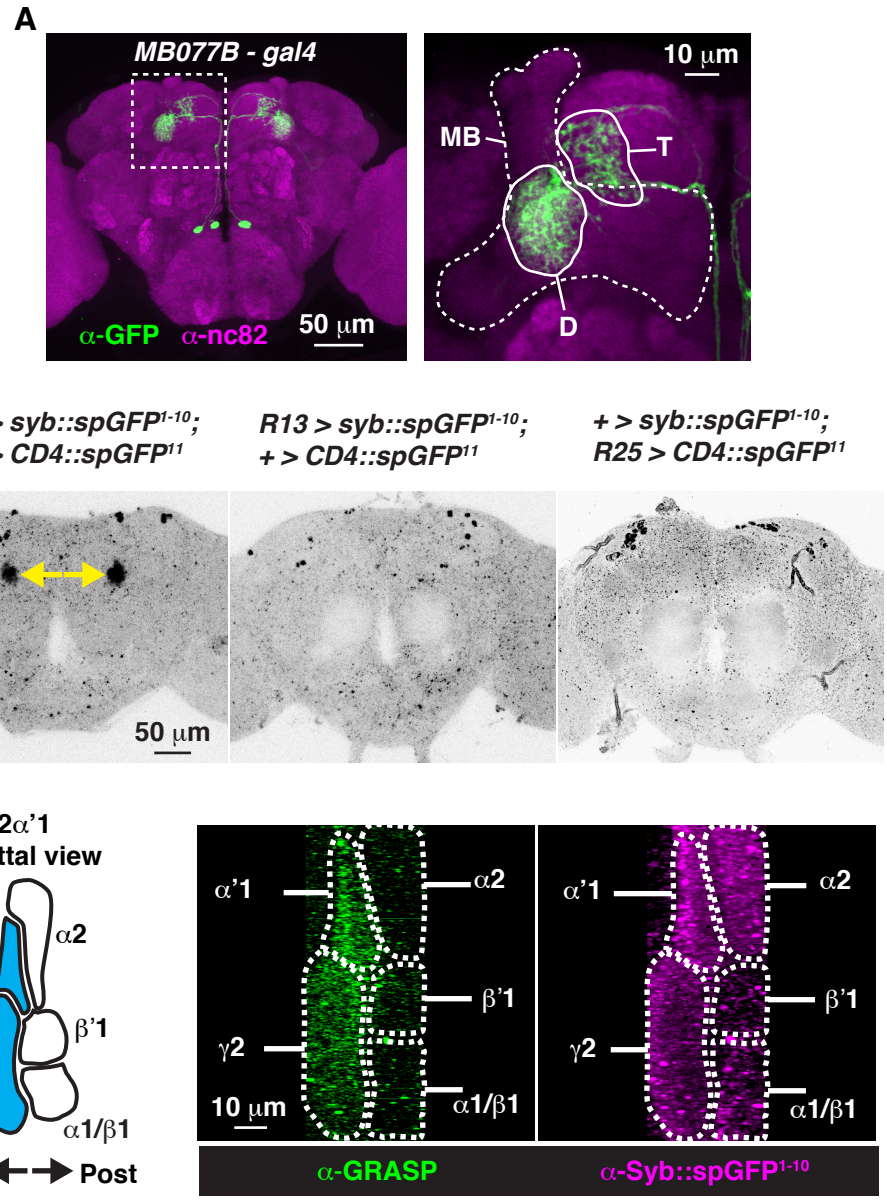
Cell Reports, Volume *25*

Supplemental Information

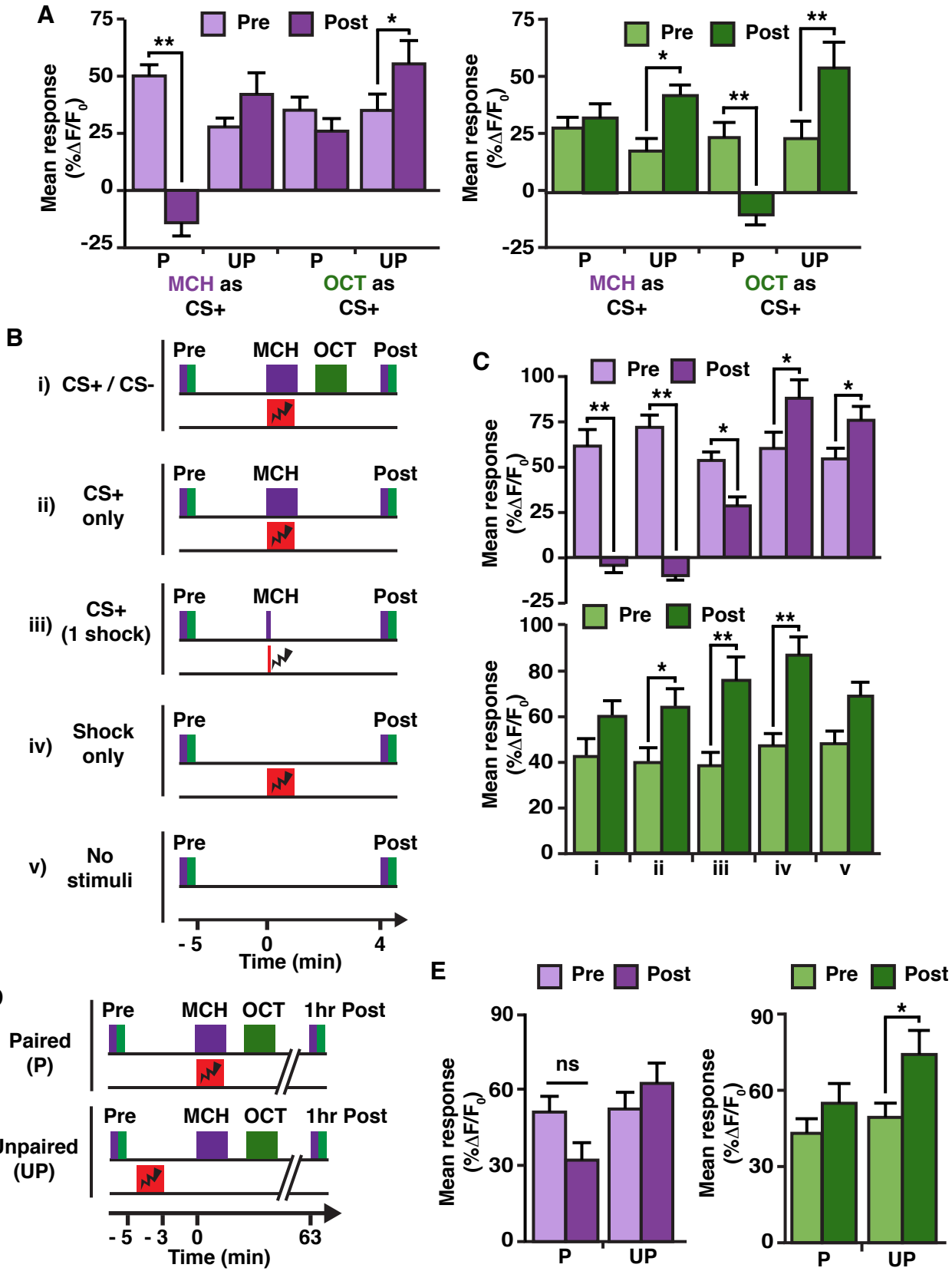
Dopamine Neurons Mediate Learning and Forgetting through Bidirectional Modulation of a Memory Trace

Jacob A. Berry, Anna Phan, and Ronald L. Davis



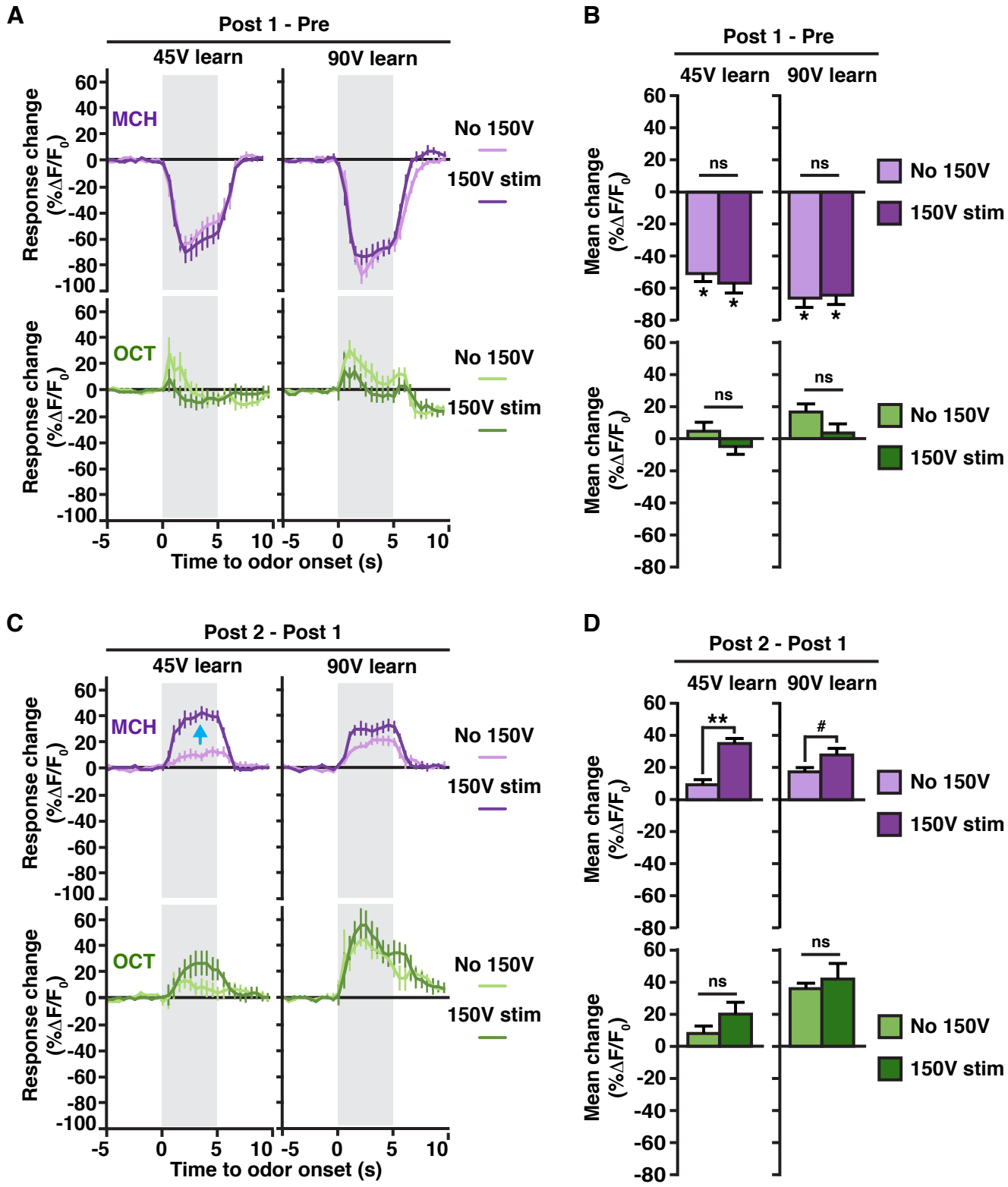
Supplemental Figure 1

Figure S1. MBns form presynaptic connections onto the dendrites of MBOn-γ2α'1, related to Figure 1. (A) *MB077B-gal4* driver expression in the whole fly brain (left) and in the region outlined surrounding the MB lobes (right; also shown in Figure 1C). D, dendrites; T, presynaptic terminals. (B) Native synaptic GRASP signal in γ2α'1 (yellow arrows) in experimental brains (left) versus genetic controls (middle, right). *R13F02-gal4* (*R13*) and *R25D02-lexA* (*R25*) were used to drive presynaptic Syb:spGFP$^{1-10}$ in MBns or postsynaptic CD4-spGFP^{11} in MBOn-γ2α'1, respectively. (C) Reconstituted GFP signal (middle, via anti-GRASP immunostaining) specifically in α'1 and γ2 compartments and related to MBn presynaptic terminals (right, via anti-syb:GFP1-10 immunostaining) after SIM processing and rotation to a sagittal perspective (left).



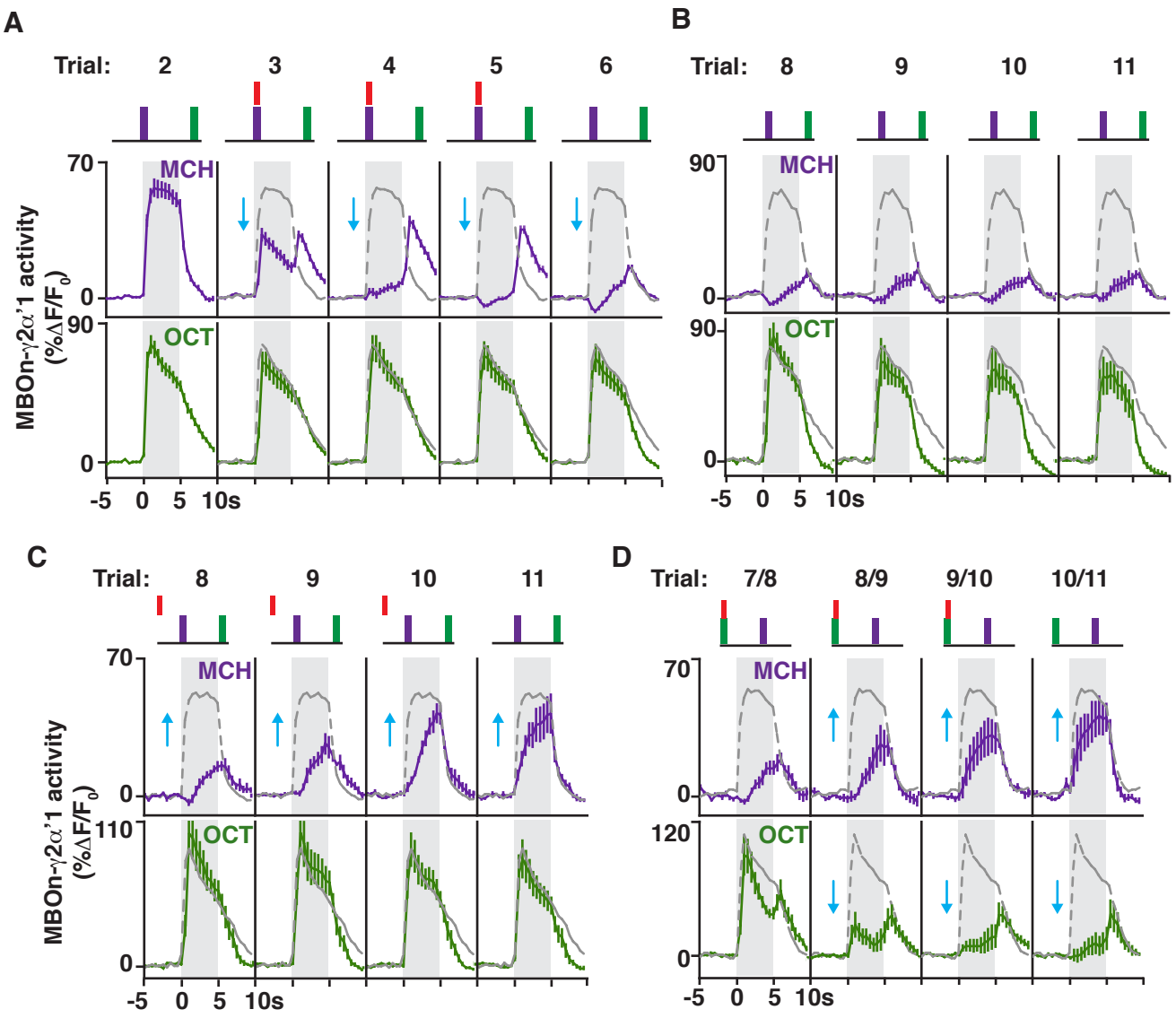
Supplemental Figure 2

Figure S2. MBOn-γ2α'1 axonal or dendritic plasticity after various conditioning protocols, related to Figure 2. (A) Mean axonal response to 5 sec MCH or OCT exposure from MBOn-γ2α'1 before and after paired (P) or unpaired protocols (UP) shown in Figure 2G. (**, $P < 0.001$; *, $P < 0.05$, $n = 8$-10). (B) Conditioning protocols used to collect data shown in panel C. Timeline (bottom) indicates the start of CS/US association and the start of Pre and Post responses. (C) Mean dendritic response during a 5 sec exposure to MCH or OCT before and after the conditioning protocol illustrated in panel B (**, $P < 0.001$; *, $P < 0.05$, $n = 7$-11). (D) Conditioning protocols used to collect 1 hr data shown in panel E. Timeline (bottom) indicates the start of CS/US association, start of Pre and Post responses, and end of unpaired shock. (E) Mean dendritic response during a 5 sec exposure to MCH or OCT before and 1 hr after paired (P) and unpaired (UP) protocols illustrated in panel in D (*, $P < 0.05$; "ns", not significant, $n = 8$-10). All statistics in this figure: Two-way repeated measures ANOVA with Bonferroni posthoc tests.



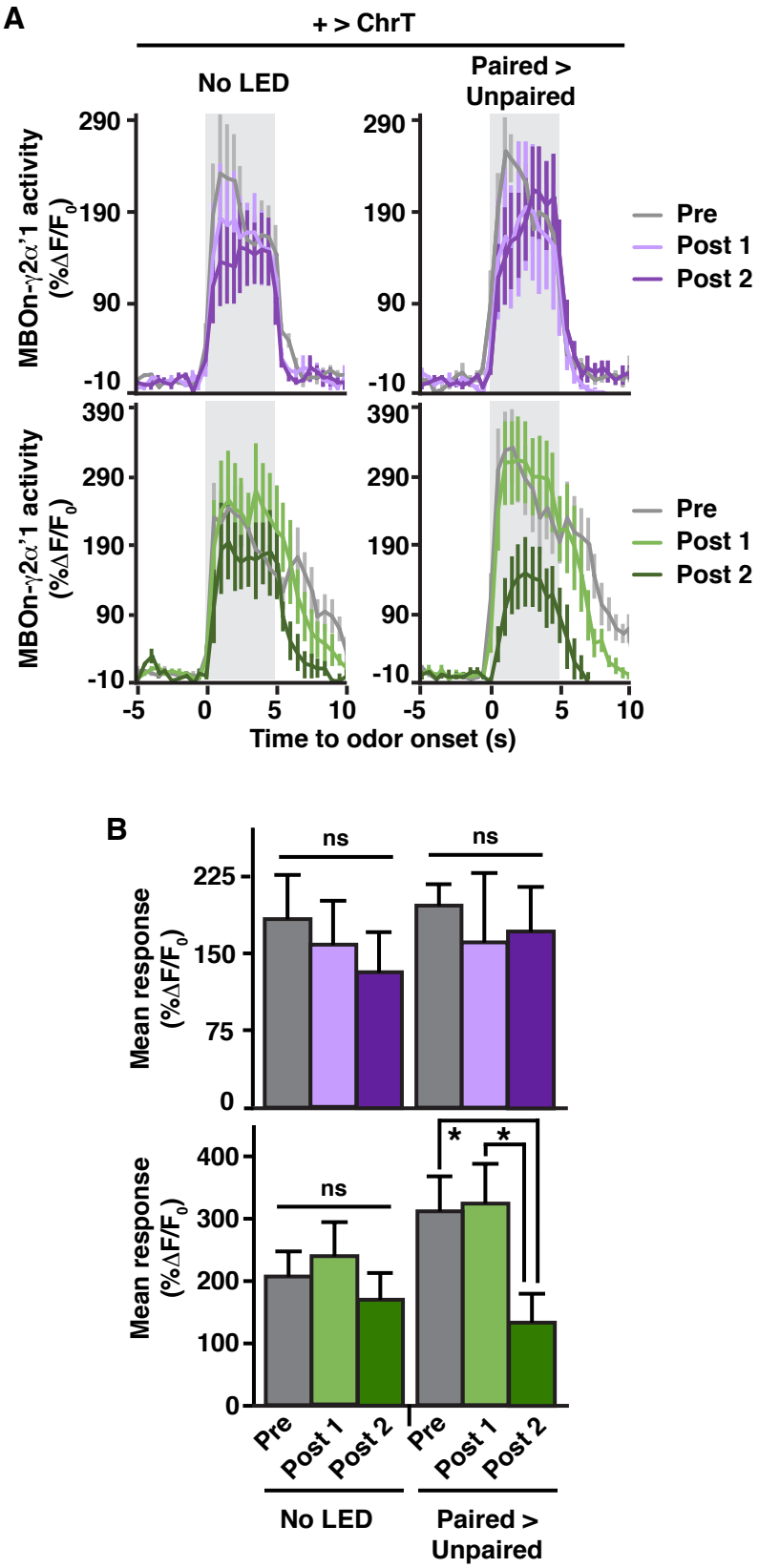
Supplemental Figure 3

Figure S3. Strong US pairing with odor produces MBOn-γ2α'1 plasticity resistant to subsequent electric shock stimuli, related to Figure 3. (A) Time course of the change in dendritic Ca^{2+} response of MBOn-γ2α'1 during a 5 sec odor exposure after MCH odor-shock pairing ("Post1 – Pre") using either 45V or 90V (12x) as the US, from protocols illustrated in Figure 3A. Groups receiving a 150V shock stimulus after the first test (dark colored lines) are compared to a group without this stimulus (light colored lines). (B) Mean change in response (depression) to odors from data shown in panel A. Statistics: Mann Whitney T-test for comparisons across conditions ("ns", not significant); Wilcoxon signed-rank test for comparisons with zero (*, significantly different from zero, $P = 0.002$, $n = 10$). (C) Time course of the change in dendritic Ca^{2+} response of MBOn-γ2α'1 during a 5 sec odor exposure ("Post 2 – Post1") for 45V and 90V conditioning groups from protocols in Figure 3A. Groups receiving a 150V shock stimulus after Post1 (dark colored lines) are compared to a group without this stimulus (light colored lines). (D) Mean change in response (restoration) during odor exposure from data shown in panel C. Statistics: Mann Whitney T-test for comparisons across conditions (**, $P < 0.007$; #, $P = 0.0569$; "ns", not significant; $n = 10$-11).



Supplemental Figure 4

Figure S4. MBOn-γ2α'1 odor responses during initial learning and subsequent forgetting, related to Figure 4. (A) Time course of mean dendritic GCaMP^{6f} responses in MBOn-γ2α'1 during a 5 sec odor exposure to MCH and OCT across trials 2-6 and pooled for all three conditioning protocols shown in Figure 4B,C. The mean trace for trial 2 is shown as a gray dashed line as a reference for the initial response level. Blue arrows indicate either depressed or potentiated responses across all panels. (B) Time course of mean dendritic GCaMP^{6f} responses in MBOn-γ2α'1 during a 5 sec odor exposure to MCH and OCT across trials 8-11 for the control group (P → ----) plotted in Figure 4C. The mean response of this group (P → ----) during trial 2 is shown as a reference for the initial response level. (C) Time course of mean dendritic GCaMP^{6f} responses in MBOn-γ2α'1 to MCH and OCT across trials 8-11 for the Unpaired group (P → UP) plotted in Figure 4C. The mean response of this group (P → UP) during trial 2 is shown as a reference for the initial response level. (D) Time course of mean dendritic GCaMP^{6f} responses in MBOn-γ2α'1 to MCH and OCT across trials 7-10 for OCT and 8-11 for MCH for the Reversal group (P → Rev) plotted in Figure 4C. The mean response of this group (P → Rev) during trial 2 is shown as a reference for the initial response level to MCH and trial 6 for the response level to OCT just before OCT:shock pairings during trials 7-9.



Supplemental Figure 5

Figure S5. Light exposure paired or unpaired with odors fails to depress or restore MBOn-γ2α'1 responses without Chrimson expression in DAn-γ2α'1, **related to Figure 5.** (A) Time course of axonal MBOn-γ2α'1 odor responses during a 5 sec exposure (gray shaded region) to MCH or OCT at the Pre (gray lines), Post 1 (light colored lines), and Post 2 (dark colored lines) time points indicated in Figure 5G. The flies expressed GCaMP^{6f} in MBOn-γ2α'1 using *R25D02-lexA* but were without ChrT expression in DAn-γ2α'1. (B) Mean responses of MBOn-γ2α'1 during odor exposure colored to match the data in panel A. Statistics: Two-way repeated measures ANOVA with Bonferonni post hoc tests (*, $P < 0.05$, "ns", not significant, $n = 7$). The significantly depressed response at Post2 to the control odor in the Paired>Unpaired group was unexpected. However, this significant depression was not observed in the experimental group (Figure 5I) and does not alter the overall interpretations.