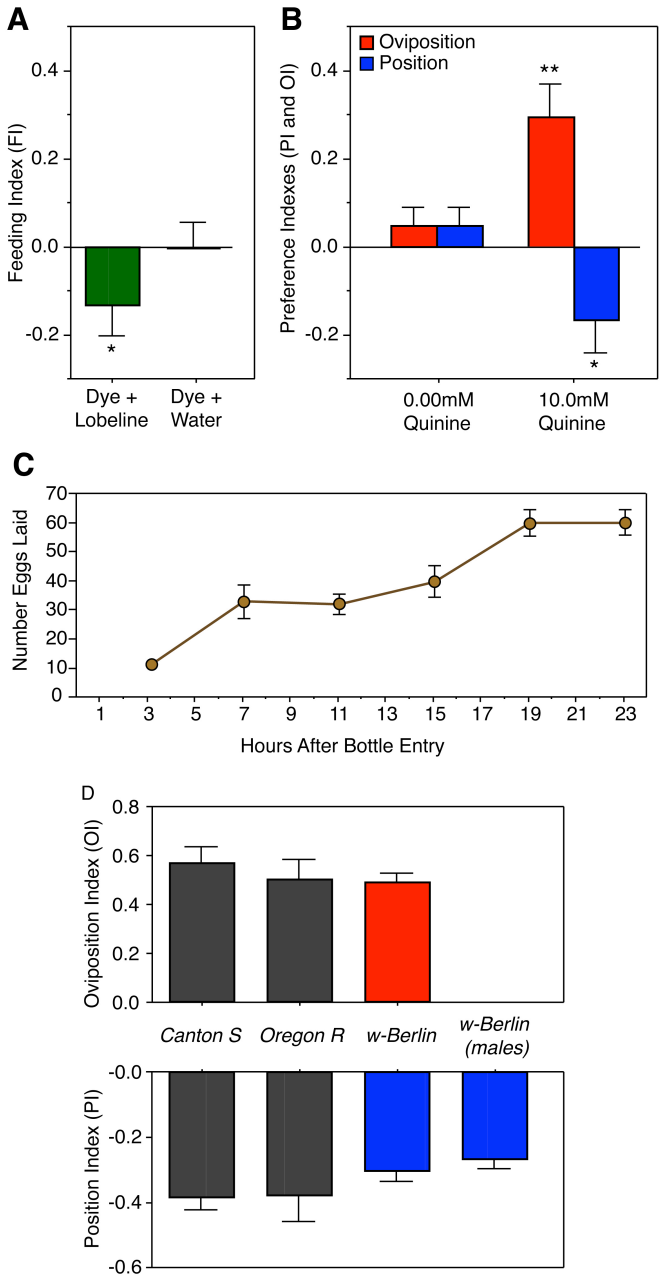

Figure S1 Additional characterization of bitter-induced responses in female *Drosophila*. (A) Females demonstrated aversion to lobeline in two-choice feeding assays. Feeding preference indexes (FI) were obtained for dishes containing either (i) blue dye + 0.50 mM lobeline / green dye + water, or reciprocal (ii) blue dye + water / green dye + 0.50 mM lobeline two-choice combinations. Variation due to day-to-day preferences for dye alone was corrected for using paired FI values obtained from blue dye + water / green dye + water controls to normalize indexes. FI values for the reciprocal two-choice dye + lobeline experiments were then pooled and averaged for comparison to the mean FI of the corrected no-choice blue dye + water / green dye + water controls (*, $P<0.05$; paired t-test (two tailed); n=16). (B) Females also exhibited positional aversion and egg-laying attraction to 10 mM quinine, another bitter tasting compound, when compared to no-quinine controls (*, $P<0.05$; **, $P<0.01$; unpaired t-test (two-tailed); $n\geq7$). (C) Average number of total eggs laid at different time-intervals by females assayed in experiments from (Figure 1C). Groups of females needed to lay more than 10 eggs per assay for reliable oviposition indexes; thus groups were allowed to lay eggs overnight. (D) Females from the *Canton S, Oregon R*, and w^{1118} *Berlin* genetic backgrounds exhibit similar positional aversion and egg-laying attraction responses to 0.50 mM lobeline ($P>0.05$, 1-way ANOVA; $n\geq7$). Additionally, w^{1118} *Berlin* males are equally repulsed to 0.50 mM as w^{1118} *Berlin* females.

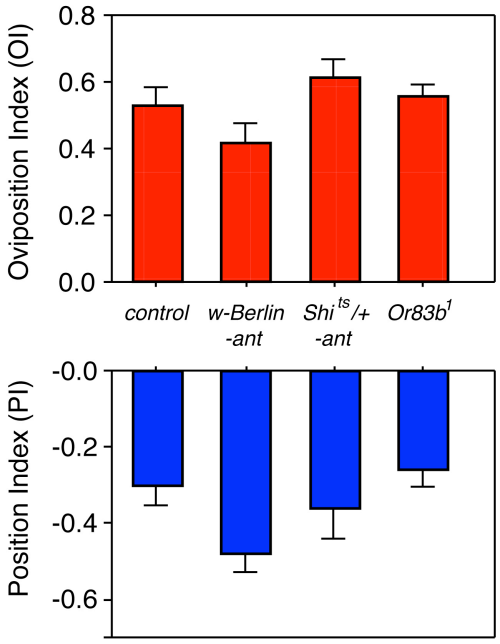

Figure S2 Females with disrupted olfactory systems exhibit normal lobeline-induced behavioral responses. *w*^{1118} *Berlin* females lacking antenna, mixed background *UAS-Shi*^{ts}*/+* females lacking antenna, and *w*^{1118} *Berlin Or83b*^{1}*/Or83b*^{1} mutant flies exhibit positional aversion and egg-laying attraction for 0.50 mM that is not significantly different from wild-type *w*^{1118} *Berlin* control females (P>0.05; 1-way ANOVA, Dunnett's multiple comparison post-test; n≥10).

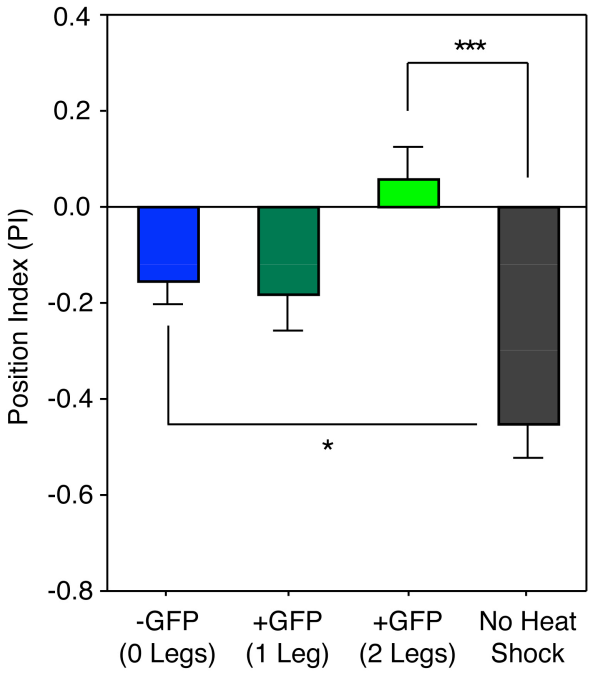

Figure S3 Single females with silenced *Gr66a* neurons in the legs have diminished positional aversion to lobeline. (A) Mean PI values for females grouped as either *–GFP* heat shocked females that lacked clones in the legs (blue bar); possessing a *+GFP*, *UAS-TeTx* silenced clone on one leg (teal bar); possessing *+GFP*, *UAS-TeTx* silenced neurons on both legs (green bar); or controls of the same genotype that did not undergo heat shock (gray bar). Females with silenced *Gr66a* neurons on both legs trended towards a loss of positional aversion, but a significant difference was only seen when compared to the no heat shock controls, likely due to the increased variability associated with obtaining PI values in single fly assays. Of note, the no heat shock controls were significantly more repulsed than *–GFP* females, suggesting that the heat shock itself could have some effects on positional responses in our assay. (*, P<0.05; ***, P<0.001; 1-way ANOVA, Bonferroni post-test; n=59 for –GFP, n=21 for +GFP 1-leg, n=9 for +GFP 2-legs, n=18 for no heat shock).

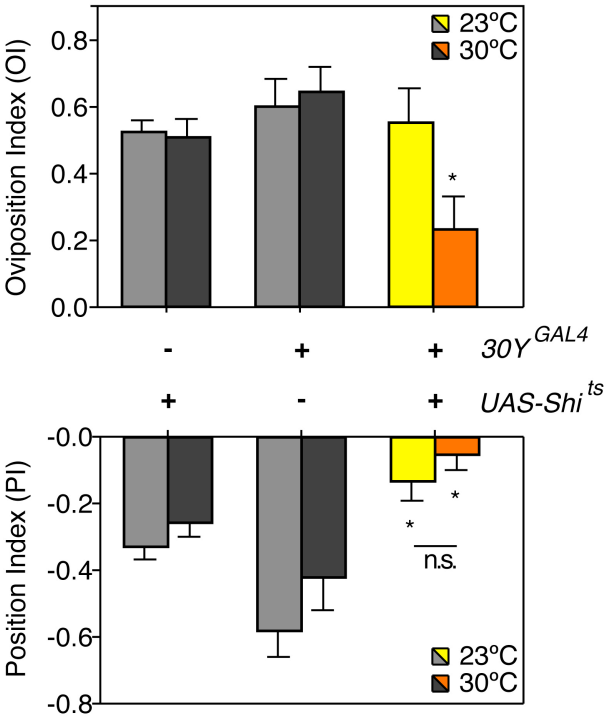

Figure S4 Silencing the mushroom body in *30Y^{GAL4}* females disrupts aversive positional and attractive egg-laying responses. (A) At the non-permissive temperature (30°C), *30Y^{GAL4}* females expressing *UAS-Shi^{ts}* in the mushroom body lose both positional aversion and egg-laying attraction to 0.50 mM lobeline when compared to relevant controls (gray bars). (*, P<0.05; 1-way ANOVA, Bonferroni post-test for comparison between columns within the 23°C or 30°C groups; 2-way ANOVA, Bonferroni post-test for comparison between temperatures within same genotypes; n≥8). Of note, the positional aversion between *30Y^{GAL4}/UAS-Shi^{ts}* females at 25°C and 30°C were not significantly different in the 2-way ANOVA Bonferoni post-test, likely due to the fact that leaky activity of the *UAS-Shi^{ts}* transgene also caused a decrease of positional aversion at the permissive temperature. Additionally, *30Y^{GAL4}/+* females demonstrated a significant increase in positional aversion at 23°C, when compared to *UAS-Shi^{ts}/+* (*, P<0.05). However, this increase in positional aversion resulting from *30Y^{GAL4}* construct in the heterozygote did not affect the *30Y^{GAL4}/UAS-Shi^{ts}* females, which still lost positional aversion.