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Supplementary Information for

Meeting a threat of the Anthropocene: taste avoidance of metal ions by *Drosophila melanogaster*

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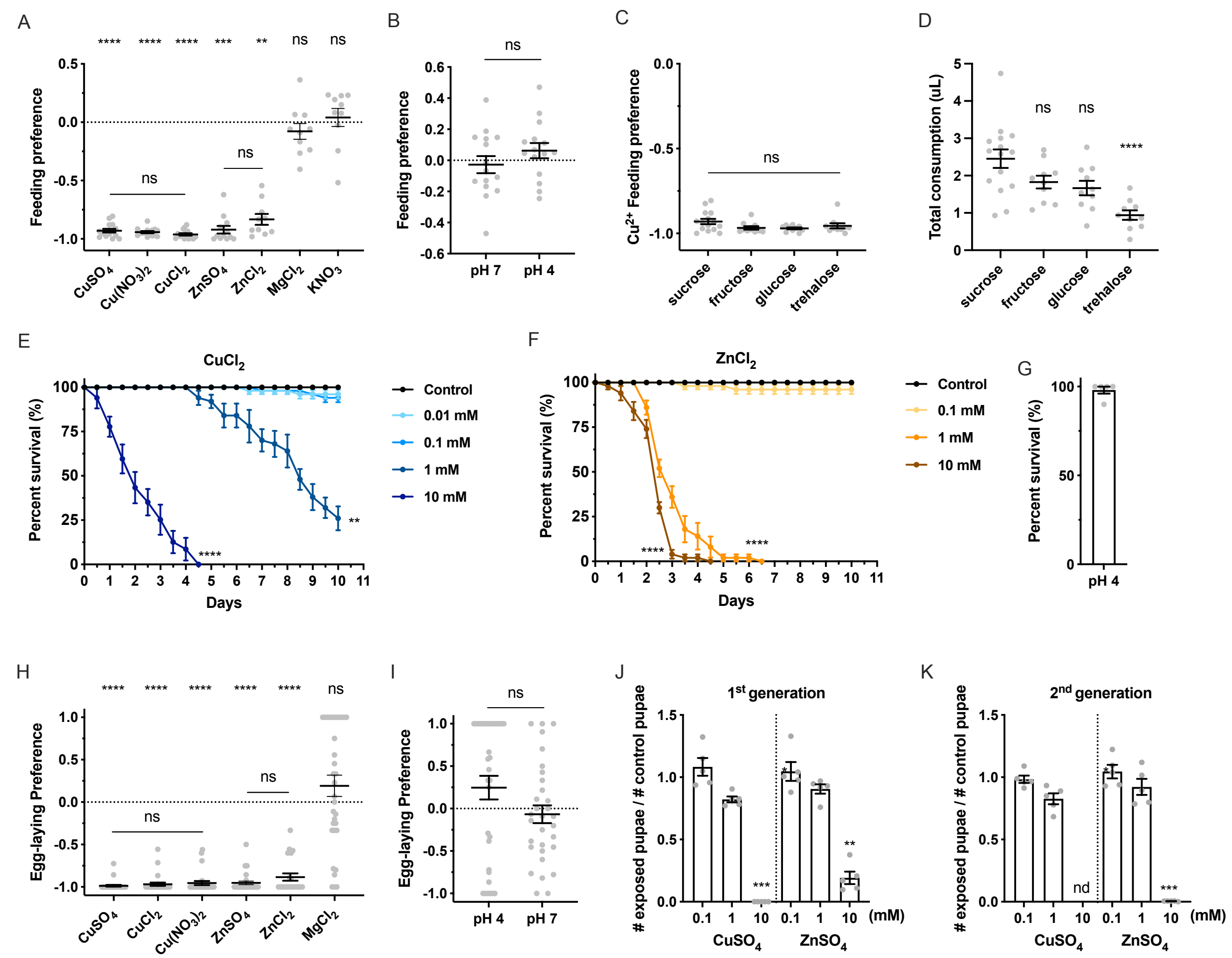

Fig. S1. Cation-specific taste avoidance to copper and toxic effects of copper and zinc.

(A) Feeding preferences to 10 mM $CuSO_4$, $CuCl_2$, $Cu(NO_3)_2$, $ZnSO_4$, $ZnCl_2$, $MgCl_2$, and KNO_3. Asterisks on top indicate significant differences from 0. n = 10-15. ****$p < 0.0001$, Wilcoxon signed tank test. No significant differences were found among different copper salts. Kruskal-Wallis test. (B) Feeding preferences to 10 mM sucrose at neutral pH or pH 4, acidified with HCl, tested against 10 mM sucrose alone. n = 15. p = 0.436, Mann-Whitney U test. (C) Feeding preferences to 10 mM $CuSO_4$ in CAFE assay, with 10 mM sucrose replaced with 10 mM other sugars as indicated above. n = 10-15. p = 0.176, Kruskal-Wallis test. (D) Total volume consumed by 10 flies in the CAFE assay within 3 hours. Conditions were the same as in (C). n = 10-15. ****$p < 0.0001$, Kruskal-Wallis test followed by uncorrected Dunn's test. (E) Survival rate of flies raised on normal food and then transferred to 100 mM sucrose mixed with $CuCl_2$ at the indicated concentrations for 10 consecutive days. n = 5. **$p < 0.01$, ****$p < 0.0001$. Friedman test followed by uncorrected Dunn's test. (F) Survival rate of flies raised on 100 mM sucrose mixed with $ZnCl_2$ at the indicated concentrations for 10 consecutive days. n = 5. ****$p < 0.0001$. Friedman test followed by uncorrected Dunn's test. (G) Survival rate of flies raised on 100 mM sucrose acidified to pH 4 with HCl for 10 days. n = 5. (H) Egg-laying preferences to 10 mM $CuSO_4$, $CuCl_2$, $Cu(NO_3)_2$, $ZnSO_4$, $ZnCl_2$, and $MgCl_2$. Asterisks on top indicate significant differences from 0. n = 22-33. ****$p < 0.0001$, Wilcoxon signed-rank test. No significant differences were found among different copper salts. Kruskal-Wallis test. (I) Egg-laying preferences to 100 mM sucrose at neutral pH or pH 4 acidified with HCl against 100 mM sucrose alone. n = 31-36. p = 0.075, Mann-Whitney U test. (J) Pupae number in metal-supplemented vials as compared to control vials for the first generation. n = 5. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, Kruskal-Wallis test followed by uncorrected Dunn's test. (K) Pupae number in metal-supplemented vials as compared to control vials for the second generation. "nd", not determined, as no viable adults were available in this condition. n = 5. * $p < 0.05$, *** $p < 0.001$, Kruskal-Wallis test followed by uncorrected Dunn’s test. Error bars are SEM.

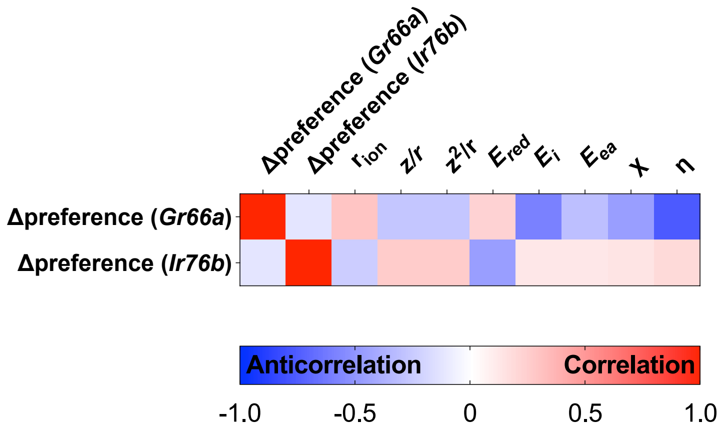

Fig. S2. Spearman correlation matrix between the chemical properties of metal ions and the dependence of the corresponding avoidance responses on *Gr66a* or *Ir76b*

Δpreference (*Gr66a*) and Δpreference (*Ir76b*) are defined below. r_{ion}, effective ionic radii; values were taken from (1). *z* refers to charge number. *z/r*, ratio of charge to radius. z^2/r is directly related to hydration energy of a cation. E_{red}, reduction potential; values were taken from (2). E_i, ionization energy. E_{ea}, electron affinity. χ, absolute electronegativity. η, absolute hardness; values were taken from (3). The analysis was performed with the 9 metal ions tested in Fig. 3: Cu^{2+}, Zn^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Ag^{+}, and Cd^{2+}. The strongest correlation was between Δpreference (*Gr66a*) and η (Spearman correlation r = -0.767, p = 0.021). In two cases the preference was slightly more negative for the mutant than the control and in these two cases we have simplified by using 0 as a value for the Δpreference; thus we use the larger ("max") of the Δpreference or 0. abs, absolute value.

$$\Delta\text{preference}\ (\textit{receptor}) = \frac{\text{max}((\text{preference}(\textit{receptor})\text{-preference}(\text{Control})), 0)}{\text{abs}(\text{preference}(\text{Control}))}$$

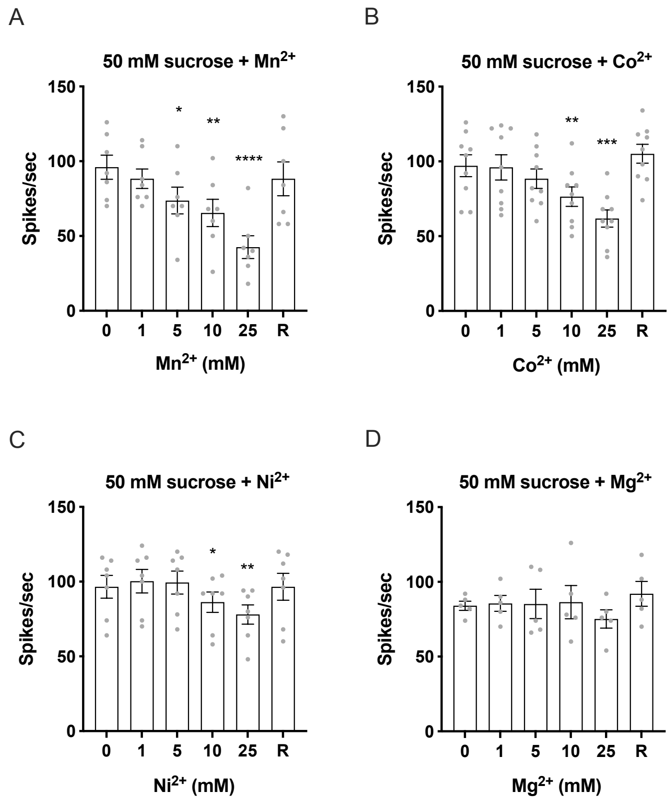

Fig. S3. Effects of different concentrations of $MnSO_4$, $CoSO_4$, $NiSO_4$, and $MgCl_2$ on sugar responses in L sensilla.

Mixtures of 50 mM sucrose with increasing concentrations of metal ions were applied to the same L sensilla at 2 min intervals. “R” denotes “recovery”, *i.e.* the response to 50 mM sucrose after the sensilla were stimulated with sucrose-metal mixtures. (A) Responses to sucrose and sucrose-manganese mixtures from L sensilla. n = 7, *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, Friedman test followed by uncorrected Dunn’s test. (B) Responses to sucrose and sucrose-cobalt mixtures from L sensilla. n = 9, **$p < 0.01$, ***$p < 0.001$, Friedman test followed by uncorrected Dunn’s test. (C) Responses to sucrose and sucrose-nickel mixtures from L sensilla. n = 7, *$p < 0.05$, **$p < 0.01$, Friedman test followed by uncorrected Dunn’s test. (D) Responses to sucrose and sucrose-magnesium mixtures from L sensilla. n = 5, $p = 0.374$, Friedman test. Error bars are SEM.

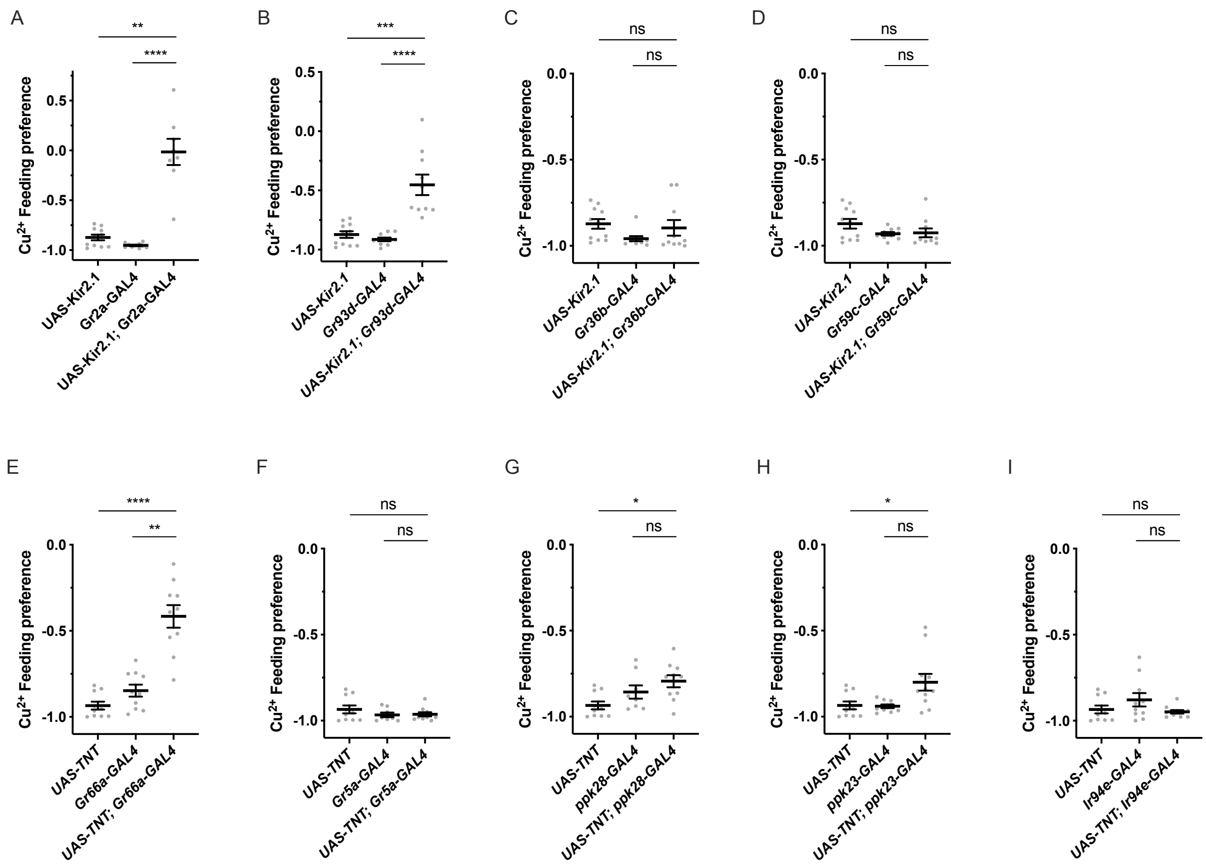

Figure S4. Feeding preferences to 10 mM $CuSO_4$ when signaling from subsets of taste neurons is impaired. (A-D) Effect of expression of Kir2.1 with different receptor-GAL4 drivers on feeding preference to copper. n = 10-11. **p < 0.01, ***p < 0.001, ****p < 0.0001, Kruskal-Wallis test followed by Dunn's tests. (E-I) Effect of expression of TNT with different receptor-GAL4 drivers on feeding preference to copper. n = 8-10. *p < 0.05, **p < 0.01, ****p < 0.0001, Kruskal-Wallis test followed by Dunn's tests. Error bars are SEM.

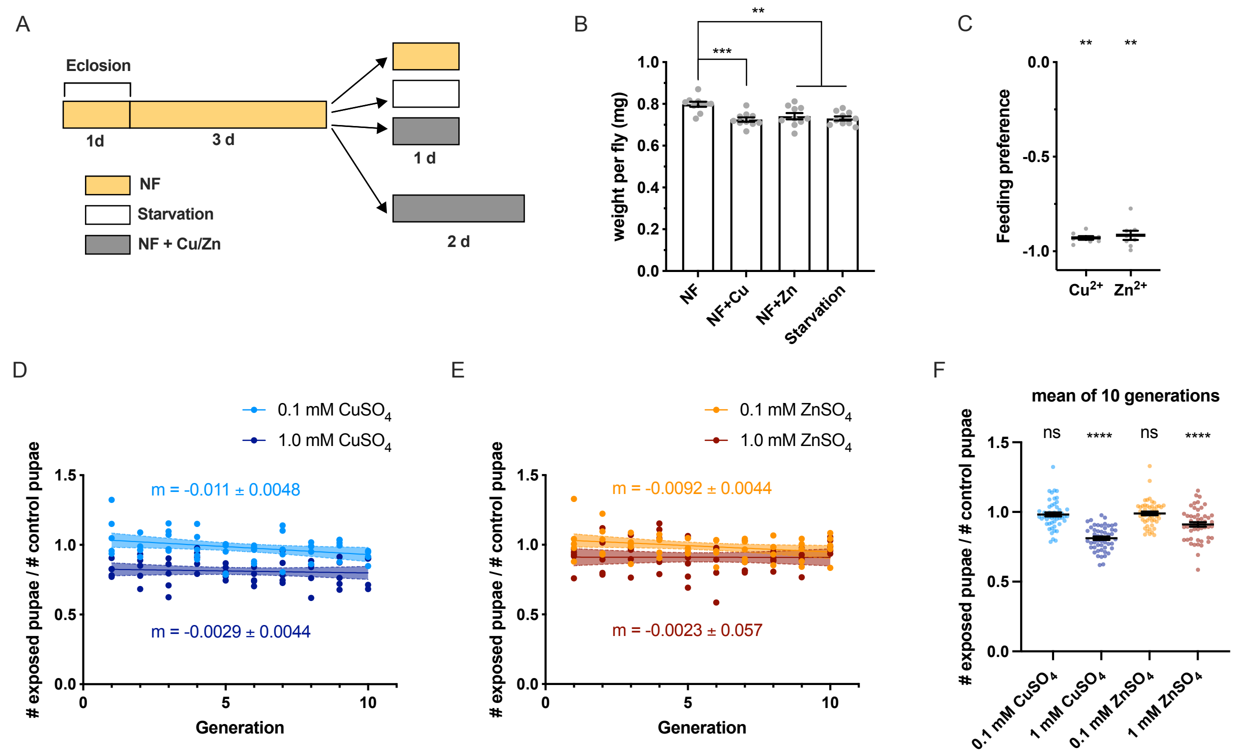

Fig. S5. Effect of short- and long-term exposure of copper and zinc.

(A) Exposure regime. “NF” denotes normal food. "Cu/Zn" indicates either Cu or Zn. B) Weight per male fly after short exposure of copper or zinc, or starvation. n = 10. **p < 0.01, ***p < 0.001, Kruskal-Wallis test followed by uncorrected Dunn’ tests. (C) Feeding avoidance of 10 mM CuSO4 and 10 mM ZnSO4 in male flies. (D) Ratio of pupae in copper-supplemented vials as compared to control vials for 10 generations. n = 5. Simple linear regression was plotted with 95% confidence intervals. m, the slope is given with SEM. (E) Ratio of pupae in zinc-supplemented vials as compared to control vials for 10 generations. n = 5. Simple linear regression was plotted with 95% confidence intervals. m, the slope was given with SEM. (F) Means of ratios of pupae in metal-supplemented vials to control vials across 10 generations. n = 50, i.e. 5 vials per generation x 10 generations. Asterisks indicate significant differences from 1. ****p < 0.0001, Wilcoxon signed-rank test. Error bars are SEM.

Table S1. Number of action potentials in Fig. 4B (mean ± SEM)

	Zn^{2+}	Co^{2+}	Mn^{2+}	Ni^{2+}	Cd^{2+}	Cu^{2+}
S5	37.8 ± 1.8	34.4 ± 6.4	31.7 ± 2.5	29.0 ± 5.3	24.4 ± 2.6	12.0 ± 1.9
S3	34.2 ± 4.4	29.0 ± 9.5	24.0 ± 9.0	16.6 ± 7.3	21.6 ± 4.4	8.4 ± 2.0
S9	34.0 ± 6.6	18.3 ± 2.8	16.2 ± 3.5	20.2 ± 4.9	16.8 ± 1.0	8.8 ± 2.7
S1	4.0 ± 1.8	1.2 ± 0.6	3.5 ± 1.1	3.2 ± 2.0	0.8 ± 0.8	3.8 ± 0.9
S10	2.2 ± 1.2	2.5 ± 2.5	9.0 ± 4.4	4.5 ± 2.1	1.2 ± 1.0	7.0 ± 2.0
S7	10.2 ± 2.7	13.3 ± 4.8	14.4 ± 2.1	10.9 ± 2.2	1.3 ± 1.0	8.0 ± 2.3
S2	5.2 ± 1.9	9.7 ± 2.8	8.6 ± 4.3	8.4 ± 4.6	8.4 ± 1.9	9.7 ± 2.6
S6	6.9 ± 2.9	13.6 ± 6.2	6.3 ± 2.0	5.8 ± 3.1	7.6 ± 2.4	10.9 ± 1.7

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