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Supplemental Data

$GABA_A$ Receptor RDL Inhibits *Drosophila* Olfactory Associative Learning

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Figure S1. The expression pattern of c772-Gal4 and inhibition by MB{Gal80}.

(A) The c772-Gal4 transgene driving the expression of a membrane bound green fluorescence protein, mCD8-GFP. Expression was observed in the mushroom bodies (MB), the antennal lobes (AL), and the ellipsoid body (behind and masked by MB expression).

(B) The c772-Gal4, MB{Gal80} recombined chromosome driving mCD8-GFP. The MB expression was absent, revealing the expression in the ellipsoid body (EB) located more posterior than the MBs.

(C) Quantification of AL size revealed no difference between the two drivers as calculated from the areas of interest (dashed region in A and B) in pixel numbers.

(D) Quantification of mCD8-GFP expression revealed no decrease in expression in the antennal lobes. For both (C) and (D), n = 16 for c772 and n = 22 for c772-Gal4, MB{Gal80}. Means ± SEM are shown.

Figure S2. The expression pattern of c772-Gal4 in the MB lobes and calyx.

The c772-Gal4 (panels A through E) or c772-Gal4, MB{Gal80} (panels F through K) transgenes driving the expression of mCD8-GFP. Panels A, B, C, F, I: anti-mCD8 staining; panels D, G, J: anti-Discs Large (DLG) counter staining; panels E, H, K: merge of the two channels.

(A) The c772-Gal4 transgene driving mCD8-GFP imaged at the level of α/β lobes.

(B) Higher magnification of box in (A). Arrow shows weak expression in α' lobe.

(C) c772-Gal4 driving mCD8-GFP imaged at the level of the calyx. The mushroom body cell bodies (cb), the calyx (ca) and the origin of peduncle (pd) are identified. No projections from extrinsic neurons were detected in the region of the calyx.

(D) Anti-DLG counter staining for (C) showing the calyx (ca) and the protocerebral bridge (pb).

(E) Merge of (C) and (D).

(F) c772-Gal4, MB{Gal80} driving mCD8-GFP showing the central brain, posterior view.

(G) Anti-DLG counter staining for (F).

(H) Merge of (F) and (G).

(I) Higher magnification of box in (F). No signal was detected, indicating that all signal in the calyx region of the c772-Gal4 driver was due to expression in the MB neurons.

(J) Anti-DLG counter staining for (I) showing the calyx (ca) and the protocerebral bridge (pb).

(K) Merge of (F) and (G).

Figure S3. Flies imaged for assaying responses to electric shock responded normally to odor.

The flies used for measuring responses to electric shock shown in Figure 7 (E) and (F) were subsequently tested for their response towards benzaldehyde (BEN) at the MB lobes (A) or 3-octanol (OCT) at the MB calyx (B). The differential responses observed among the groups in Figure 7 (C) and (B) were again observed. Means ± SEM are shown. Asterisks indicate significant differences from the c772/+ control group, *: $P<0.05$; **: $P<0.01$.

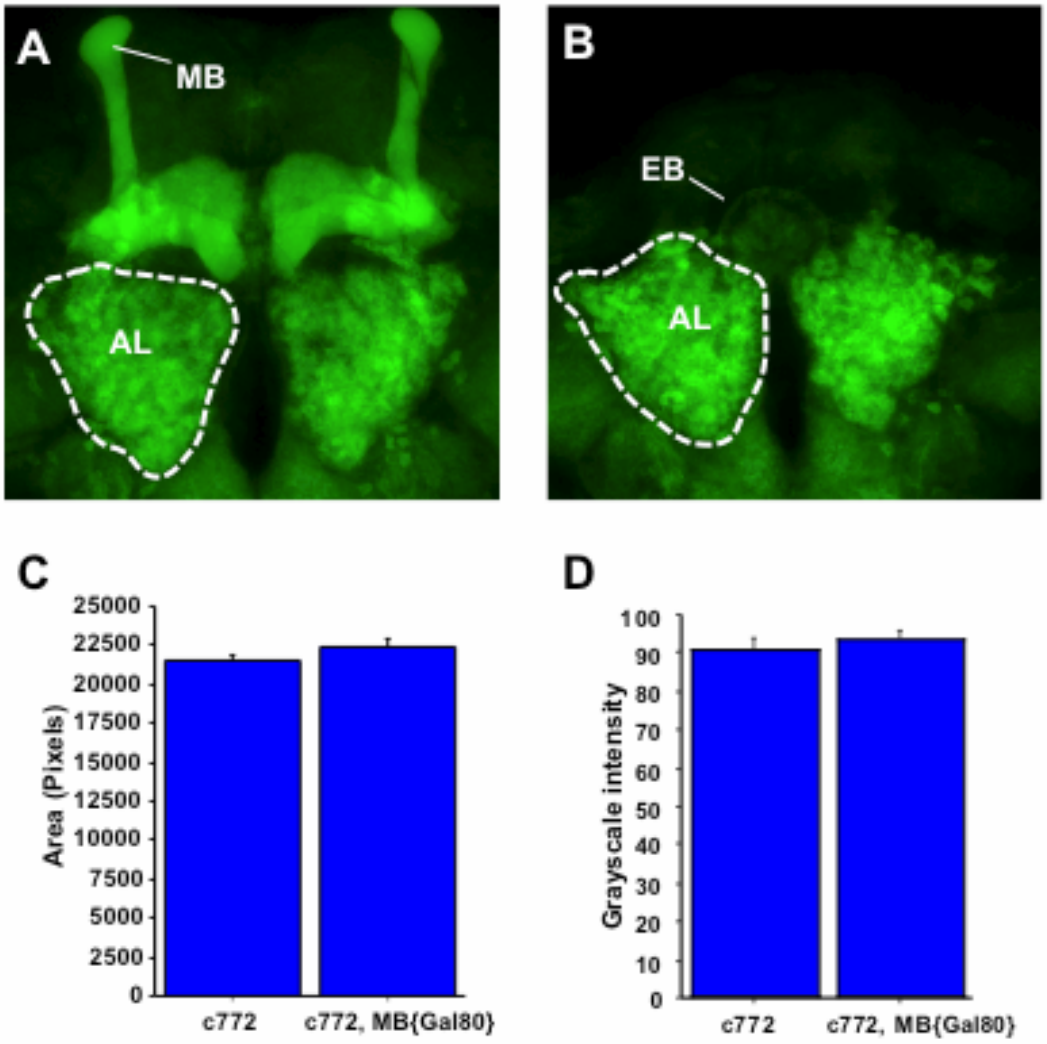

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Figure S1

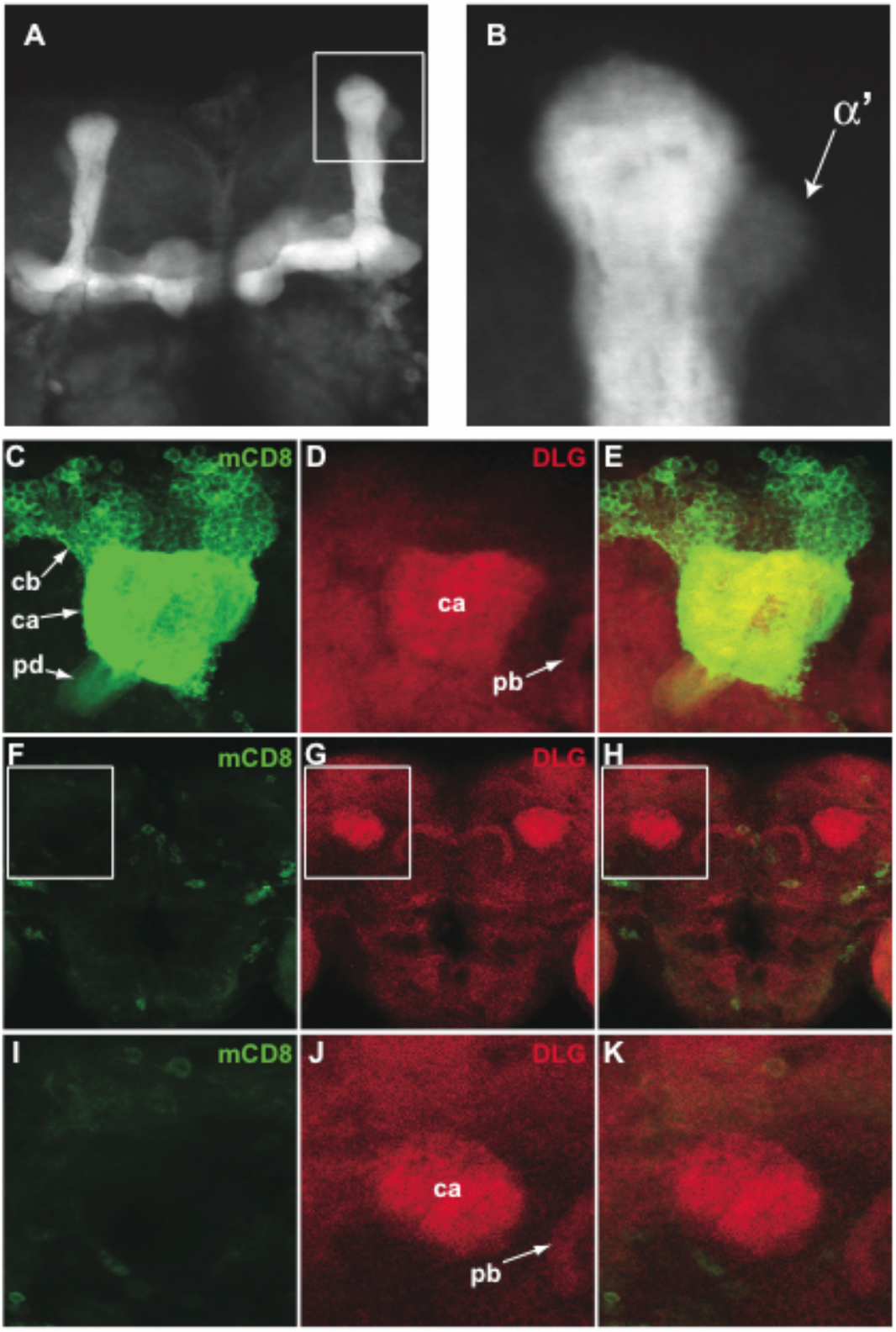

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Figure S2

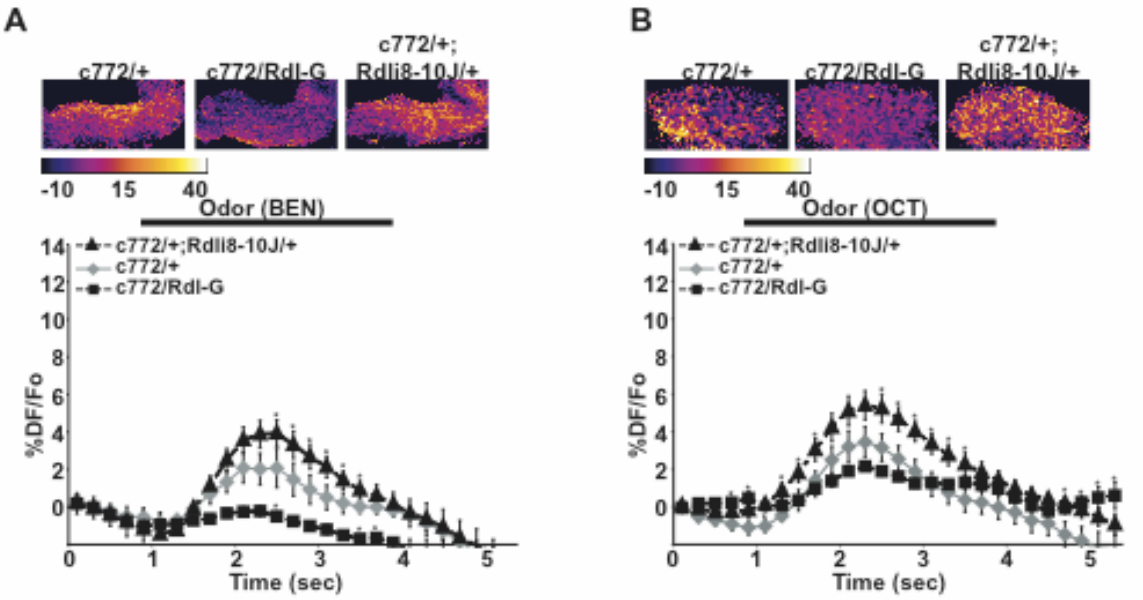

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Figure S3