

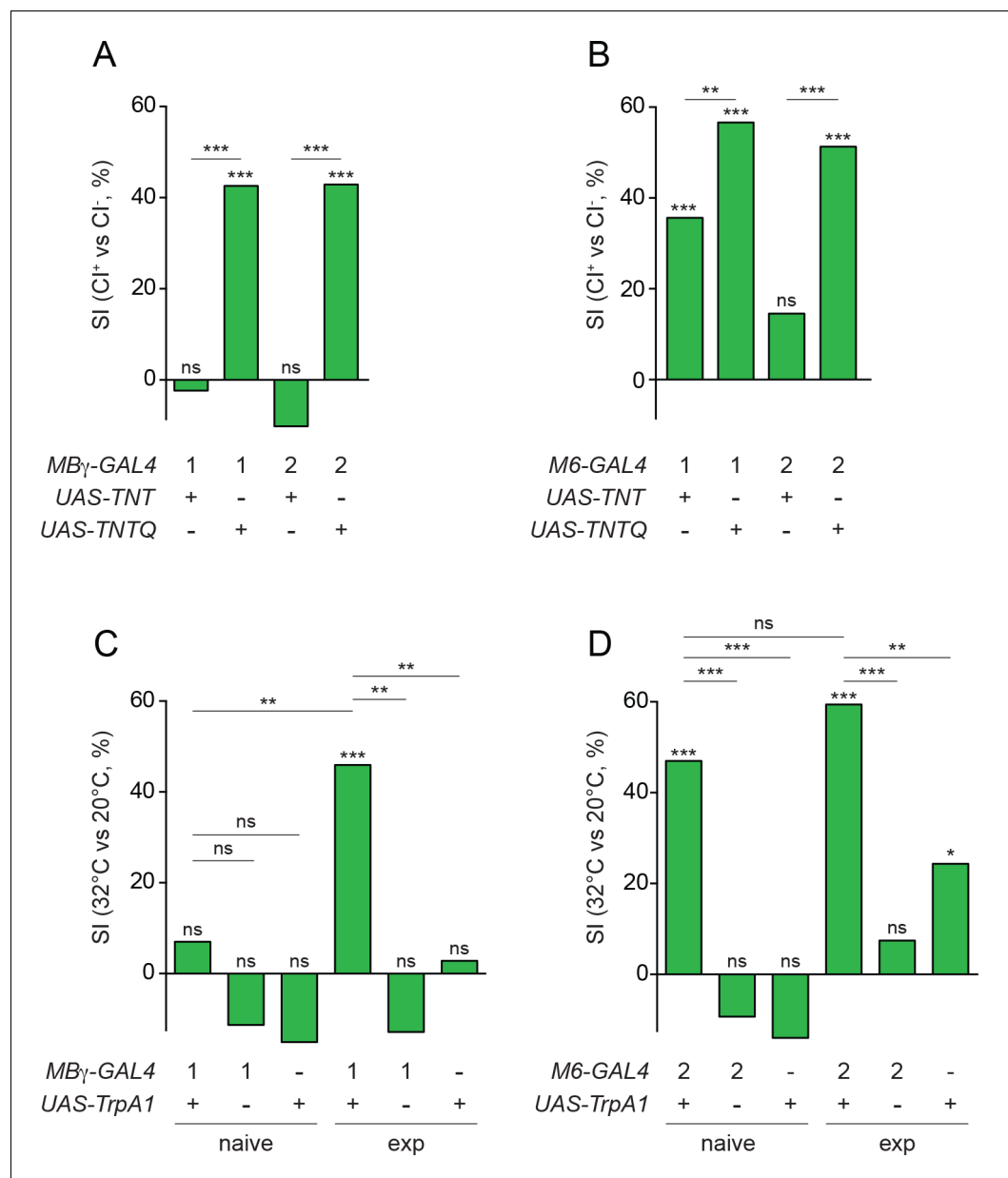


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## Figures and figure supplements

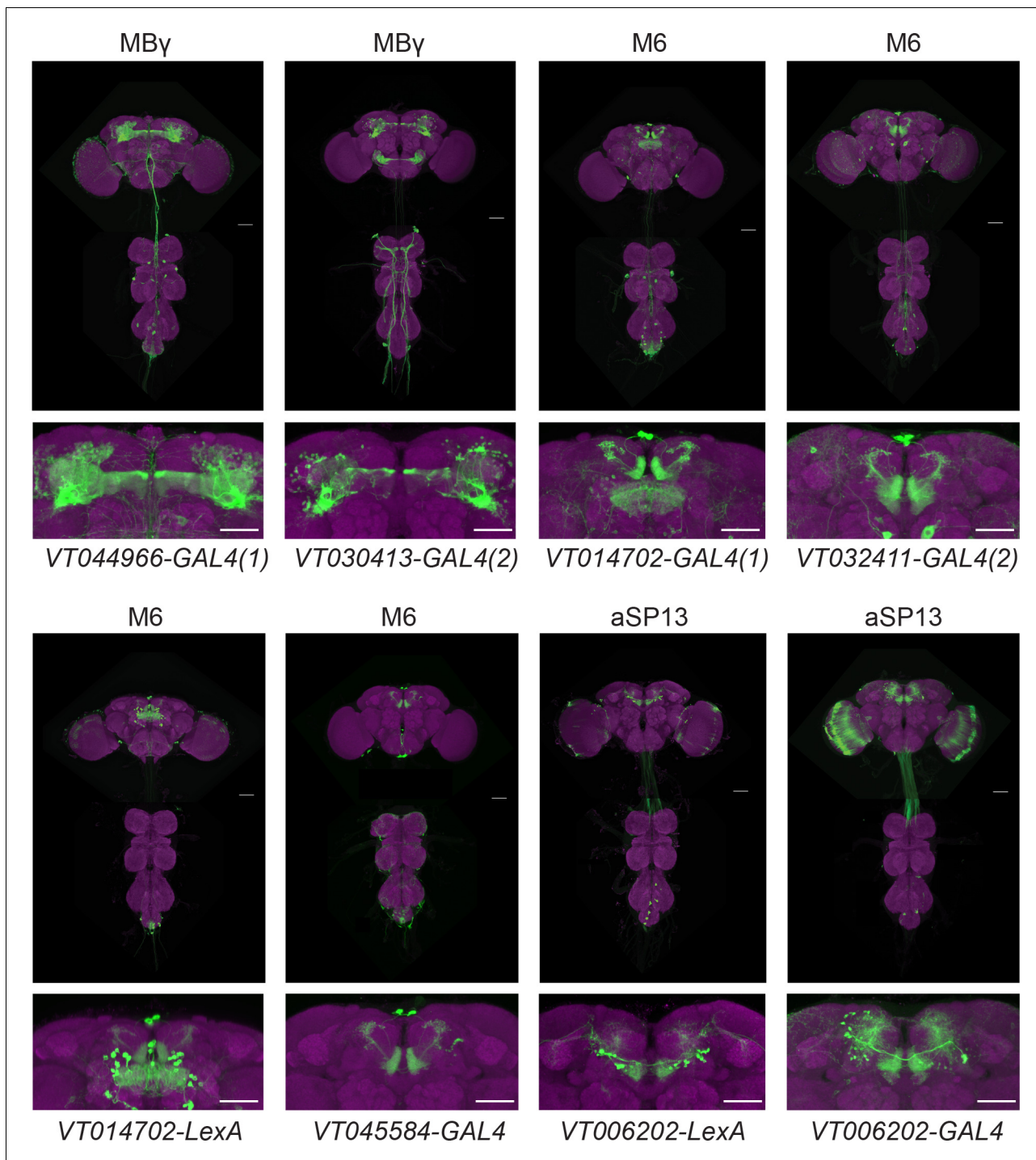
Persistent activity in a recurrent circuit underlies courtship memory in *Drosophila*

**Xiaoliang Zhao et al**



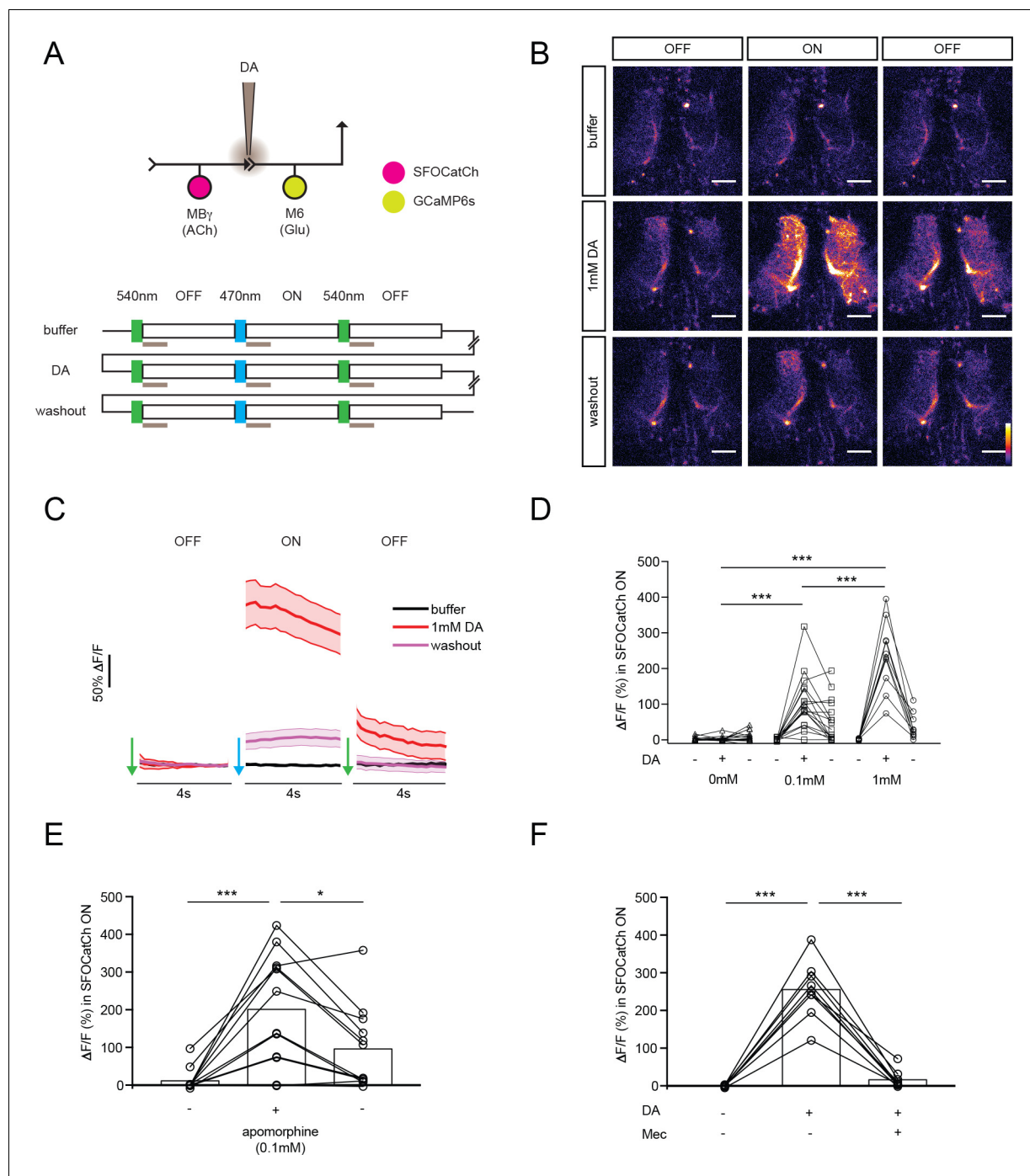
**Figure 1.** Experience modulates circuit properties between MBy and M6 neurons. (A) Suppression indices (SI), calculated from mean courtship indices of male flies in which active (UAS-TNT) or inactive (UAS-TNTQ) tetanus toxin is expressed in MBy neurons (1, VT044966-GAL4; 2, VT030413-GAL4). In this and other panels, statistical significance of differences from zero or from control groups is indicated as follows: \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ , n.s.  $p > 0.05$ , permutation tests, see **Supplementary file 1**. (B) Suppression indices (SI), calculated from mean courtship indices of male flies in which active (UAS-TNT) or inactive (UAS-TNTQ) tetanus toxin is expressed in M6 neurons (1, VT014702-GAL4; 2, VT032411-GAL4). See **Supplementary file 1**. (C) Suppression indices (SI) of naïve or experienced (exp) male flies upon thermogenetic activation of MBy neurons (1, VT044966-GAL4). See **Supplementary file 1**. (D) Suppression indices (SI) of naïve or experienced (exp) male flies upon thermogenetic activation of M6 neurons (2, VT032411-GAL4). See **Supplementary file 1**.

DOI: <https://doi.org/10.7554/eLife.31425.003>



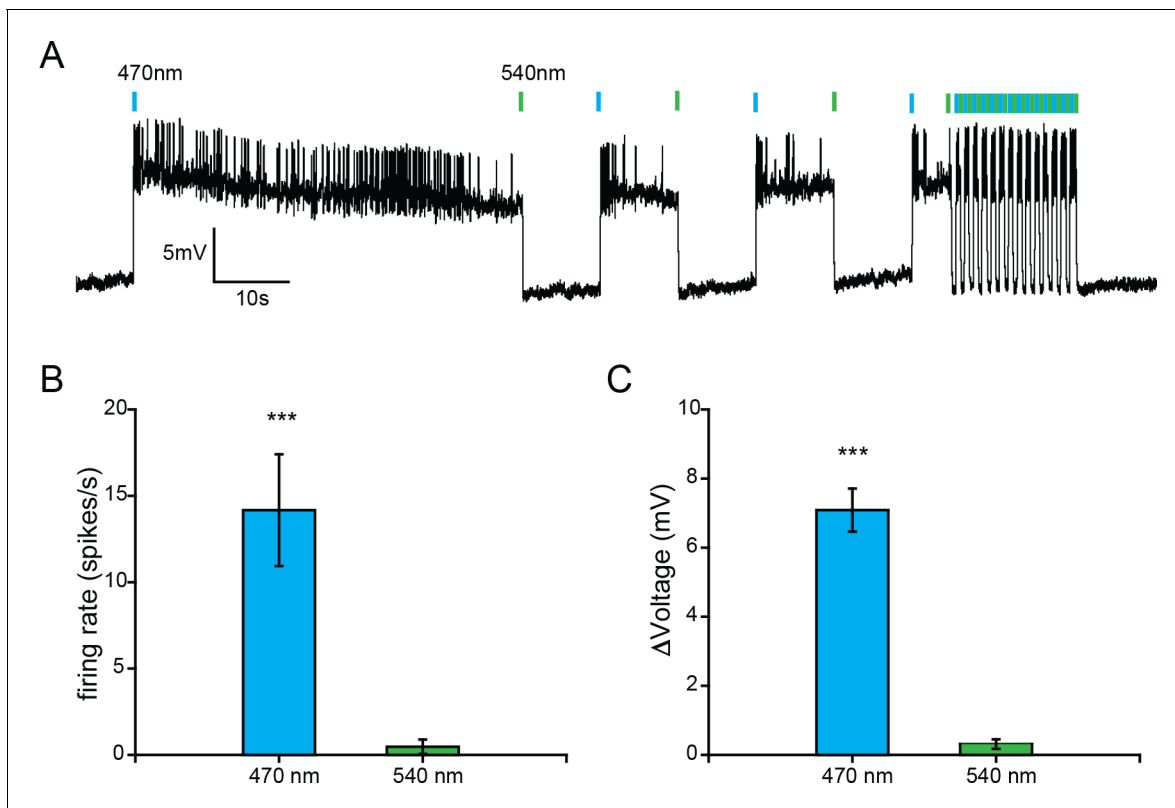
**Figure 1—figure supplement 1.** MBY, M6 and aSP13 GAL4 driver lines. Maximum intensity projections from confocal images of whole central nervous systems of VT-GAL4 UAS-mCD8-GFP or VT-LexA LexAop-mCD8-GFP males, stained for presynaptic sites (magenta, mAb nc82) and anti-GFP (green). Scale bars, 50  $\mu$ m.

DOI: <https://doi.org/10.7554/eLife.31425.004>



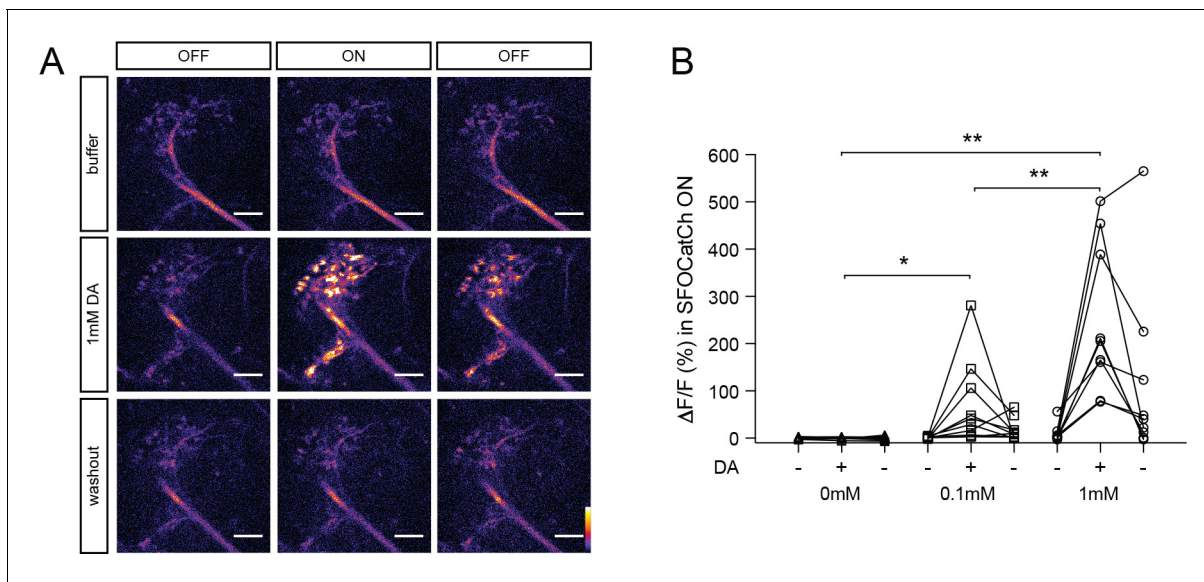
**Figure 2.** Dopamine modulates synaptic transmission from MBy to M6 neurons. **(A)** Experimental protocol. OFF and ON indicate 4 s imaging periods, preceded by 100 ms pulses of 540 nm or 470 nm light to switch SFOCatCh OFF and ON, respectively. Gray bars indicate 1 s focal perfusion into the  $\gamma 5$  compartment. Buffer, dopamine injection (DA) and washout trials are separated by 3 min intervals. **(B)** Representative calcium responses in M6 dendrites in the  $\gamma 5$  compartment. Scale bar, 10  $\mu$ m. **(C)** Average  $\Delta F/F$  responses in M6 dendrites.  $n = 10$ . Mean  $\pm$  s.e.m. **(D)** Average  $\Delta F/F$  responses during the SFOCatCh ON periods of successive buffer, DA, and washout trials.  $n = 15, 17, 10$  for 0, 0.1, and 1.0 mM DA, respectively. \*\*\* $p < 0.001$ , t-test. **(E)** Average  $\Delta F/F$  responses during the SFOCatCh ON periods of successive buffer, apomorphine, and washout trials.  $n = 12$ . \* $p < 0.05$ , \*\*\* $p < 0.001$ , t-test. **(F)** Average  $\Delta F/F$  responses during the SFOCatCh ON periods of successive trials with buffer only, 1 mM DA, and DA plus 0.15 mM mecamylamine (Mec).  $n = 9$ . \*\*\* $p < 0.001$ , t-test.

DOI: <https://doi.org/10.7554/eLife.31425.005>



**Figure 2—figure supplement 1.** SFOCatCh, a step-function optogenetic activator. (A) Patch-clamp recording from an olfactory projection neuron of a *GH146-GAL4 UAS-SFOCatCh* male. Blue and green bars indicate 100 ms pulses of 470 nm and 540 nm light. (B) and (C) Firing rate (B),  $n = 11$  cell-attached recordings) and membrane potential (C),  $n = 4$  whole-cell recordings of olfactory projection neurons during the first 5 s after stimulation with 470 nm or 540 nm light. Mean  $\pm$  s.e.m. \*\*\* $p < 0.001$  for comparisons to zero, t-test.

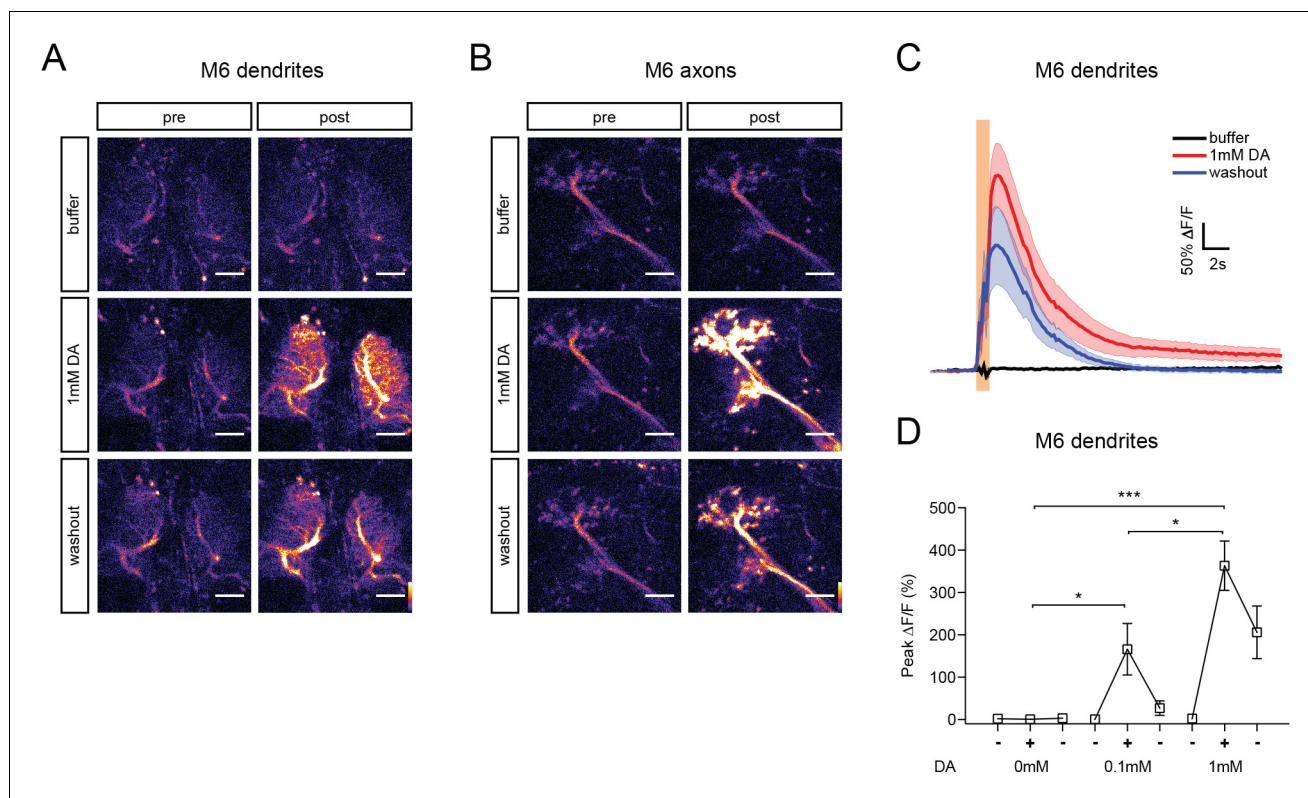
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**Figure 2—figure supplement 2.** Calcium responses in M6 axons upon optogenetic stimulation of MBy neurons using SFOCatCh. **(A)** Representative calcium responses in M6 axons. Experimental protocol as in **Figure 2A**. Scale bar, 10  $\mu$ m. **(B)** Average  $\Delta F/F$  responses in M6 axons during the SFOCatCh ON periods of successive buffer, DA, and washout trials.  $n = 11, 10, 9$  for 0, 0.1, and 1.0 mM DA, respectively. \* $p < 0.05$ , \*\* $p < 0.01$ , one-way ANOVA test.

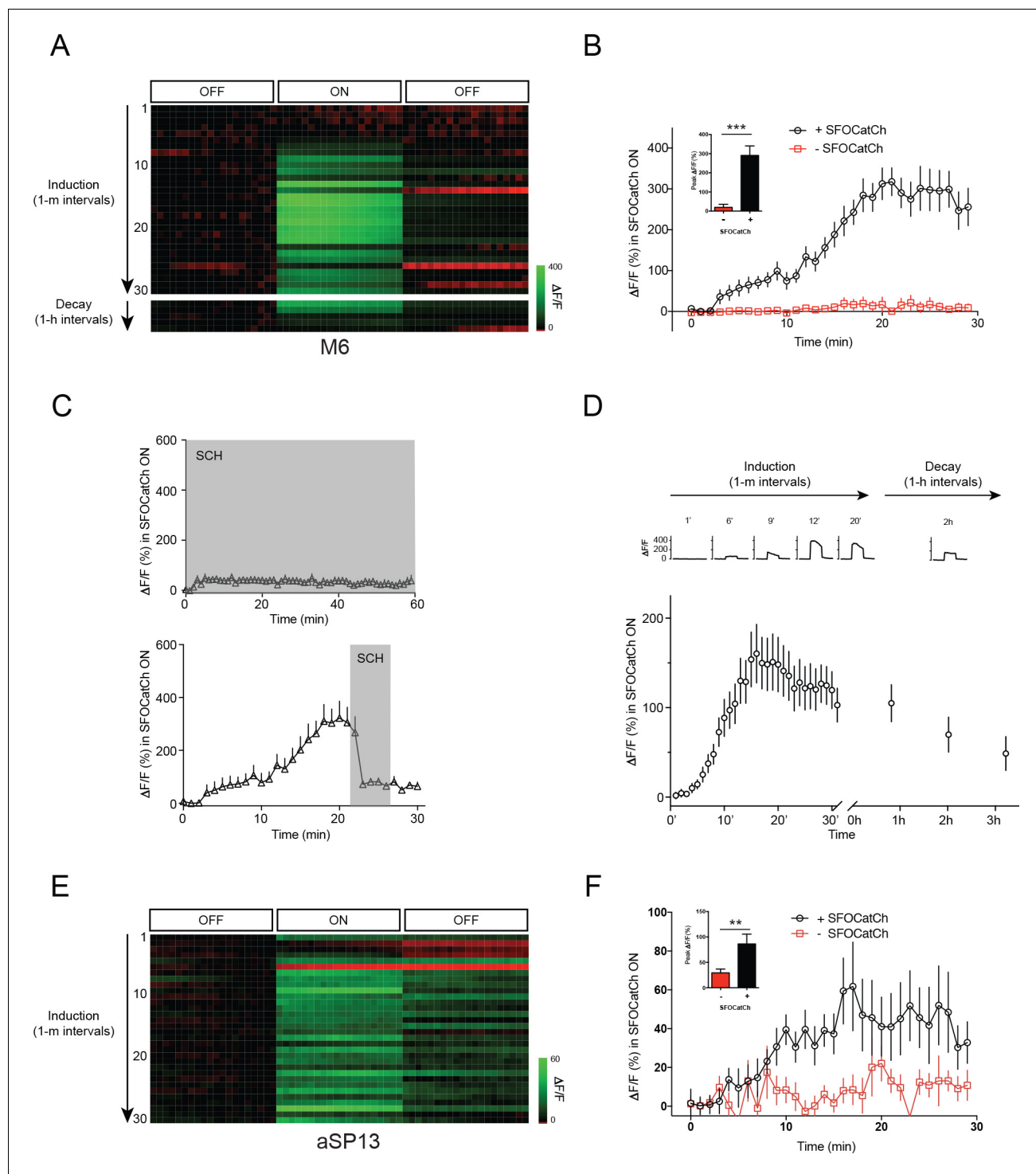
DOI: <https://doi.org/10.7554/eLife.31425.007>





**Figure 2—figure supplement 3.** Calcium responses in M6 neurons upon optogenetic stimulation of MB $\gamma$  neurons using CsChrimson. (A) and (B) Representative calcium responses in M6 dendrites (A) and axons (B) of male brains expressing GCaMP6s in M6 neurons and CsChrimson in MB $\gamma$  neurons, prior to (pre) and after (post) stimulation with 635 nm LED light. Scale bar, 10  $\mu$ m. Buffer, DA, and washout trials were performed at 3 min intervals. (C) Average  $\Delta F/F$  responses in M6 dendrites during successive buffer, DA, and washout trials. Light red bar indicates stimulation with 30 2 ms LED pulses at 30 Hz. Mean  $\pm$  s.e.m.  $n = 12$ . (D) Average  $\Delta F/F$  responses in M6 dendrites during successive buffer, DA, and washout trials.  $n = 12, 7, 12$  for 0, 0.1, and 1.0 mM DA, respectively. \* $p < 0.1$ , \*\*\* $p < 0.001$ , one-way ANOVA test.

DOI: <https://doi.org/10.7554/eLife.31425.008>



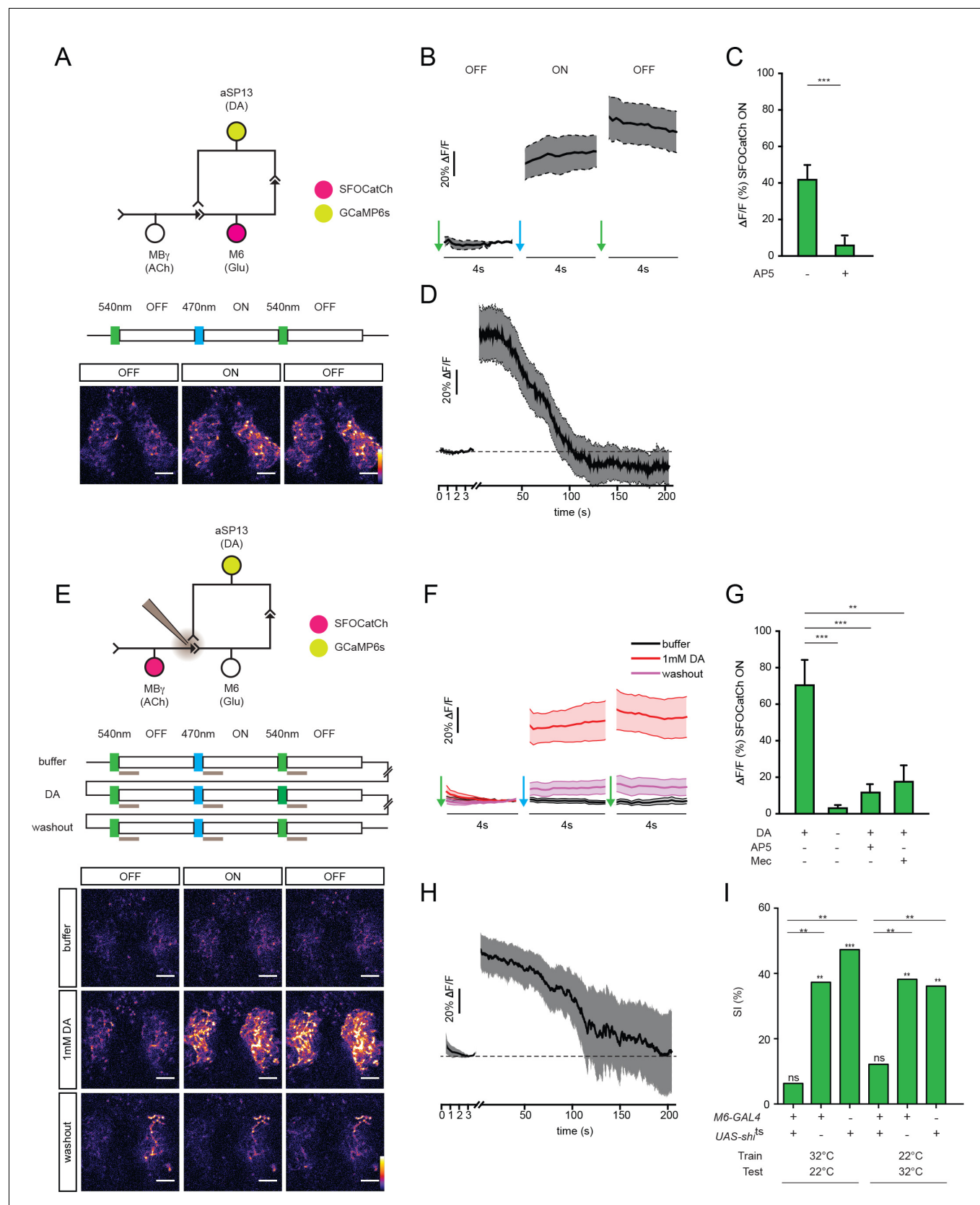
**Figure 3.** Repetitive stimulation of MBy potentiates MBy to M6 transmission. (A) Representative calcium responses in M6 dendrites in the  $\gamma 5$  compartment upon repetitive optogenetic stimulation of MBy neurons with SFOCatCh. (B) Time course of average  $\Delta F/F$  responses in M6 dendrites during potentiation, mean  $\pm$  s.e.m.  $n = 16$  for +SFOCatCh,  $n = 6$  for -SFOCatCh. Inset, peak  $\Delta F/F$  responses, mean  $\pm$  s.e.m. \*\*\* $p < 0.001$ , t-test. (C) Average  $\Delta F/F$  responses during the SFOCatCh ON periods in M6 dendrites in the  $\gamma 5$  compartment in trials with SCH23390 present (grey shading) either during (top) or after (bottom) induction. (D) Time course of average  $\Delta F/F$  responses in M6 dendrites during potentiation (30 stimuli at 1 m intervals) and decay (stimulation at  $\sim 1$  hr intervals), mean  $\pm$  s.e.m.  $n = 19$ . Top, representative calcium responses at various time points, during the  $3 \times 4$  s OFF/ON/OFF imaging periods. (E) Representative calcium responses in aSP13 axons in the  $\gamma 5$  compartment upon repetitive optogenetic stimulation. Figure 3 continued on next page



Figure 3 continued

of MBy neurons with SFOCatCh. (F) Time course of average  $\Delta F/F$  responses in aSP13 axons during potentiation, mean  $\pm$  s.e.m.  $n = 10$  for +SFOCatCh,  $n = 7$  for –SFOCatCh. Inset, peak  $\Delta F/F$  responses, mean  $\pm$  s.e.m.  $**p < 0.01$ , t-test.

DOI: <https://doi.org/10.7554/eLife.31425.009>

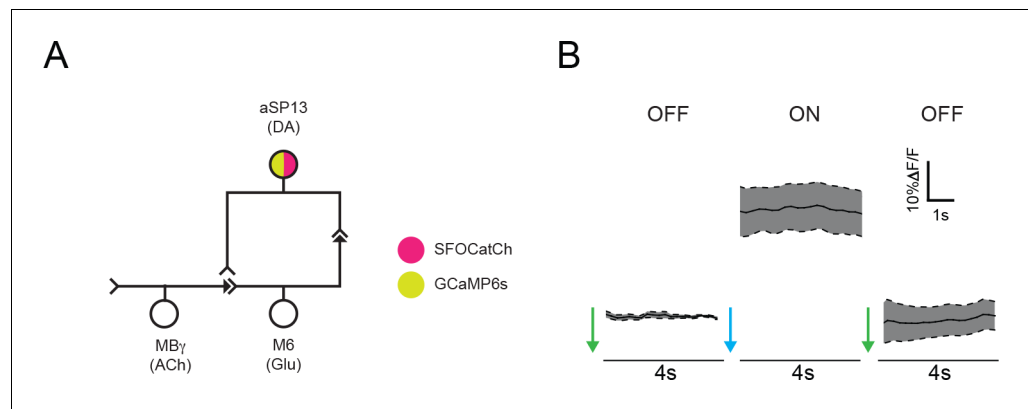


**Figure 4.** M6 or MB $\gamma$  activation induces a persistent calcium response in aSP13. (A) Experimental protocol for M6 activation and aSP13 imaging, and representative calcium responses in aSP13 axons in the  $\gamma$ 5 compartment. Scale bar, 10  $\mu$ m. (B) Average  $\Delta F/F$  responses in aSP13 axons, mean  $\pm$  s.e.m. Figure 4 continued on next page

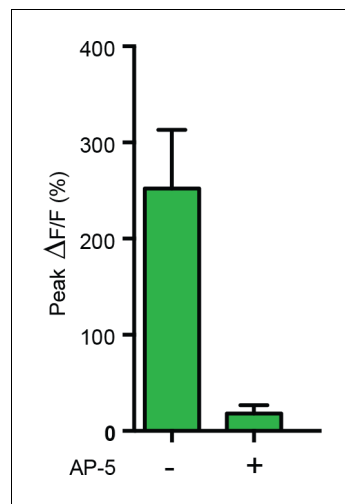
## Figure 4 continued

$n = 22$ . (C) Average  $\Delta F/F$  responses during the SFOCatCh ON periods in trials with ( $n = 9$ ) or without 50  $\mu\text{M}$  D-AP-5 ( $n = 22$ ). \*\*\* $p < 0.001$ , t-test. (D) Average  $\Delta F/F$  responses, imaged at 1 Hz after 200 s of post-stimulus section. (E) Experimental protocol for MB $\gamma$  activation and aSP13 imaging, and representative calcium responses in aSP13 axons in the  $\gamma 5$  compartment. Scale bar, 10  $\mu\text{m}$ . (F) Average  $\Delta F/F$  responses in aSP13 axons, mean  $\pm$  s.e.m.  $n = 13$ . (G) Average  $\Delta F/F$  responses during the SFOCatCh ON periods in trials with or without 1 mM DA, 50  $\mu\text{M}$  AP-5, or 150  $\mu\text{M}$  mecamylamine (Mec).  $n = 13, 13, 5, 10$ , respectively. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , t-test. (H) Average  $\Delta F/F$  responses, imaged at 1 Hz after 200 s of post-stimulus section. (I) Suppression indices (SI) of male flies in which *shi<sup>ts</sup>* is expressed in M6 neurons, shifted to 32°C during training or testing, as indicated. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ , permutation tests, see **Supplementary file 2**.

DOI: <https://doi.org/10.7554/eLife.31425.010>



**Figure 4—figure supplement 1.** Stimulation of aSP13 does not elicit a persistent autonomous calcium response. Average  $\Delta F/F$  responses in the  $\gamma 5$  compartment of males expressing both SFOCatCh and GCaMP6s in aSP13 neurons. Experimental protocol as in **Figure 3A**. Mean  $\pm$  s.e.m.  $n = 6$ .  
DOI: <https://doi.org/10.7554/eLife.31425.011>



**Figure 4—figure supplement 2.** Response in M6 after repetitive activation of MBy is blocked by NMDA-R antagonist. Average  $\Delta F/F$  responses during the SFOCatCh ON periods with or without 50  $\mu\text{M}$  AP-5. Mean  $\pm$  s.e.m.  $n = 6$  and  $9$ , respectively.

DOI: <https://doi.org/10.7554/eLife.31425.012>