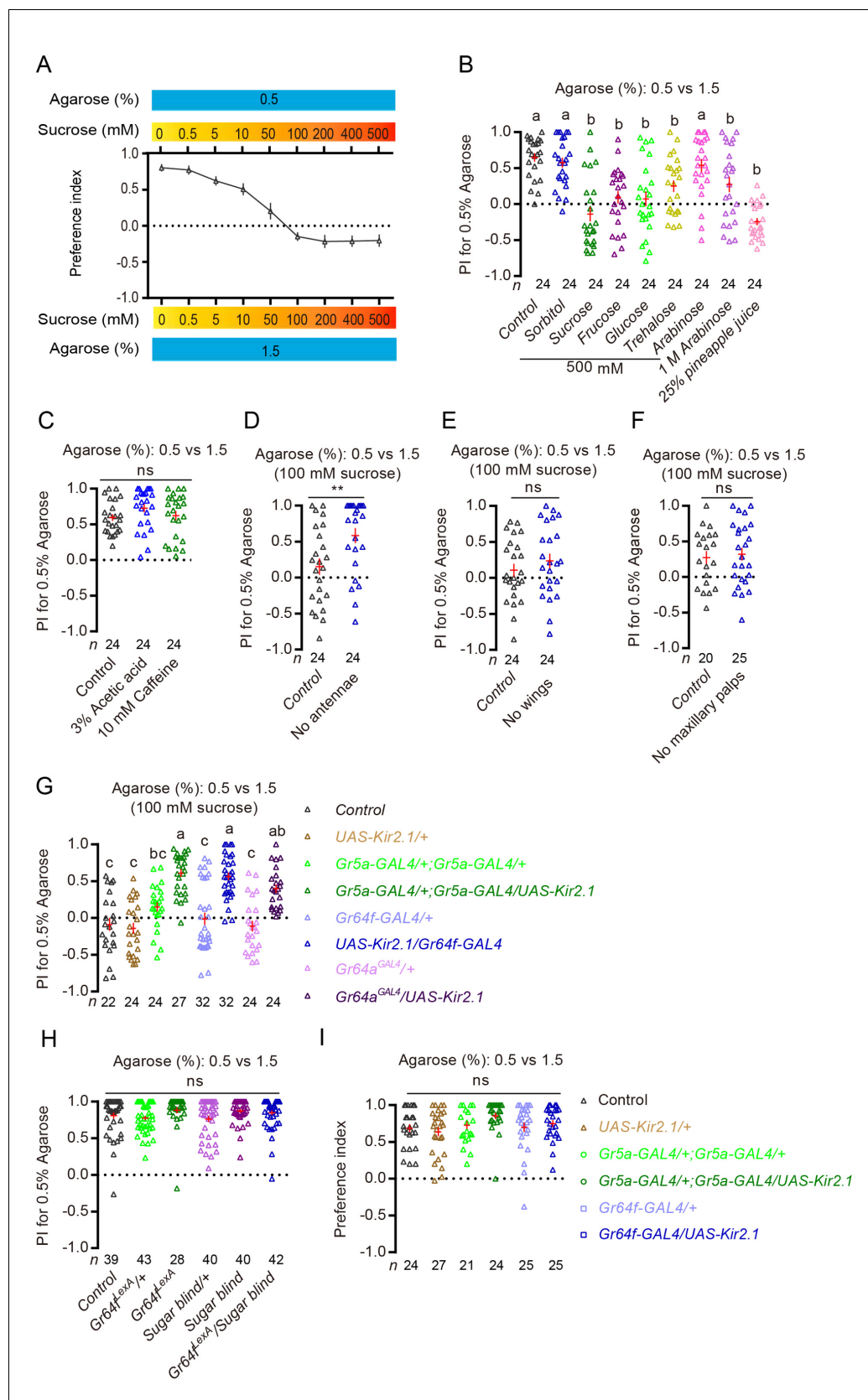


Figure 3—figure supplement 1. Sugar sensing by sweet-taste neurons is responsible for sucrose-induced indifference between substrates of different hardness. (A) Impact of sucrose on WT females' PI (for 0.5% agarose) in a 0.5% vs 1.5% two-choice assay where sucrose (from 0 mM to 500 mM) was Figure 3—figure supplement 1 continued on next page

Figure 3—figure supplement 1 continued

added to both substrates. $n = 24$. Data are mean and s.e.m. **(B)** PI (for 0.5% agarose) of WT females in a 0.5% vs. 1.5% two-choice assay where both substrates were sugar free (black) or where both substrates contained some sweet substances. Groups that share at least one letter are statistically indistinguishable; Kruskal–Wallis test followed by Dunn’s multiple comparisons test with $p < 0.01$. **(C)** PI (for 0.5% agarose) of WT females in a 0.5% vs. 1.5% two-choice assay where both substrates contained either 3% acetic acid or 10 mM caffeine. ns: not significant; Kruskal–Wallis test followed by Dunn’s multiple comparisons test. **(D–F)** PI (for 0.5% agarose) of WT females with their antennae, maxillary palps, and wings selectively severed from them in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. ns: not significant, $**p < 0.01$; Mann–Whitney test). **(G)** PI (for 0.5% agarose) of females with their sweet neurons (labeled by different *GAL4s*) silenced in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. Note that *Gr5a-GAL4* and *Gr64f-GAL4* both label sweet neurons on the labellum and legs, whereas *Gr64a-GAL4* labels only sweet neurons on the legs. Groups that share at least one letter are statistically indistinguishable; one-way ANOVA followed by Tukey’s multiple comparisons test with $p < 0.05$. **(H)** PI (for 0.5% agarose) of females that lacked either the *Gr64f* receptor (the critical co-receptor for sugar sensing) or all eight sugar receptors in a plain 0.5% vs. plain 1.5% assay. ns: not significant; Kruskal–Wallis test followed by Dunn’s multiple comparisons test. **(I)** PI (for 0.5% agarose) of females with their sweet neurons silenced in a plain 0.5% vs. plain 1.5% assay using two independently generated sweet neurons drivers. ns: not significant; Kruskal–Wallis test followed by Dunn’s multiple comparisons test.

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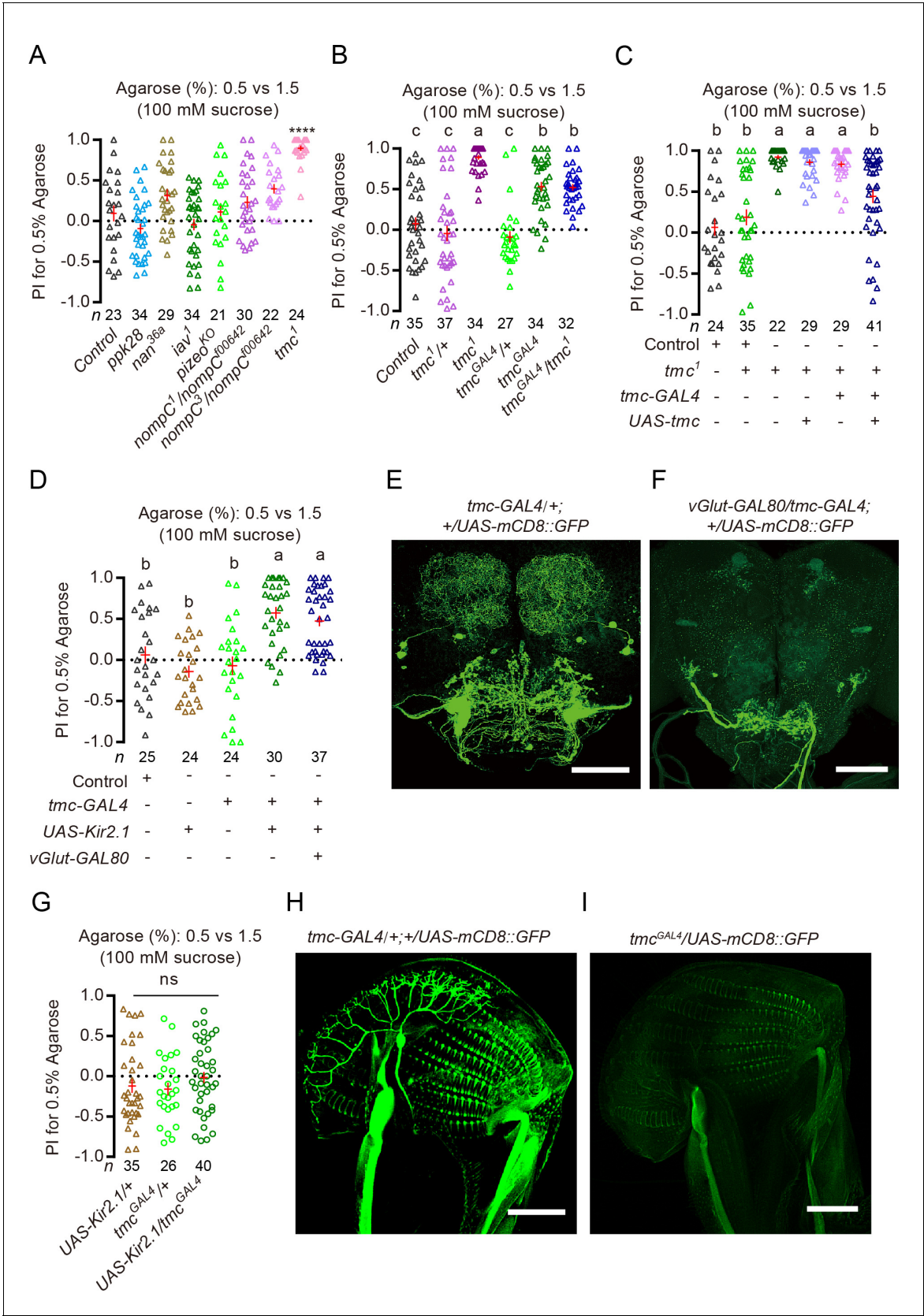


Figure 4. Sucrose-induced indifference to substrate of different hardness requires TMC and TMC-expressing neurons. (A) PI (for 0.5% agarose) of different channel mutants in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. ****p<0.0001; Mann-Whitney test, compared against control. (B and Figure 4 continued on next page

Figure 4 continued

(C) PI (for 0.5 agarose) of *tmc* mutants and mutants with *tmc* selectively rescued in *tmc-GAL4*-expressing neurons in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. *tmc*¹ and *tmc*^{GAL4} are two independently generated mutations in *tmc*. Groups that share at least one letter are statistically indistinguishable; Kruskal–Wallis test followed by Dunn’s multiple comparisons test, $p < 0.05$. Note that *tmc*^{GAL4} and *tmc-GAL4* are two independently generated GAL4s. **(D)** PI (for 0.5% agarose) of females with *tmc-GAL4*-expressing neurons selectively silenced in the presence and absence of *vGlu-GAL80* in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. Groups that share at least one letter are statistically indistinguishable; Kruskal–Wallis test followed by Dunn’s multiple comparisons test with $p < 0.05$. **(E and F)** Processes labeled by *tmc-GAL4* in the brain in the **(E)** absence and **(F)** presence of *vGlu-GAL80*. **(G)** PI (for 0.5% agarose) for females whose TMC-expressing neurons were inhibited by using *tmc*^{GAL4}, an independently generated GAL4 for *tmc*, in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. ns: not significant; Kruskal–Wallis test followed by Dunn’s multiple comparisons test. **(H and I)** Comparison of expression patterns on the labellum driven by *tmc-GAL4* vs. *tmc*^{GAL4}.

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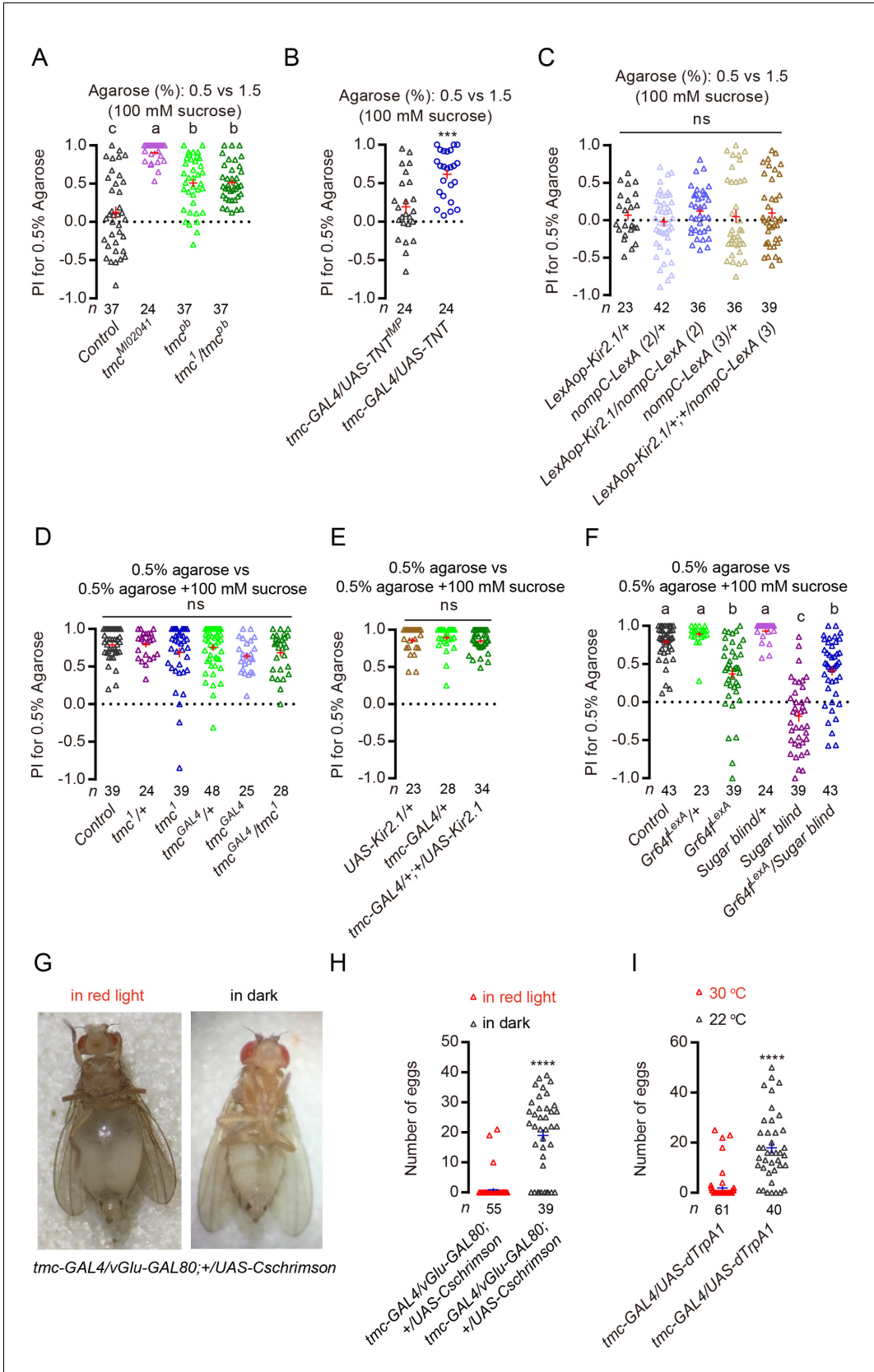


Figure 4—figure supplement 1. TMC-expressing neurons on the labellum are required for sucrose-induced inhibition of discrimination of substrate hardness. (A) PI (for 0.5% agarose) of two additional *tmc* mutants in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. Groups that share at least

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Figure 4—figure supplement 1 continued

one letter are statistically indistinguishable; Kruskal–Wallis test followed by Dunn’s multiple comparisons test with $p < 0.05$. (B) PI (for 0.5% agarose) for females with output of their TMC-expressing neurons inhibited by TNT in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. *** $p < 0.001$; Mann–Whitney test. (C) PI (for 0.5% agarose) for females with their NompC/Nan-expressing neurons inhibited in a sucrose + 0.5% vs. sucrose + 1.5% two-choice assay. We used drivers on two different chromosomes, that is *nompC-LexA* (2) and *nompC-LexA* (3), to inhibit these neurons. ns: not significant; Kruskal–Wallis test followed by Dunn’s multiple comparisons test. (D) PI (for plain 0.5% agarose) of *tmc* mutants in plain 0.5% vs. sucrose + 0.5% two-choice assay. ns: not significant; Kruskal–Wallis test followed by Dunn’s multiple comparisons test. (E) PI (for plain 0.5% agarose) of animals whose neurons labeled by the *tmc-GAL4* were selectively silenced in a plain 0.5% vs. sucrose + 0.5% two-choice assay. ns: not significant; Kruskal–Wallis test followed by Dunn’s multiple comparisons test. (F) PI (for plain 0.5% agarose) of *Gr64f* and *sugar blind* mutants in plain 0.5% vs. sucrose + 0.5% two-choice assay. Groups that share at least one letter are statistically indistinguishable; Kruskal–Wallis test followed by Dunn’s multiple comparisons test with $p < 0.05$. (G) Representative pictures of *tmc-GAL4/vGlu-GAL80/+UAS-CsChrimson* females after exposure to red light (left) or kept in darkness (right) overnight. (H) Comparison of number of eggs laid by *tmc-GAL4/vGlu-GAL80/+UAS-CsChrimson* females in red light condition vs. in dark condition. Each data point in a column denotes the number of eggs laid by a single female over 14 hr. **** $p < 0.0001$; Mann–Whitney test. (I) Comparison of number of eggs laid by *tmc-GAL4/UAS-dTrpA1* female at 30 °C vs. at 22 °C. Each data point in a column denotes the number of eggs laid by a single female over 14 hr. **** $p < 0.0001$; Mann–Whitney test.

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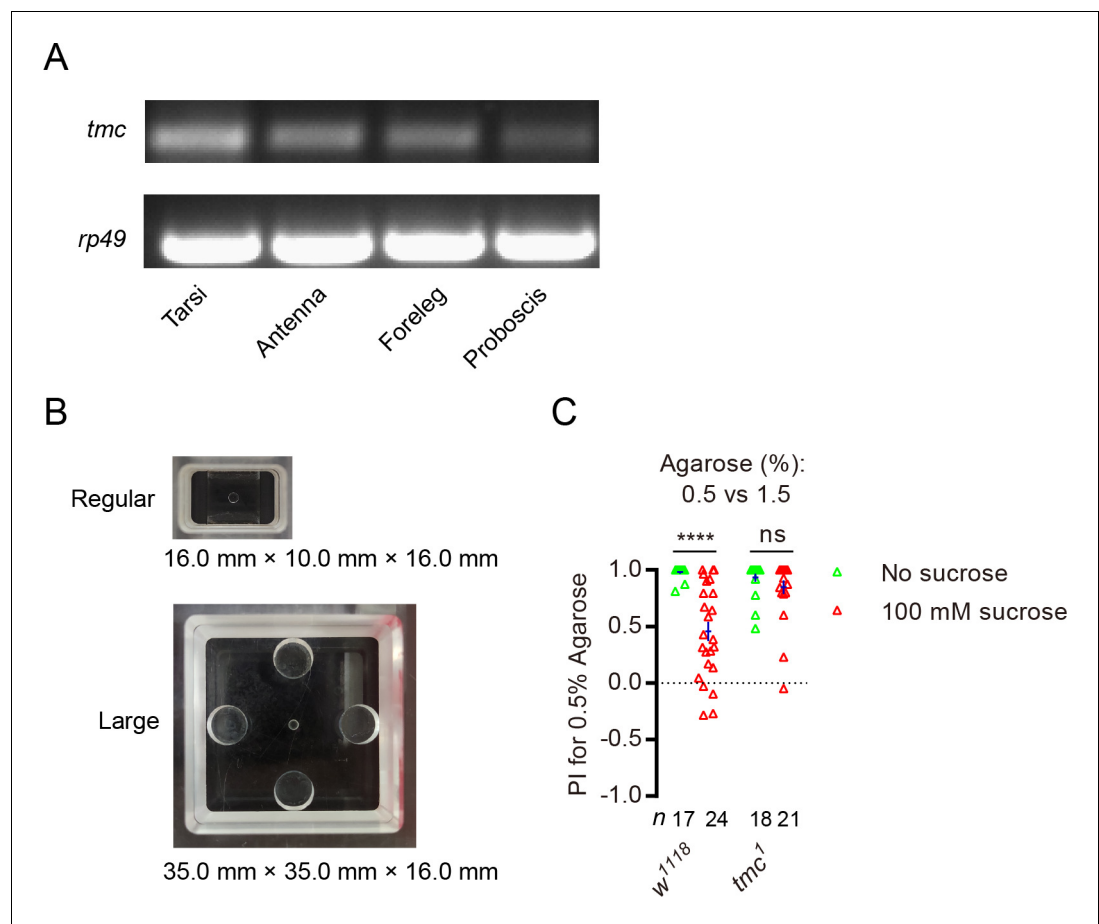


Figure 4—figure supplement 2. Additional characterizations of *tmc* expression and *tmc* mutant phenotype. (A) RT-PCR of *tmc* transcripts and *rp49* transcripts (loading control) using total RNA extracted from tarsi, antennae, foreleg, and proboscis (positive control). Note that these transcripts were sequence confirmed. (B) A comparison of the sizes of the regular arena (up) and a large arena (bottom) we used for assessing egg-laying choice. Note that for the large arenas, there are four wells per arena for housing agarose of different concentrations. (C) PI (for 0.5% agarose) of WT females in a 0.5% vs. 1.5% task in the large arenas. Green: substrates were sucrose free. Red: substrates contained 100 mM sucrose. *** $p < 0.001$, ns: not significant; Mann–Whitney test. Each data point represents one egg-laying bias from a single female.

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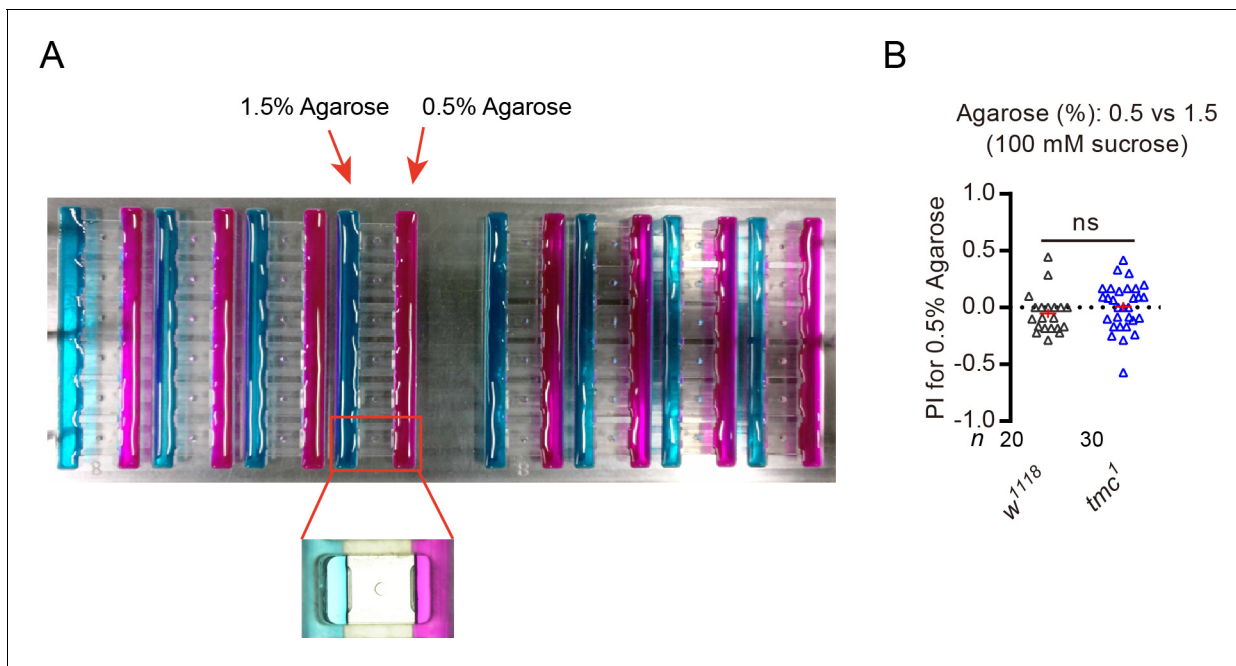


Figure 4—figure supplement 3. Feeding preference of WT and *tmc* mutants. **(A)** Our two-choice feeding assay. Up: the bottom piece of our behavior apparatus with agarose of different concentrations (in different colors) loaded into the substrate-holding troughs. Bottom: zoomed-in picture of a single arena when the apparatus was fully assembled. Note that we used the same apparatus for assessing egg-laying preferences. **(B)** Feeding preferences of WT and *tmc* mutants when given a 100 mM sucrose-containing 0.5% agarose vs. a 100 mM sucrose-containing 1.5% agarose. ns: not significant; Mann–Whitney test. See Materials and methods about how we calculated the preference index. Further, the PIs for these two groups of flies are not significantly different from 0; Wilcoxon signed-rank test ($H_0 = 0$).

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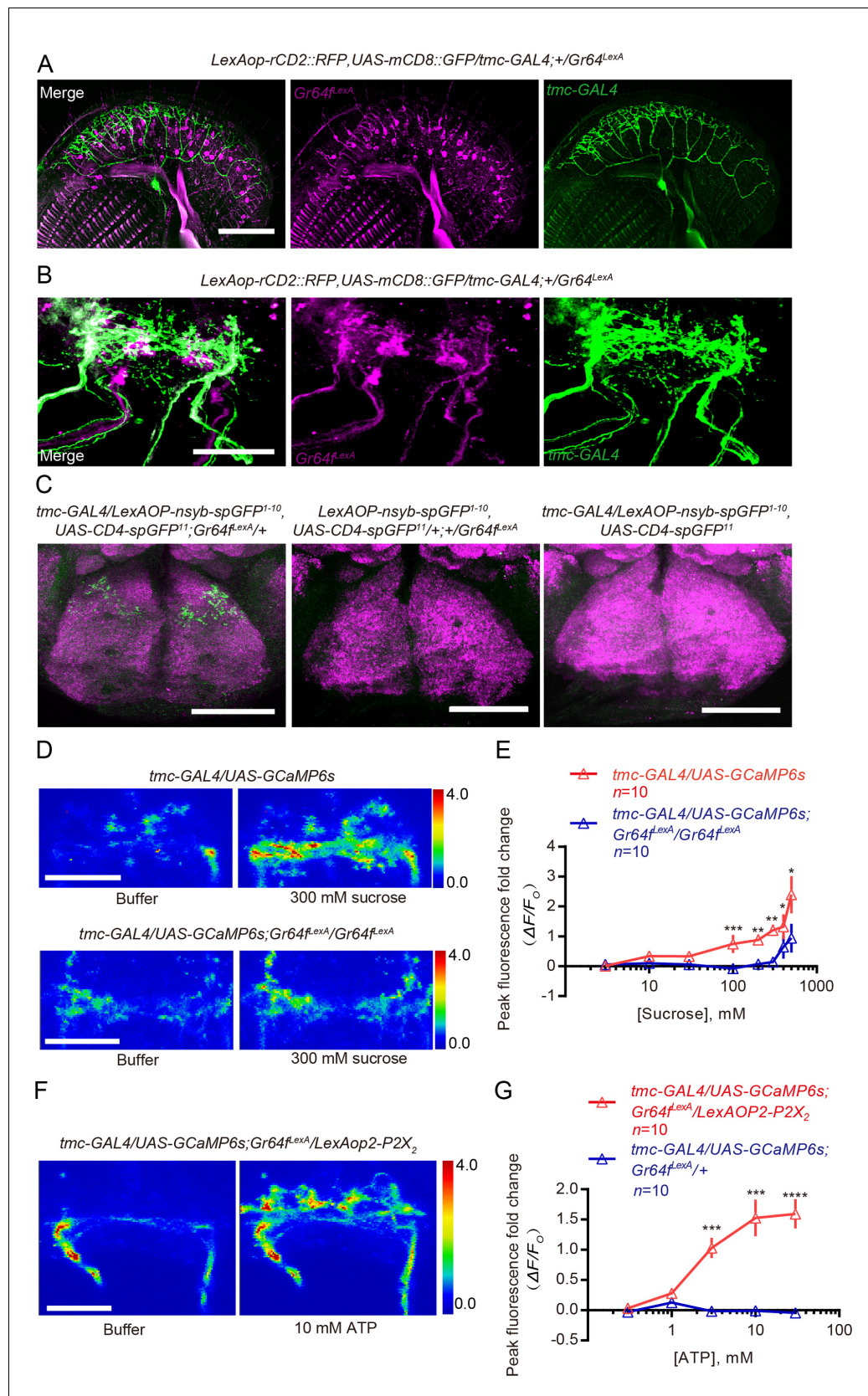


Figure 5. Axons of sweet neurons have physical contact with and can signal to axons of TMC-expressing neurons. (A and B) Double labeling of the TMC-expressing MD-L neurons and Gr64f-expressing sweet neurons on the labellum (A) and in the SEZ (B) in the brain. Scale bar: 50 μ m. (C) syb: Figure 5 continued on next page

Figure 5 continued

GRASP (green) between TMC-expressing MD-L neurons and sweet neurons. These brains were counter-stained with neuropil marker nc82 (magenta). Scale bar: 50 μm . (D) Representative images showing buffer- and sucrose-induced changes in CGaMP signal in axon termini of TMC neurons in the SEZ. Top: preparation made from WT animals; bottom: preparation made from *Gr64f* mutants (*Gr64f^{ΔexA}*). The color scale on the right shows $\Delta F/F_0$. (E) Changes in peak GCaMP intensity ($\Delta F/F_0$) in TMC axons from WT vs. *Gr64f* mutants in response to different concentrations of sucrose. ns: not significant, *** $p < 0.001$, ** $p < 0.01$; Mann–Whitney test. (F) Representative images showing buffer- and ATP-induced changes in the GCaMP signal of TMC axons in preparations made from animals that overexpressed P2X₂ in *Gr64f*-expressing sweet neurons. (G) Changes in peak GCaMP intensity ($\Delta F/F_0$) in TMC axons from animals with or without P2X₂ overexpressed in *Gr64f*-expressing sweet neurons in response to different concentrations of ATP. **** $p < 0.0001$, *** $p < 0.001$; Mann–Whitney test.

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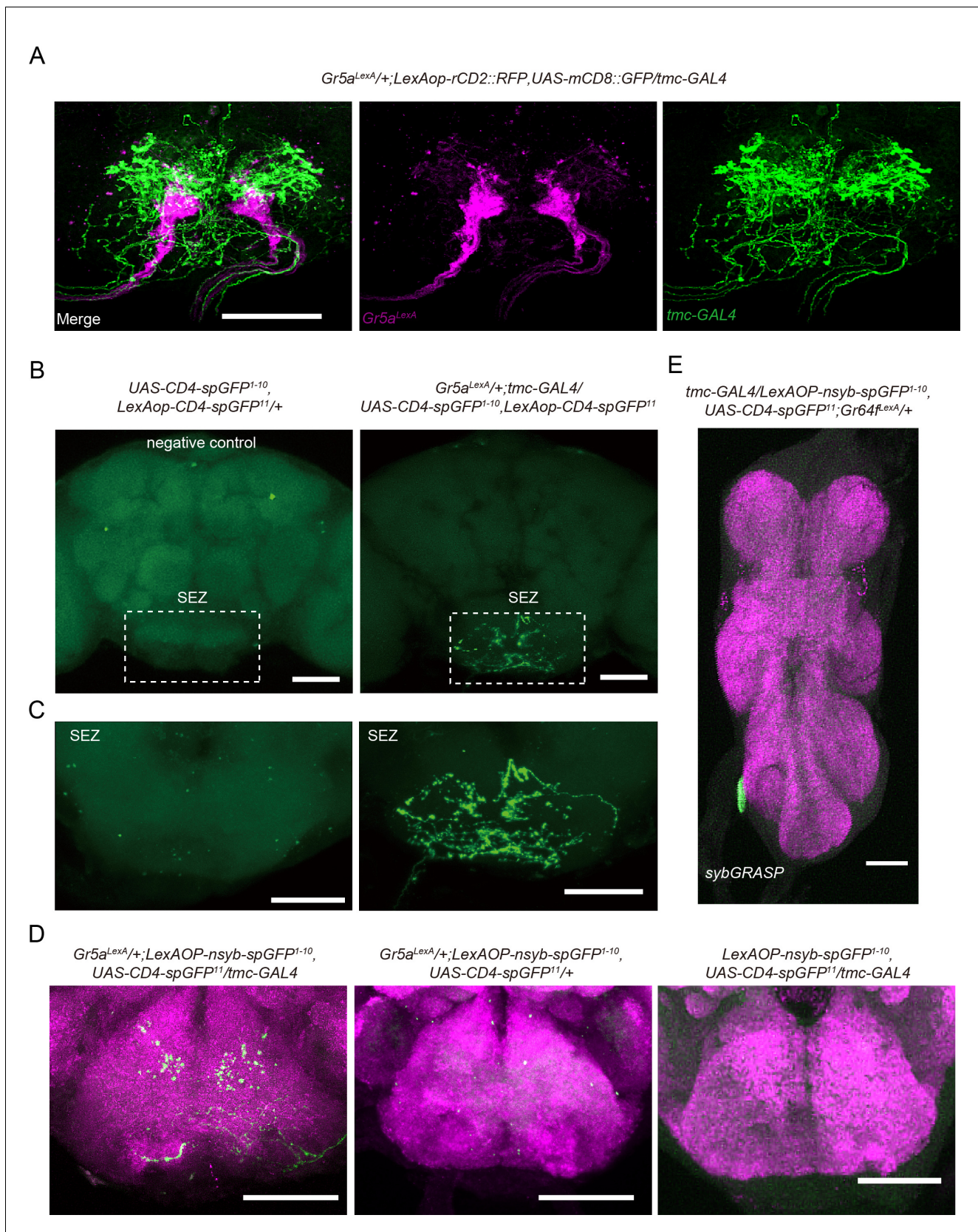


Figure 5—figure supplement 1. Axons of TMC-expressing neurons are in contact with those of sweet neurons in the SEZ. (A) Double labeling of *Gr5a^{LexA}*-labeled sweet neurons and *tmc-GAL4*-labeled neurons in the SEZ. *Gr5a^{LexA}* is an independent driver for labeling sweet neurons. Scale bar: 50 μ m. Figure 5—figure supplement 1 continued on next page

Figure 5—figure supplement 1 continued

μm. **(B and C)** Reconstituted GFP between *Gr5a^{LexA}*-labeled sweet neurons and *tmc-GAL4*-labeled neurons detected by the conventional GRASP technique in the SEZ. Scale bar: 50 μm. **(D)** Reconstituted GFP between *tmc-GAL4*-labeled neurons and *Gr5a^{LexA}*-labeled sweet neurons in the SEZ detected using the syb:GRASP technique instead. Scale bar: 50 μm. **(E)** No syb:GRASP signal can be detected in the VNC between *tmc-GAL4*-labeled neurons and *Gr64f^{LexA}*-labeled sweet neurons. Scale bar: 50 μm.

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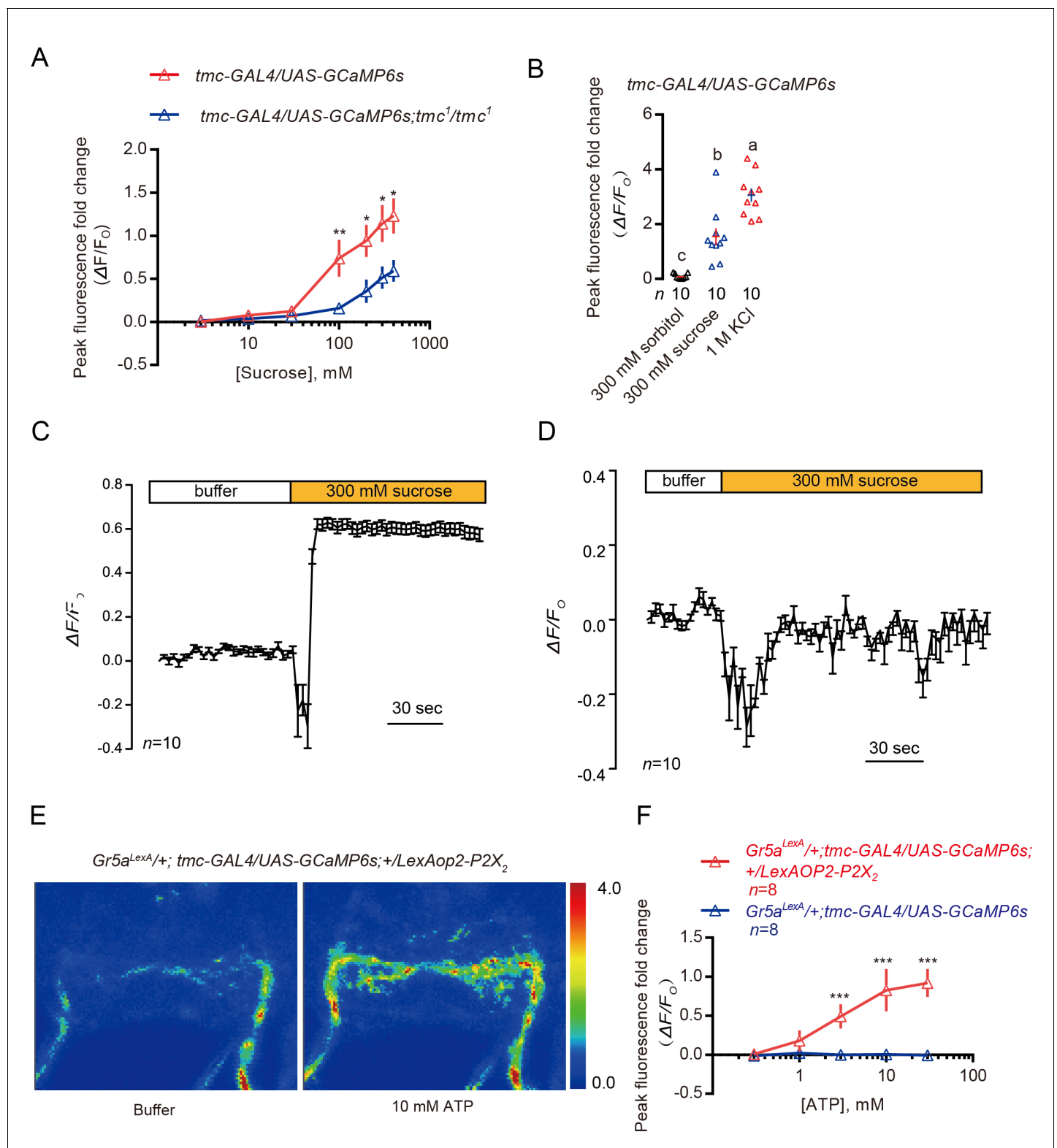


Figure 5—figure supplement 2. Axons of TMC-expressing neurons respond to sucrose but such a response is not intrinsically derived and is diminished in the absence of TMC. (A) Changes in peak GCaMP intensity ($\Delta F/F_0$) in TMC axons from WT vs. *tmc* mutants in response to different concentrations of sucrose. ns: not significant, ** $p < 0.01$, * $p < 0.05$; Mann-Whitney test. (B) Changes in GCaMP ($\Delta F/F_0$) in axons of TMC-expressing neurons in response to 300 mM sorbitol, 300 mM sucrose, and 1 M KCl in our ex vivo preparation. Groups that share at least one letter are statistically indistinguishable; one-way ANOVA followed by Tukey's multiple comparisons test with $p < 0.05$. (C) Changes in GCaMP ($\Delta F/F_0$) in the somas of a few Figure 5—figure supplement 2 continued on next page

Figure 5—figure supplement 2 continued

sweet neurons in response to 300 mM sucrose. The trace was averaged from ten samples. The solid line represents the means, and the error bars indicate s.e.m. (D) Changes in GCaMP ($\Delta F/F_0$) in somas of TMC neurons in response to 300 mM sucrose. The trace was averaged from ten samples. The solid line represents the means, and the error bars indicate s.e.m. (E) Images showing the changes in GCaMP in axons of TMC-expressing neurons in response to buffer or 10 mM ATP when P2X₂ was expressed in *Gr5a^{LexA}*-expressing neurons. (F) Changes in GCaMP signals ($\Delta F/F_0$) in axons of TMC-expressing neurons in response to different concentrations of ATP when P2X₂ was expressed in *Gr5a^{LexA}*-expressing neurons. *** $p < 0.001$; Mann–Whitney test.

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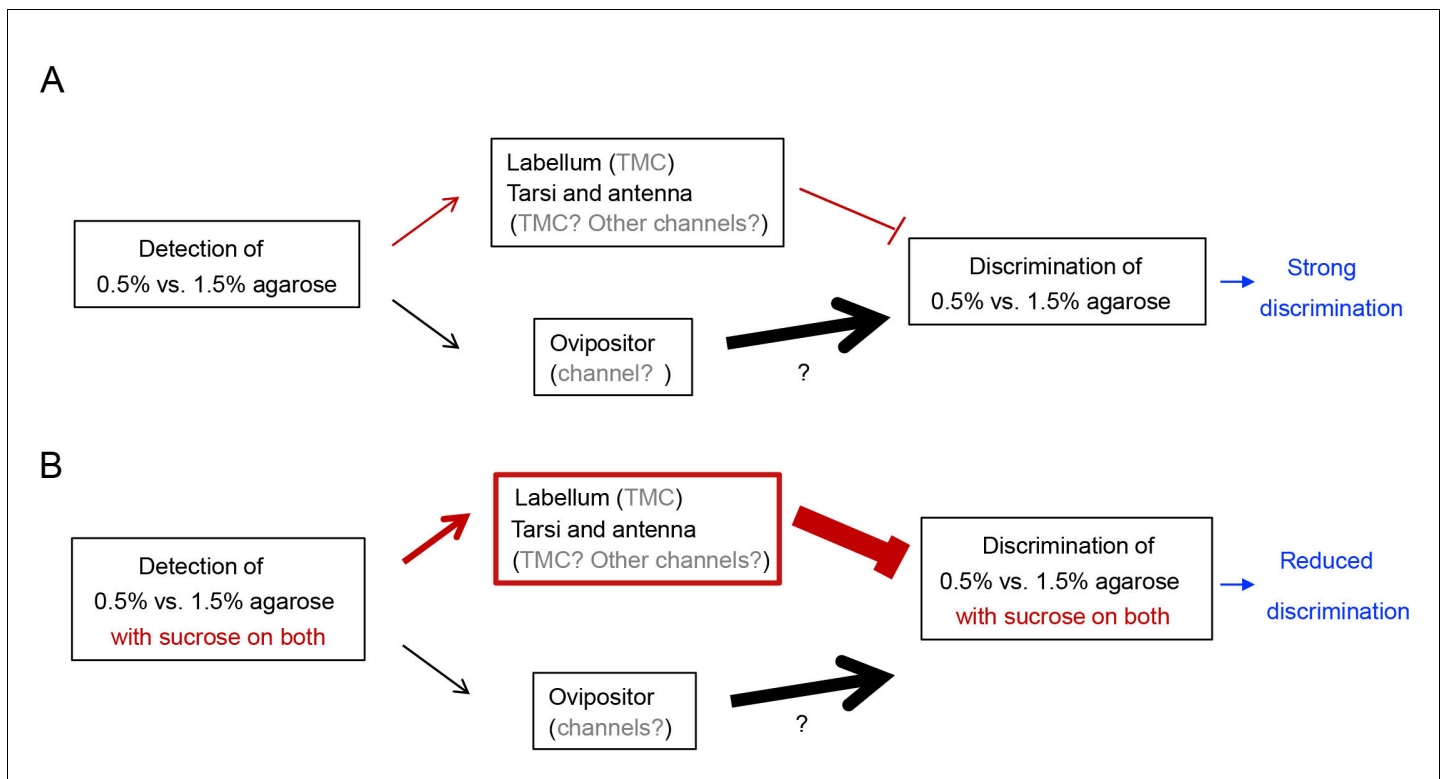


Figure 6. A tentative model explaining how discrimination of egg-laying substrates of different hardness is regulated by mechanosensory neurons on different appendages. **(A)** In the absence of sucrose, detection of hardness of egg-laying substrates by mechanosensory neurons on the ovipositor promotes discrimination whereas detection of hardness of substrates by mechanosensory neurons on the tarsi and labellum inhibits discrimination. Moreover, animals discriminate the plain 0.5% vs. 1.5% agarose well because the contribution from the mechanosensory neurons on the ovipositor dominates over that from the tarsi and labellum in this decision. (We note that while *tmc* transcripts are present on tarsi, labellum, and antenna, inhibition of hardness discrimination in the absence of sucrose may be promoted by mechanosensitive channels that we have not identified in this work. Further, we have only indirect evidence that supports the idea that the specific mechanosensitive neurons critical for egg-laying substrate discrimination are present on the ovipositor.) **(B)** In the presence of sucrose, however, contribution from the mechanosensory neurons on the labellum, tarsi, and antenna increases, partly because output of TMC neurons on the labellum can be enhanced by sucrose-induced activation of sweet neuron. Consequently, animals discriminate the two sweet substrates less well due to enhanced input from mechanosensitive neurons that inhibit discrimination. (We note that TMC-expressing neurons on the tarsi and antenna may contribute to sucrose-induced inhibition, too, given that *tmc* transcripts were present on both. However, their precise relationship with sweet neurons is not known. Further, some mechanosensitive channels that we have not identified in this work may contribute, too).

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