# Persistent, generalized hypersensitivity of olfactory bulb interneurons after olfactory fear generalization

## Supplementary Information

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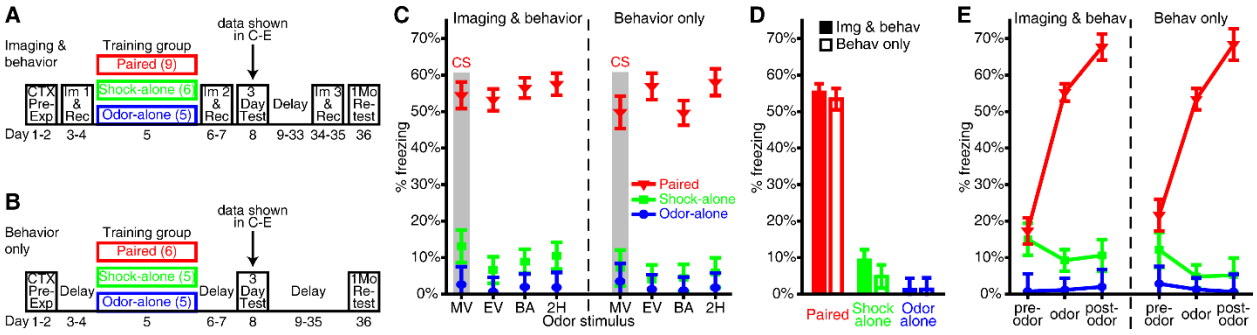



**Supplementary Figure S1: Freezing behavior was not affected by the general anesthesia and optical imaging procedures.** It was not clear if the presentation of odors (or the anesthesia that was used) during the imaging preparations would alter subsequent stimulus-evoked fear responses during the 3-day test. To address this concern, we ran a parallel "behavior only" experiment in which subjects underwent the same protocol as subjects that underwent the imaging procedures, except that the behavior only subjects had a 2-day delay in the home cage on imaging and recovery days. By comparing test sessions between the behavior only and optical imaging experiments, we were able to determine that 1 day of fear conditioning with a single CS results in stimulus-evoked freezing behavior that generalizes to novel stimuli, and that our optical imaging procedures (which include general anesthesia and surgical procedures) do not interfere with the expression of this generalized fear response.

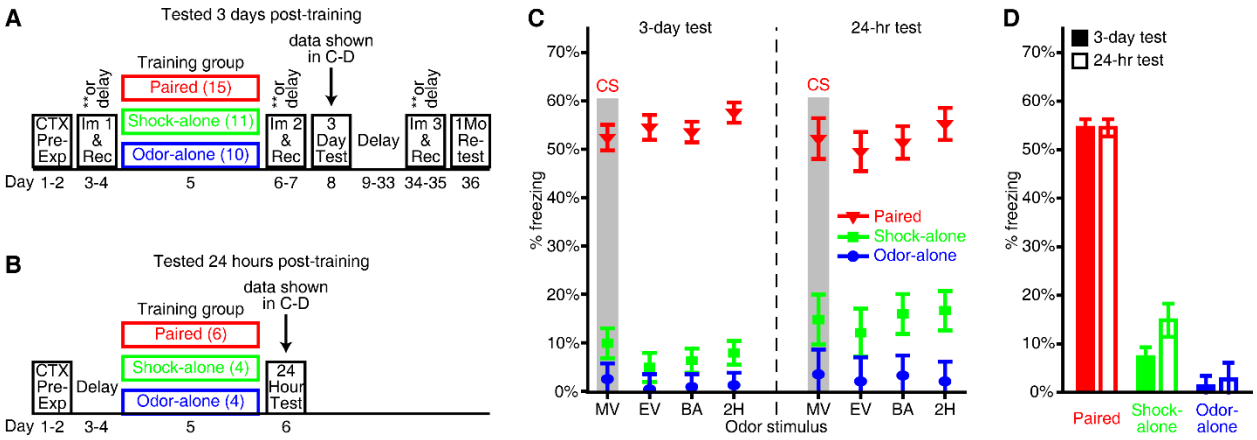
**(A-B)** Protocol summaries comparing the paradigm that included imaging and behavioral experiments **(A)** with the paradigm that only included behavioral experiments **(B)**. The arrows indicate the data that was analyzed and summarized in **C-E**. The *N*s per group are shown in parentheses next to each group label. **(C)** The percent time freezing to MV (the CS), EV, BA, and 2H are averaged across 3 trials per odor within each group and shown separately for imaging and behavior subjects (left) and behavior only subjects (right). **(D)** The freezing data are then collapsed across all odors in each group to demonstrate that odor-evoked freezing did not differ overall across experiments. **(E)** Freezing data is pooled across all 12 trials (4 odors, 3×each) and plotted

relative to trial phase (pre-odor, odor, and post-odor) for each group in each experiment to show relative changes in freezing behavior before, during, and after odor presentations. The data presented in **C-E** are shown as the mean±SEM.

The data in **C-D** were quantified as the percent of time spent freezing during each 20 sec odor presentation. Importantly, the effect of fear conditioning on stimulus-evoked freezing was not affected by the optical imaging procedures (non-significant effect of experimental paradigm, $F_{(1, 30)} = 0.706$, $p = 0.407$, $\eta_p^2 = 0.023$; non-significant group×paradigm interaction, $F_{(2, 30)} = 0.271$, $p = 0.765$, $\eta_p^2 = 0.018$). Stimulus-evoked freezing was equal across all 4 odors for the paired group (non-significant effect of odor, $F_{(3, 39)} = 1.048$, $p = 0.382$, $\eta_p^2 = 0.075$), and this was true for imaging and behavior subjects as well as behavior only subjects (non-significant paradigm×odor interaction, $F_{(3, 39)} = 0.959$, $p = 0.422$, $\eta_p^2 = 0.069$). These results also demonstrate that odor-guided freezing behavior was not affected by genotype since the imaging and behavior experiment **(A)** included subjects that expressed a genetically-encoded calcium indicator (GCaMP3 or GCaMP6f) and the behavior only experiment **(B)** included either wild-type mice that were obtained from Jackson Laboratories or littermates that did not inherit GCaMP.

The data shown in **E** were divided into 3 trial phases (pre-odor, odor, and post-odor) that were quantified as the percent of time spent freezing during 3 consecutive 20-sec bins per trial. The pattern of freezing behaviors that was observed across trial phases differed between groups (significant trial phase×group interaction, $F_{(2.7, 40.5)} = 42.704$, $p < 0.001$, $\eta_p^2 = 0.740$), but was not affected by the optical imaging procedures (non-significant trial phase×paradigm interaction, $F_{(1.4, 40.5)} = 0.400$, $p = 0.672$, $\eta_p^2 = 0.013$; and non-significant trial phase×group×paradigm interaction, $F_{(2.7, 40.5)} = 0.085$, $p = 0.958$, $\eta_p^2 = 0.006$). Freezing behavior varied across trial phases in the paired group (significant effect of trial phase, $F_{(1.3, 17.5)} = 57.812$, $p < 0.001$, $\eta_p^2 = 0.816$), such that each

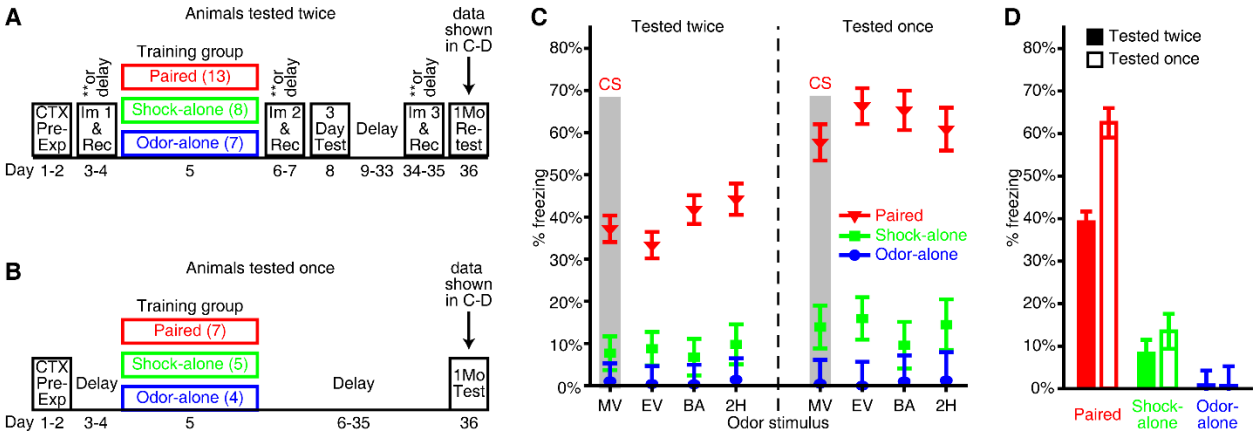
odor presentation evoked a significant increase in freezing (pre-odor versus odor, $p < 0.001$) that remained elevated immediately after odor offset (post-odor versus both pre-odor and odor, $P$s < 0.001). The stimulus-evoked enhancement of freezing relative to pre-odor baseline did not differ between animals that underwent imaging procedures and animals that did not (non-significant paradigm×trial phase interaction, $F_{(1.3, 17.5)} = 0.220$, $p = 0.716$, $\eta_p^2 = 0.017$), and it was equal across all 4 odors (non-significant odor×trial phase interaction, $F_{(3.2, 42.1)} = 1.153$, $p = 0.341$, $\eta_p^2 = 0.081$). By contrast, the change in freezing that was observed across trial phases in shock-alone controls (significant effect of trial phase, $F_{(2, 18)} = 9.082$, $p = 0.002$, $\eta_p^2 = 0.502$) was characterized by an odor-evoked suppression of freezing (pre-odor versus odor, $p = 0.009$) that remained slightly suppressed during the 20-sec interval after odor offset (post-odor versus odor, $p = 1.00$, post-odor versus pre-odor, $p = 0.071$). This trial phase-dependent modulation of freezing behavior was not affected by the optical imaging procedures (non-significant paradigm×trial phase interaction, $F_{(2, 18)} = 1.717$, $p = 0.208$, $\eta_p^2 = 0.160$). There was no change in behavior across trial phases in odor-alone controls, as this group exhibited near-zero levels of freezing before, during, and after each odor presentation (non-significant effect of trial phase, $F_{(2, 16)} = 0.389$, $p = 0.684$, $\eta_p^2 = 0.046$), regardless of which paradigm they were included in (imaging and behavior versus behavior only) and which odor was presented (non-significant paradigm×odor×trial phase interaction, $F_{(1.8, 14.3)} = 0.761$, $p = 0.604$, $\eta_p^2 = 0.087$). In sum, these results demonstrate that our optical imaging procedures did not have an effect on freezing behavior during odor presentations or during odor-free intervals.




**Supplementary Figure S2: Fear generalizes to non-threatening odors within 24 hours of olfactory fear conditioning.** Subjects that were in the imaging and behavior experiment were tested for stimulus-evoked freezing 3 days after training because they could not be tested prior to imaging (to prevent potential extinction effects), and then a 1-day rest period was needed after imaging to ensure full recovery from the effects of anesthesia. Due to those limitations, we added a parallel experiment in which subjects were tested 24 hours after training.

**(A-B)** Protocol summaries comparing the paradigms in which the behavioral test was performed either 3 days **(A)** or 24 hours **(B)** after training. The arrows indicate the data that was analyzed and summarized in **C-D**. The *N*s per group are shown in parentheses next to each group label. Because the imaging procedures did not have an effect on freezing behavior (Supplementary Figure S1) data from the 3-day test **(A)** was pooled across the "Imaging and behavior" and "Behavior only identical" paradigms (Supplementary Figure S1A-B). **(C)** The percent time freezing to MV (the CS), EV, BA, and 2H are averaged across 3 trials per odor within each group and shown separately for the 3-day (left) and 24-hour (right) test sessions. **(D)** Odor-evoked freezing is pooled across all odors and trials in each group and shown separately for subjects that were tested 3 days after training (solid bars) and subjects that were tested 24 hours after training (open bars). The data in **C-D** are presented as the mean±SEM. There was an overall effect of group

($F_{(2, 44)} = 234.436$, $p < 0.001$, $\eta_p^2 = 0.914$), such that the paired group exhibited significantly more odor-evoked freezing than the shock-alone and odor-alone control groups (*P*s < 0.001), regardless of the odor that was being presented (non-significant effect of odor, $F_{(3, 132)} = 1.120$, $p = 0.343$, $\eta_p^2 = 0.025$; non-significant group×odor interaction, $F_{(6, 132)} = 0.521$, $p = 0.792$, $\eta_p^2 = 0.023$). Importantly, there was no difference in freezing behavior between animals that were tested 3 days after training and animals that were tested 24 hours after training (non-significant effect of test time, $F_{(1, 44)} = 1.019$, $p = 0.318$, $\eta_p^2 = 0.023$; non-significant group×test time interaction, $F_{(2, 44)} = 1.873$, $p = 0.166$, $\eta_p^2 = 0.078$). These data demonstrate that odor-cued fear conditioning results in conditioned fear that generalizes to non-threatening odors within 1 day of training, which is the same behavioral phenotype expressed by subjects that were tested 3 days after training.



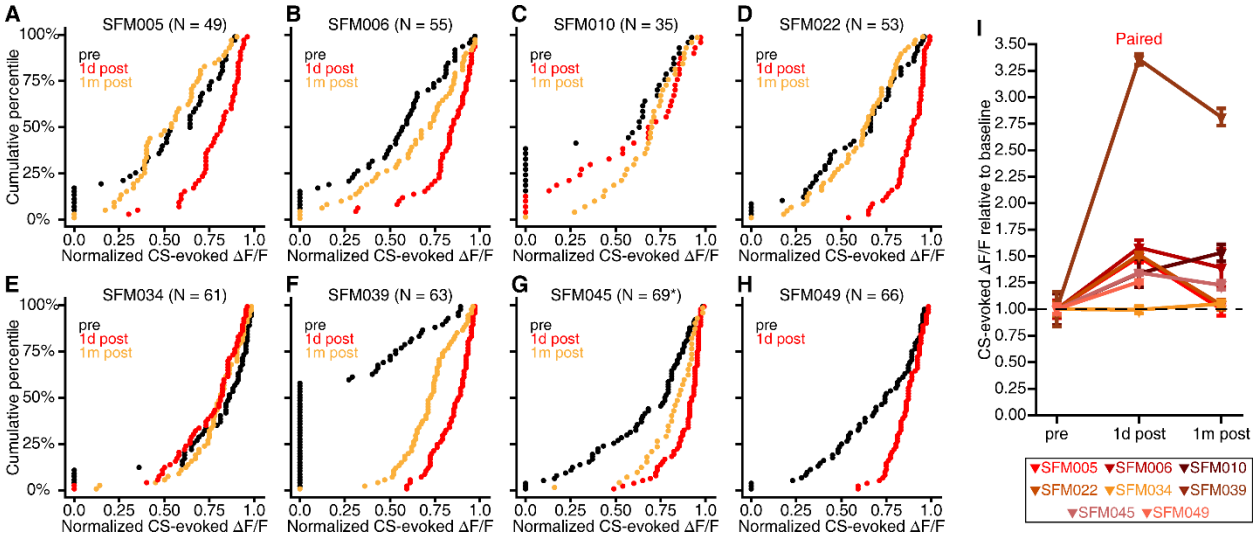

**Supplementary Figure S3: Olfactory fear conditioning results in broad fear generalization across odors that lasts up to at least 1 month after learning.** Subjects underwent behavioral testing twice (Figure 1A) because we were primarily concerned with whether or not the effects of fear conditioning could persist up to 1 month after learning, rather than evaluating potential differences in the strength of the initial memory over time. However, no shocks are delivered during behavioral test sessions, so it is possible for subjects in the paired group to undergo some form of extinction learning during the 3-day test, and consequently the results obtained during the 1 month retest could be difficult to interpret. As an attempt to parse apart any behavioral extinction effects that might be induced by the 3-day test from any potential forgetting that might occur during the month-long delay prior to the 1-month retest, we included an additional cohort of behavior only animals that underwent the same fear conditioning protocol and timeline as the imaging and behavior experiment, except that these subjects were only tested once 1 month after the initial training. The results from this control experiment show that the generalized fear response that is expressed 1 month after training is equal to that expressed 3 days (or 24 hours) after learning, suggesting that broad generalization of conditioned fear can be relatively persistent.

**(A-B)** Protocol summaries comparing the paradigm in which subjects were being retested 1 month after training **(A)** with the paradigm in which subjects were being tested for the first time

1 month after training **(B)**. The arrows indicate the data that was analyzed and summarized in **C-D**. The *N*s per group are shown in parentheses next to each group label. Because generalization of conditioned fear was not affected by the optical imaging procedures (Supplementary Figure S1) we pooled data across the 1-month retests **(A)** from animals in the "Imaging and behavior" and "Behavior only" experiments (Supplementary Figure S1A-B). **(C)** The percent time freezing to MV (the CS), EV, BA, and 2H are averaged across 3 trials per odor within each group and shown separately for the subjects being retested (tested twice, left) and the subjects being tested for the first time (tested once, right). **(D)** Odor-evoked freezing is pooled across all odors and trials in each group and shown separately for subjects in each experiment (closed versus open bars). All data are shown as the mean±SEM.

During the 1-month retest (**C**, left), the paired group exhibited significantly more freezing to odors (*P*s < 0.001) than the shock-alone and odor-alone control groups (which did not differ from each other, $p = 0.469$), suggesting that the generalized fear response is somewhat long-lasting. However, as shown in Figure 1E-F, the paired group also exhibited a reduction in odor-evoked freezing during the 1-month retest relative to their own freezing during the 3-day test. To address this, we directly compared freezing data from subjects being *retested* 1 month after training (**C**, left) with freezing data from subjects being tested for the first time 1 month after training (**C**, right). There was a significant interaction between training group and testing history ($F_{(2, 38)} = 6.563$, $p = 0.004$, $\eta_p^2 = 0.257$), such that the paired subjects that were being retested exhibited less odor-evoked freezing than the paired subjects that were being tested for the first time ($F_{(1, 18)} = 17.800$, $p = 0.001$, $\eta_p^2 = 0.497$). However, freezing from shock-alone ($F_{(1, 11)} = 1.360$, $p = 0.268$, $\eta_p^2 = 0.110$) and odor-alone ($F_{(1, 9)} = 0.074$, $p = 0.791$, $\eta_p^2 = 0.008$) control groups during the 1-month retest did not differ from the same-group counterparts that were tested for the first time 1

month after training. Additionally, there was no difference in odor-evoked freezing in the paired group ($F_{(1, 18)} = 2.303$, $p = 0.147$, $\eta_p^2 = 0.147$) when we compared data from the 3-day test (data shown in Supplementary Figure S1) with data from the test that was performed 1 month after training. These results indicate that 1 month after training paired subjects can express a generalized fear response that is equivalent in magnitude to the response that is expressed 1-3 days after training (Supplementary Figure S2). This suggests that it is unlikely for the modest reduction in freezing that occurred between the 3-day test and the 1-month retest (Figure 1E-F) to be attributable to forgetting during the month-long delay in between tests. It further demonstrates that the maladaptive behavioral consequences (i.e., freezing to odors that have not been associated with shock) of our odor-cued fear conditioning paradigm are relatively long-lasting, which is consistent with the persistent nature of maladaptive behaviors that are associated with pathological fear in humans.

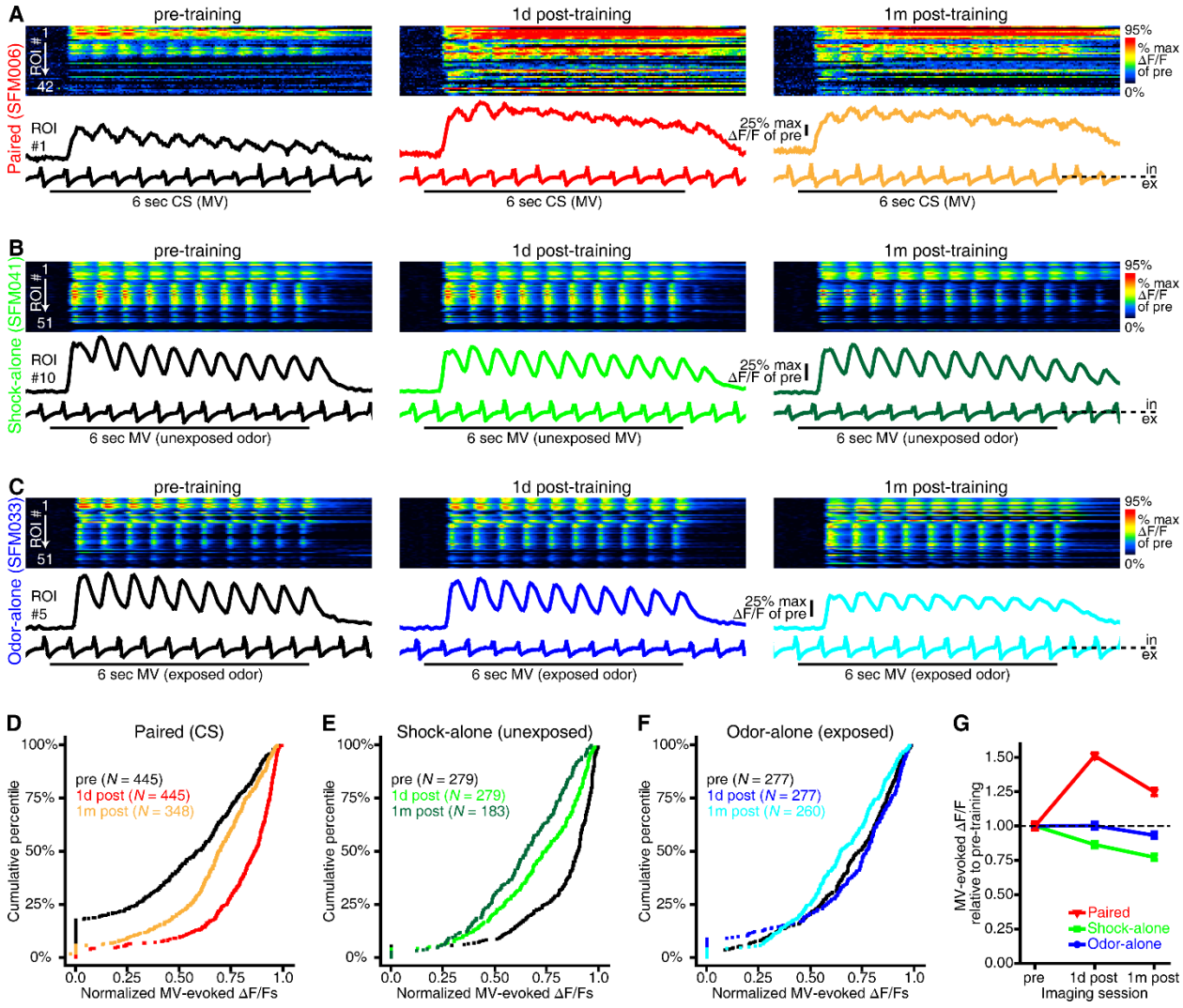



**Supplementary Figure S4: Individual variability in the effect of fear conditioning on CS-evoked PG interneuron activity during the first inhalation of odor.** Data that were pooled across all subjects in the paired group exhibited an increase in CS-evoked PG cell activity after training (Figure 1M, left and Figure 1N). After identifying this overall group effect, we performed a mouse-by-mouse analysis to assess individual variability in the extent of the training-induced facilitation. **(A-H)** Cumulative probability plots from individual mice in the paired group showing the distributions of GCaMP signals that were evoked by the first inhalation of the CS 1 day before (pre), 1 day after (1d post), and 1 month after (1m post) fear conditioning. An example of an inhalation 1-evoked response to the CS can be seen in Figure 1J. *N*s indicate the population of glomeruli per mouse that responded to the CS during any of the imaging preparations. The asterisk in **G** indicates that 1m post was only able to include data from 1 olfactory bulb. **(I)** Data are pooled across glomeruli for each subject and plotted as the mean±SEM ratio of pre-training baseline (dashed lines) for each imaging preparation to show relative changes in CS-evoked PG cell activity after fear conditioning. Individual subjects are color-coded per the corresponding key.

**Supplementary Table S1: Results from statistical analyses that were performed on the first inhalation of the CS in all individual paired subjects.**

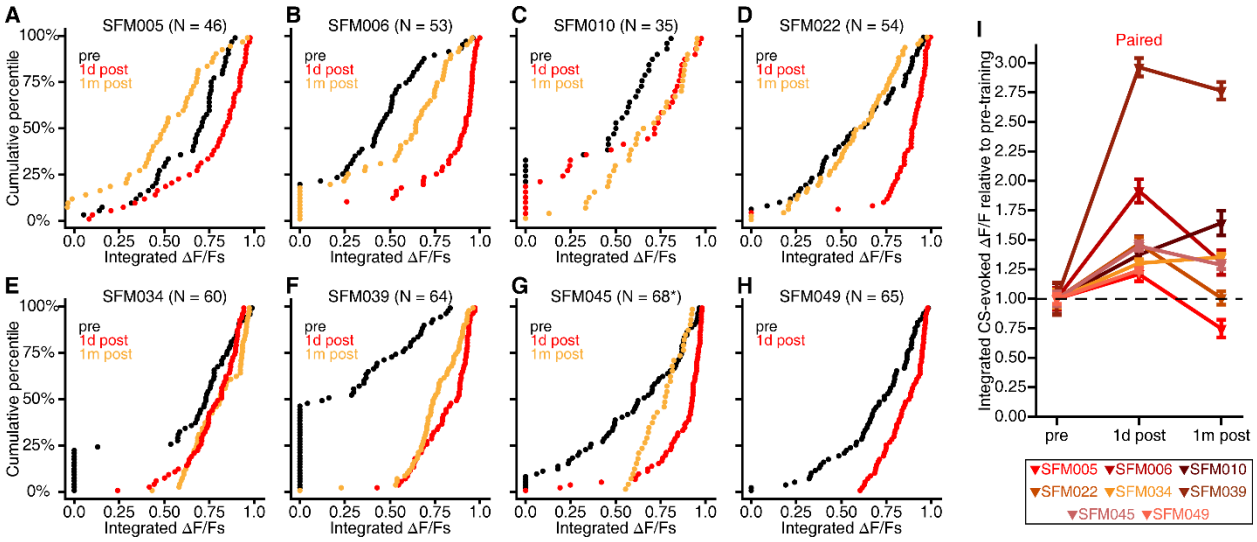
| Animal ID | Group | Data shown in Figure: | *P* values from pairwise comparisons between imaging preparations | | |
|---|---|---|---|---|---|
| | | | Pre vs. 1d Post | Pre vs. 1m Post | 1d Post vs. 1m Post |
| SFM005 | Paired | Figure S4A | < 0.001 | = 1.00 | < 0.001 |
| SFM006 | Paired | Figure S4B | < 0.001 | = 0.085 | = 0.011 |
| SFM010 | Paired | Figure S4C | = 0.091 | = 0.015 | = 0.265 |
| SFM022 | Paired | Figure S4D | < 0.001 | = 1.00 | < 0.001 |
| SFM034 | Paired | Figure S4E | = 0.581 | = 0.782 | = 0.628 |
| SFM039 | Paired | Figure S4F | < 0.001 | < 0.001 | = 0.001 |
| SFM045 | Paired | Figure S4G | < 0.001 | = 0.593 | = 0.451 |
| SFM049 | Paired | Figure S4H | = 0.002 | NA | NA |

Supplementary Table S1 lists the adjusted *P* values from all planned pairwise comparisons between imaging sessions for each mouse. These analyses accompany the cumulative frequency histograms in Supplementary Figure S4A-H.




**Supplementary Figure S5: CS-evoked PG interneuron activity is enhanced throughout the duration of an odor presentation after paired fear conditioning, but reduced after shock-alone control training. (A-C)** GCaMP signals across a population of GAD65-expressing PG interneurons during individual trials consisting of 6-sec presentations of MV. Each set of 3 pseudocolored heat maps corresponds to a population of glomerular ROIs from 1 olfactory bulb from a paired subject **(A)**, a shock-alone subject **(B)**, and an odor-alone subject **(C)**. Each row in a heat map corresponds to a single glomerular ROI (ROI #1 → ROI #*N*), with each population of ROIs being matched across imaging sessions that were performed 1 day before (left), 1 day after (middle), and 1 month after (right) fear (or control) conditioning. The 3 traces placed immediately

below each heat map show an example fluorescence record (top) from 1 of the ROIs in the corresponding heat map, the respiration record that was recorded from the piezosensor (middle), and the time of the 6-sec MV presentation during that trial (bottom). All heat maps and traces are aligned to 0.5 sec prior to MV onset. **(D-F)** Cumulative frequency histograms showing the distributions of GCaMP signals that are integrated across the entire 6-sec MV presentation 1 day before, 1 day after, and 1 month after paired **(D)**, shock-alone **(E)**, or odor-alone **(F)** training. Distributions for each group are pooled across MV-responsive glomeruli from all subjects. **(G)** The mean±SEM integrated MV-evoked ΔF/F is pooled across glomeruli from each group, plotted as a function of imaging preparation, and shown as a ratio of pre-training baseline (dashed line). Integrated PG cell activity was affected by paired and shock-alone training, but not by odor-alone training (interaction between group and imaging session, $F_{(4, 30)} = 5.568$, $p = 0.002$, $\eta_p^2 = 0.426$). Specifically, there was an effect of paired training across the population of CS-responsive glomeruli (**D**, $\chi^2_{(df = 2)} = 155.162$, $p < 0.001$), such that integrated PG cell activity was robustly enhanced 1 day after fear conditioning (pre vs. 1d post, $p < 0.001$), and it remained enhanced 1 month later (pre vs. 1m post, $p < 0.001$), albeit to a lesser extent than the initial increase (1d post vs. 1m post, $p < 0.001$). By contrast, the effect of shock-alone control exposure (**E**, $\chi^2_{(df = 2)} = 155.162$, $p < 0.001$) consisted of a persistent reduction in integrated MV-evoked PG cell activity (pre vs. 1d post, $p < 0.001$; pre vs 1m post, $p < 0.001$).

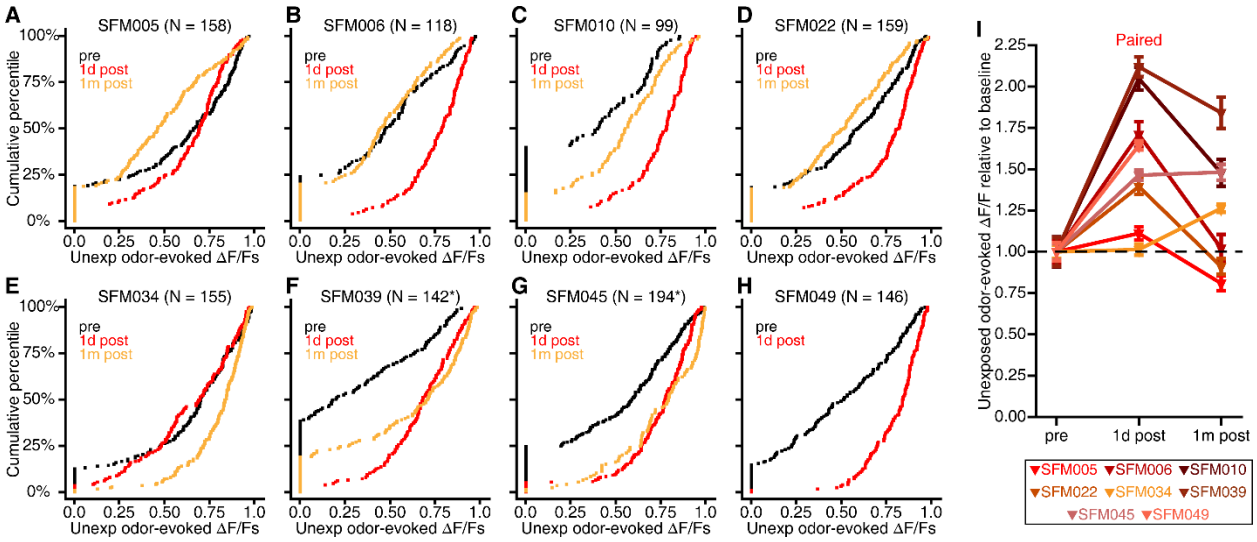



**Supplementary Figure S6: Individual variability in the effects of olfactory fear conditioning on integrated CS-evoked PG interneuron activity. (A-H)** Cumulative probability plots from individual mice in the paired group showing the distributions of integrated GCaMP signals that were evoked in PG interneurons by 6-sec presentations of the CS 1 day before (pre), 1 day after (1d post), and 1 month after (1m post) fear conditioning. Integrated GCaMP signals include multiple inhalations of odor across each 6-sec presentation, and an example of integrated CS-evoked activity can be seen in Supplementary Figure S5A. *N*s indicate the population of glomeruli per mouse that responded to the CS during any of the imaging preparations. The asterisk in **G** indicates that 1m post was only able to include data from 1 olfactory bulb. **(I)** Data are pooled across glomeruli per subject and displayed as the mean±SEM ratio of pre-training baseline (dashed line) to show relative changes in integrated CS-evoked PG cell activity after fear conditioning. Since each individual subject exhibited a significant enhancement of CS-evoked PG cell activity, the effect of fear conditioning did not appear to differ between GCaMP3-expressing mice (SFM005, SFM006, SFM010, and SFM022) and GCaMP6f-expressing mice (SFM034, SFM039, SFM045, and SFM049).

**Supplementary Table S2: Integrated CS-evoked PG interneuron activity was significantly enhanced by olfactory fear conditioning in all 8 paired subjects.**

| Animal ID | Data shown in Figure: | *P* values from pairwise comparisons between imaging preparations | | |
|---|---|---|---|---|
| | | Pre vs. 1d Post | Pre vs. 1m Post | 1d Post vs. 1m Post |
| SFM005 | Figure S6A | = 0.027 | = 0.011 | < 0.001 |
| SFM006 | Figure S6B | < 0.001 | = 0.026 | < 0.001 |
| SFM010 | Figure S6C | = 0.031 | = 0.026 | = 1.0 |
| SFM022 | Figure S6D | < 0.001 | = 1.00 | < 0.001 |
| SFM034 | Figure S6E | < 0.001 | < 0.001 | = 1.00 |
| SFM039 | Figure S6F | < 0.001 | < 0.001 | = 0.231 |
| SFM045 | Figure S6G | < 0.001 | = 0.987 | = 0.683 |
| SFM049 | Figure S6H | < 0.001 | NA | NA |

Supplementary Table S2 lists the adjusted *P* values from all planned pairwise comparisons between imaging sessions for each mouse. These analyses accompany the cumulative frequency histograms in Supplementary Figure S6A-H.

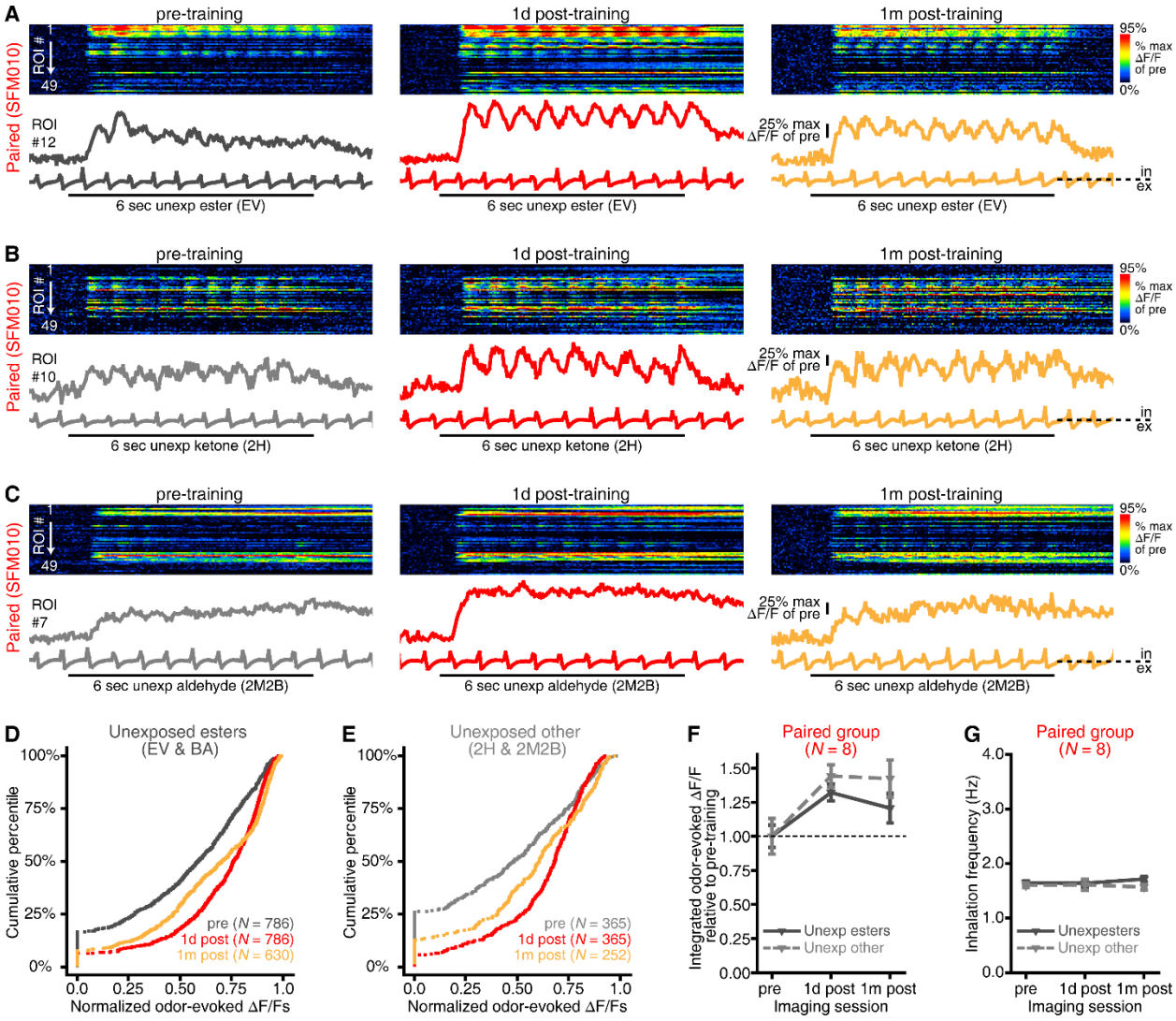



**Supplementary Figure S7: Fear learning-induced plasticity in PG interneurons generalizes to non-threatening odors. (A-H)** Cumulative probability plots from individual mice in the paired group showing the distributions of GCaMP signals that were evoked by the first inhalation of all unexposed odors 1 day before (pre), 1 day after (1d post), and 1 month after (1m post) odor-cued fear conditioning. These data are pooled across all 4 unexposed odors (EV, BA, 2H, and 2M2B) for each mouse because the effect of fear conditioning on PG cell activity was the same across all stimuli. An example of an inhalation 1-evoked response to an unexposed odor can be seen in Figure 2D. *N*s indicate the number of response amplitudes (ΔF/Fs) that are pooled across glomeruli and odors per mouse. Asterisks in **F,G** indicate that the distributions shown for 1 month post-training do not contain the same number of ΔF/Fs as the other 2 distributions. **(I)** Data are pooled across glomeruli per subject and displayed as the mean±SEM ratio of pre-training baseline (dashed line) to show the relative effect of fear conditioning on inhalation 1-evoked PG cell activity that was stimulated by unexposed odors. The generalized facilitation of odor-evoked PG interneuron activity after fear learning did not appear to be influenced by genotype because it was observed in individual mice expressing either GCaMP3 (SFM005, SFM006, SFM010, and SFM022) or GCaMP6f (SFM034, SFM039, SFM045, and SFM049).

**Supplementary Table S3: Olfactory fear conditioning results in a generalized enhancement of odor-evoked PG interneuron activity in individual paired subjects.**

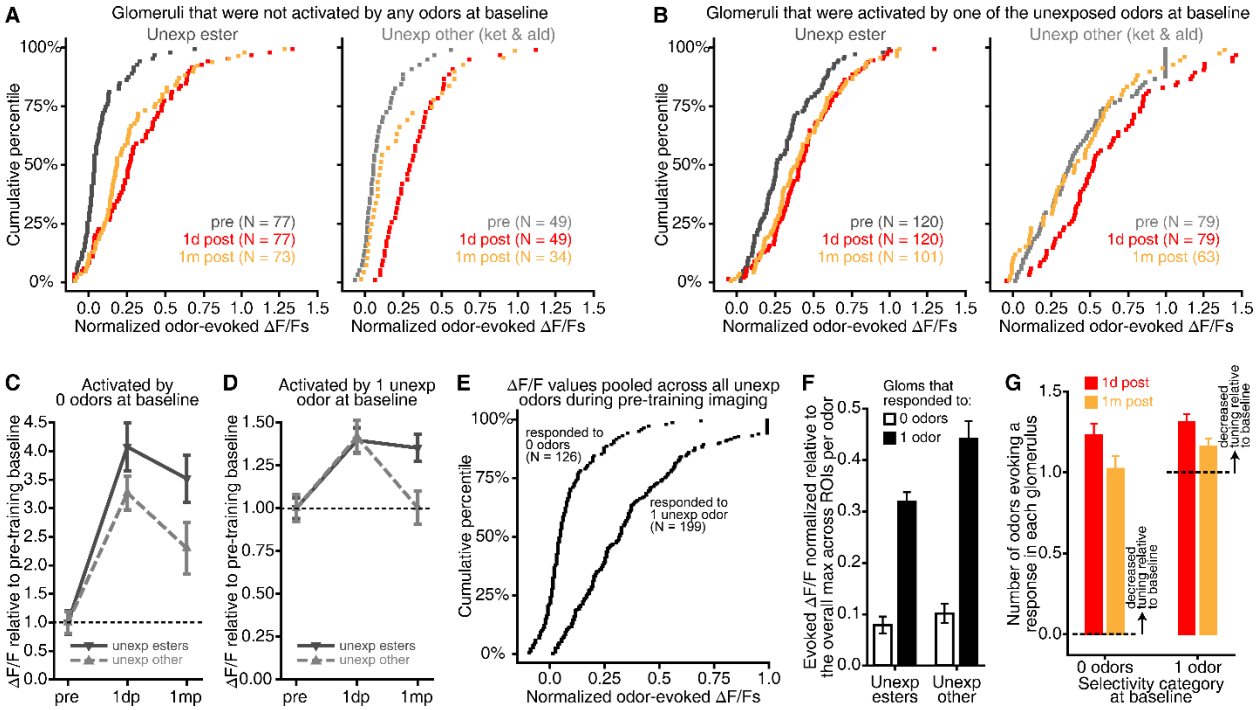
| Animal ID | Group | Data shown in Figure: | *P* values from pairwise comparisons between imaging preparations | | |
|---|---|---|---|---|---|
| | | | Pre vs. 1d Post | Pre vs. 1m Post | 1d Post vs. 1m Post |
| SFM005 | Paired | Figure S7A | = 0.004 | = 0.052 | = 0.874 |
| SFM006 | Paired | Figure S7B | < 0.001 | = 0.299 | < 0.001 |
| SFM010 | Paired | Figure S7C | < 0.001 | = 0.009 | = 0.013 |
| SFM022 | Paired | Figure S7D | < 0.001 | = 0.001 | < 0.001 |
| SFM034 | Paired | Figure S7E | = 0.759 | < 0.001 | < 0.001 |
| SFM039 | Paired | Figure S7F | < 0.001 | <0.001 | = 1.00 |
| SFM045 | Paired | Figure S7G | < 0.001 | = 0.716 | = 0.006 |
| SFM049 | Paired | Figure S7H | < 0.001 | NA | NA |

Supplementary Table S3 provides a summary of the results from the statistical analyses that accompany the cumulative probability plots in Supplementary Figure S7A-H. Adjusted *P* values from planned pairwise comparisons between imaging sessions are listed for each mouse. These analyses were performed on a mouse-by-mouse basis to look for individual variability in the extent to which fear conditioning resulted in a generalized enhancement of odor-evoked PG cell activity.




**Supplementary Figure S8: The generalized enhancement of PG interneuron activity occurs throughout the entire length of a trial. (A-C)** GCaMP signals across a population of GAD65-expressing PG interneurons during individual trials consisting of 6-sec presentations of EV **(A)**, 2H **(B)**, and 2M2B **(C)**. Each set of 3 pseudocolored heat maps shows the same population of glomerular ROIs from 1 olfactory bulb from a paired subject. Each row in a heat map corresponds to a single glomerular ROI (ROI #1 → ROI #49), with all ROIs being matched across imaging sessions that were performed 1 day before (left), 1 day after (middle), and 1 month after (right) fear conditioning. The 3 traces placed immediately below each heat map show an example

fluorescence record (top) from 1 of the ROIs in the corresponding heat map, the respiration record that was recorded from the piezosensor (middle), and the time of the 6-sec odor presentation during that trial (bottom). All heat maps and traces are aligned to 1 sec prior to odor onset. **(D-E)** Cumulative frequency histograms showing the distributions of integrated GCaMP signals that were evoked by unexposed esters **(D)** and by unexposed odors of other chemical classes **(E)** 1 day before, 1 day after, and 1 month after fear conditioning. Distributions are pooled across integrated ΔF/Fs from all 8 paired subjects. **(F)** The mean±SEM integrated odor-evoked ΔF/F was calculated across individual subjects, plotted as a function of imaging preparation for both unexposed odor categories, and shown as a ratio of pre-training baseline (dashed line). On average **(F)**, integrated PG cell activity was enhanced after paired training ($F_{(2, 14)} = 6.355$, $p = 0.011$, $\eta_p^2 = 0.476$), and the effect of fear conditioning generalized equally across the CS and unexposed odors (non-significant interaction between imaging preparation and odor category, $F_{(2.4, 17.0)} = 1.203$, $p = 0.331$, $\eta_p^2 = 0.147$). The generalized effect of fear conditioning is also exemplified by the distributions of integrated GCaMP signals **(D,E**; $\chi^2_{(df = 2)} = 224.487$, $p < 0.001$**)**, which were enhanced the day after fear conditioning (pre vs. 1d post, $p < 0.001$), and remained enhanced 1 month later (pre vs. 1m post, $p = 0.002$), albeit to a lesser extent (1d post vs. 1m post, $p < 0.001$). **(G)** Importantly, the respiration rates that were recorded during trials with unexposed odors did not differ across unexposed odor categories or imaging sessions in the paired group (non-significant effect of imaging session, $F_{(2, 14)} = 0.051$, $p = 0.950$, $\eta_p^2 = 0.007$; non-significant imaging session×odor category interaction, $F_{(1.1, 7.8)} = 1.371$, $p = 0.286$, $\eta_p^2 = 0.164$), so the non-specific enhancement of odor-evoked PG cell activity is likely the result of learning-induced sensory plasticity and not changes in respiration.

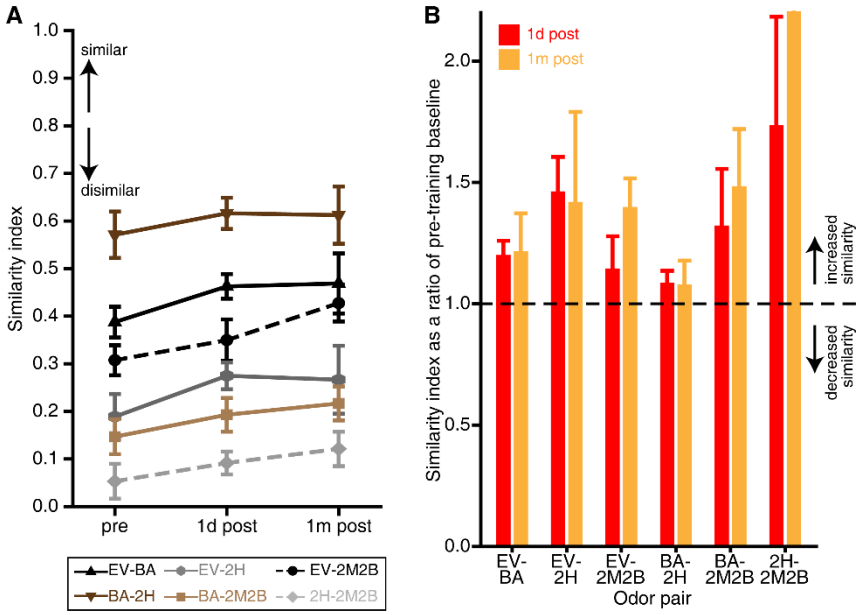



**Supplementary Figure S9: The generalized enhancement of PG interneuron activity that occurred after fear conditioning was observed in glomeruli that did not respond to the CS at baseline. (A-B)** Cumulative frequency histograms showing the distributions of response amplitudes (ΔF/Fs) that were evoked by the first inhalation of an unexposed odor 1 day before (pre), 1 day after (1d post), and 1 month after (1m post) fear conditioning in glomeruli that did not respond to any odors at baseline **(A)** and glomeruli that only responded to 1 of the 4 unexposed (unexp) odors at baseline **(B)**. Note that the glomeruli that did not respond to any odors at baseline **(A)** had below- or near-zero response amplitudes that did not exceed our statistical thresholding criterion. The activity that was evoked by unexposed esters (left; EV and BA) and by unexposed odors of "other" chemical classes (right; 2H and 2M2B) is displayed separately in each panel. **(C-D)** The distributions in **A-B** were averaged across glomeruli and are plotted here as a ratio of pre-training baseline (dashed line) to show relative changes across imaging sessions in PG cell activity that was evoked by unexposed odors. **(E-F)** Cumulative frequency histogram **(E)** and mean±SEM

summary plot **(F)** comparing the normalized unexposed odor-evoked ΔF/Fs between glomeruli that did not respond to any odors and glomeruli that only responded to 1 unexposed odor during pre-training baseline. **(G)** The mean±SEM number of odors evoking a measurable response in each glomerulus 1 day (red) and 1 month (orange) after fear conditioning is shown for populations of glomeruli that are separated by their baseline selectivity (left, responded to 0 odors; right, responded to 1 unexposed odor). The dashed lines indicate baseline odor response selectivity for each selectivity category. Values above and below the dashed lines respectively indicate decreases and increases in odor tuning relative to baseline.

The results that are summarized above in Supplementary Figure S9 show that there were glomeruli that were not activated by the CS at baseline, but were nonetheless facilitated after fear conditioning **(A-D)**, confirming a generalized enhancement of odor-evoked PG interneuron activity. This generalized enhancement was equal across unexposed odor categories (i.e., unexposed esters vs. unexposed other) and was even observed in glomeruli that did not exhibit a measurable response to any of the odors in our panel at baseline, but that began to respond to at least 1 of the unexposed odors after fear conditioning **(A,C)**. Notably, the facilitation was larger in glomeruli that did not respond to any odors at baseline **(C)** than in glomeruli that responded to 1 unexposed odor at baseline **(D)**. The population that did not respond to any odors at baseline consisted of glomeruli that had below- or near-zero amplitudes that did not exceed our statistical thresholding criterion, and were therefore substantially smaller than the baseline amplitudes that were measured from glomeruli that responded to 1 unexposed odor **(E-F)**. We found that fear conditioning differentially affected CS-evoked PG interneuron activity based on pre-training response amplitudes, such that glomeruli with the smallest CS-evoked amplitudes at baseline exhibited the largest facilitation after fear conditioning (Figure 5). We extended those findings

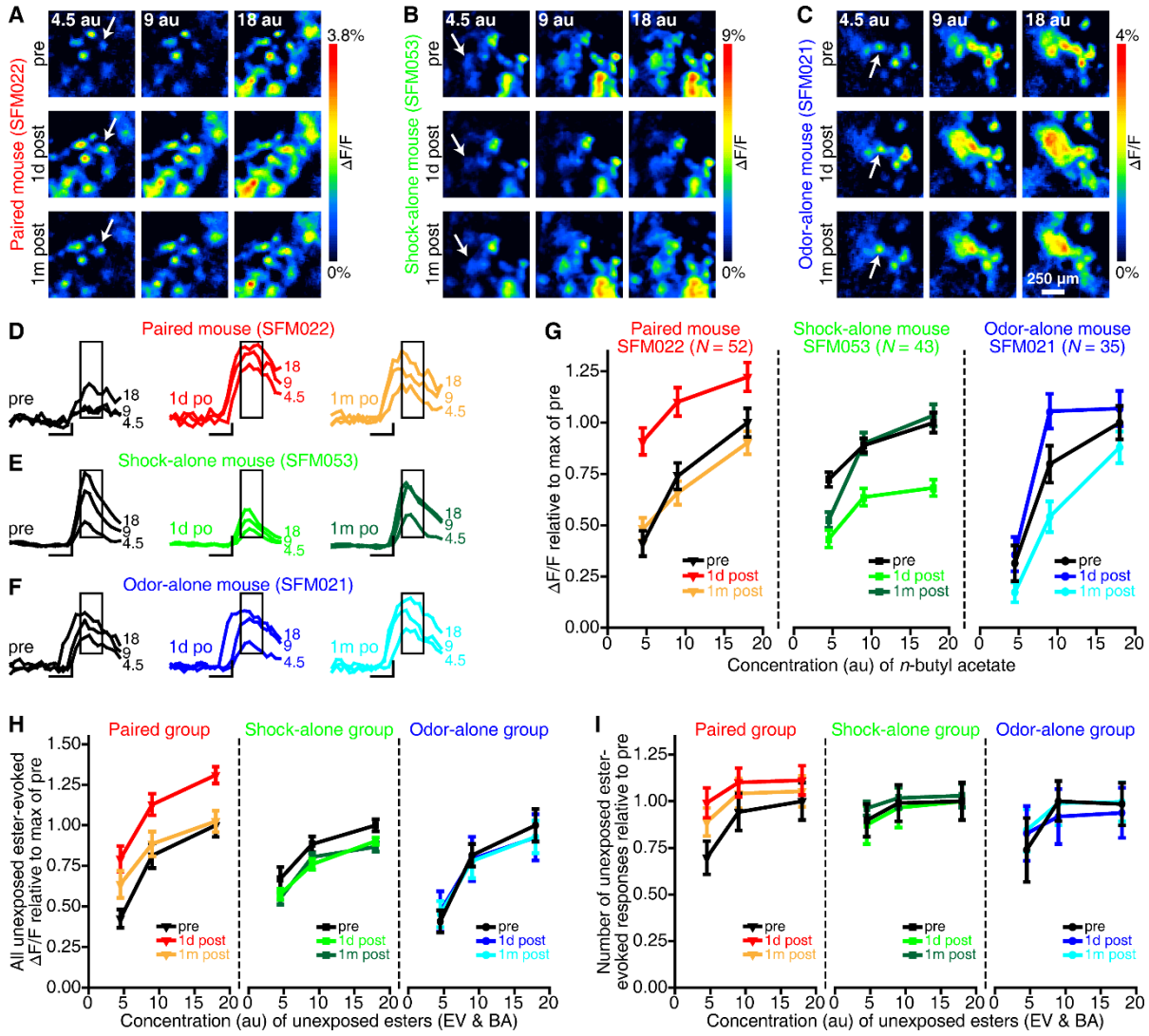
here by demonstrating that the generalized enhancement of PG interneuron activity was more robust in the glomeruli that did not respond to any odors and thus had the weakest responses at baseline. The generalized facilitation of odor-evoked PG cell activity may be related to changes in odor tuning (see Figure 4 for more extensive selectivity analysis) because glomeruli that did not respond to the CS at baseline exhibited a decrease in odor tuning (i.e., responded to more odors) after fear conditioning, regardless of their baseline odor selectivity category **(G)**.




**Supplementary Figure S10: Activity maps that are evoked by non-threatening odors tend to become more similar to each other after olfactory fear conditioning.** Mice that underwent fear conditioning exhibited a generalized behavioral fear response to novel odors that was paralleled by a generalized enhancement of odor-evoked PG interneuron activity and by a decrease in glomerular odor response selectivity. It is possible that these parallel changes in PG interneuron physiology increase the similarity (i.e., decrease the discriminability) between neural representations of the CS and unexposed odors (Figure 4K-L), and also between unexposed odors versus each other. To assess this possibility, we calculated similarity indexes for all possible pairwise comparisons of PG cell activity that was evoked by each of the 5 odors in our panel, yielding 10 odor pairs (see Figure 4K-L for CS versus unexposed odors).

**(A)** The mean±SEM similarity between pairs of unexposed odors was calculated across all 8 paired subjects and is plotted as a function of imaging preparation. The similarity index ranged from 0-1, with 0 indicating complete dissimilarity between integrated odor-evoked PG cell activity and 1 indicating complete similarity. The 6 odor pairs differed in similarity at baseline – for example, at baseline (labeled "pre" on *x*-axis) EV- and BA-evoked PG activity maps were more

similar to each other than EV- and 2H-evoked PG activity maps. **(B)** Because of these baseline differences in odor map similarity we calculated ratios between the similarity indexes from 1 day and 1 month post-training relative to the similarity indexes from pre-training. This permitted a qualitative evaluation of changes in PG activity map discriminability relative to baseline (dashed line) for responses that were integrated across the entire 6-sec odor. After fear conditioning, most odor pairs tended to be more similar to each other than they were during baseline. However, the increase in similarity was not equal for all odor pairs, and seemed to be inversely related to baseline similarity. For example, there was almost no relative change in the similarity between BA- and 2H-evoked activity maps after fear conditioning **(B)**, but out of all of the unexposed odor pairs, BA and 2H were the most similar at baseline **(A)**. Overall, these data suggest that PG activity maps that are evoked by novel odors may become more similar to each other after fear learning.




**Supplementary Figure S11: The enhanced sensitivity generalizes to PG interneuron activity that is evoked by unexposed odors. (A-F)** Pseudocolored activity maps **(A-C)** and corresponding response amplitudes **(**ΔF/Fs; **D-F)** from representative subjects that underwent either paired **(A,D)**, shock-alone **(B,E)**, or odor-alone **(C,F)** training. These examples illustrate activity that was evoked by the first inhalation of each of 3 concentrations of BA during individual trials that were presented 1 day before (pre), 1 day after (1d post), and 1 month after (1m post) conditioning. Boxed regions in **D-F** note the frames that were used for activity maps **(A-C)** and concentrations analyses **(G-I)**. Traces from each individual subject **(D-F)** are scaled relative to the overall max of pre-training

across concentrations (scale bars: horizontal, 200 ms; vertical, 25% max ΔF/F of pre). **(G)** BA-evoked concentration response functions were calculated across the population of glomeruli that responded to BA (indicated by *N*s) in the example paired (left), shock-alone (middle), and odor-alone (right) subjects. PG interneuron activity that was evoked by all 3 concentrations of BA was enhanced the day after fear conditioning, but returned to baseline levels 1 month later in the example paired subject (left, SFM022). The reverse effect occurred in this example shock-alone subject (middle, SFM053), because PG interneuron activity exhibited a decrease in sensitivity to BA the day after shock exposure, but returned to baseline levels 1 month later. The example odor-alone mouse (right, SFM021) did not exhibit a consistent shift in sensitivity to BA after training. **(H-I)** The mean±SEM response amplitudes **(H)** and number of glomerular responses **(I)** that were evoked by 2 unexposed esters (EV and BA) are pooled across all subjects in the paired (left), shock-alone (middle), and odor-alone (right) groups. Data are plotted relative to the overall max of pre-training and shown as a function of concentration for each imaging preparation. On average for the paired group, the number of glomerular responses, and the amplitudes of those responses, increased across a 4-fold range of unexposed ester concentrations after fear conditioning. Unexposed ester-evoked response amplitudes tended to decrease slightly across concentrations after shock-alone training, though the number of glomerular responses stayed relatively constant. In contrast with the paired and shock-alone groups, the odor-alone group exhibited relatively stable unexposed ester-evoked PG cell activity across imaging sessions.