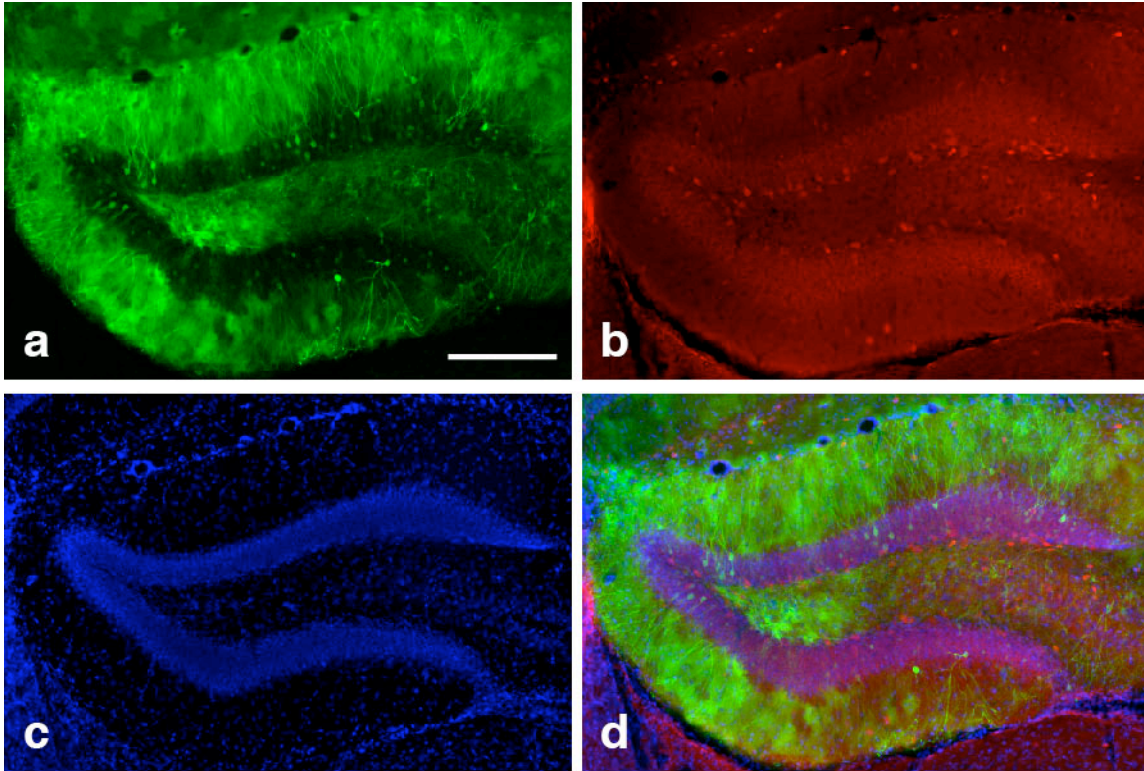


e

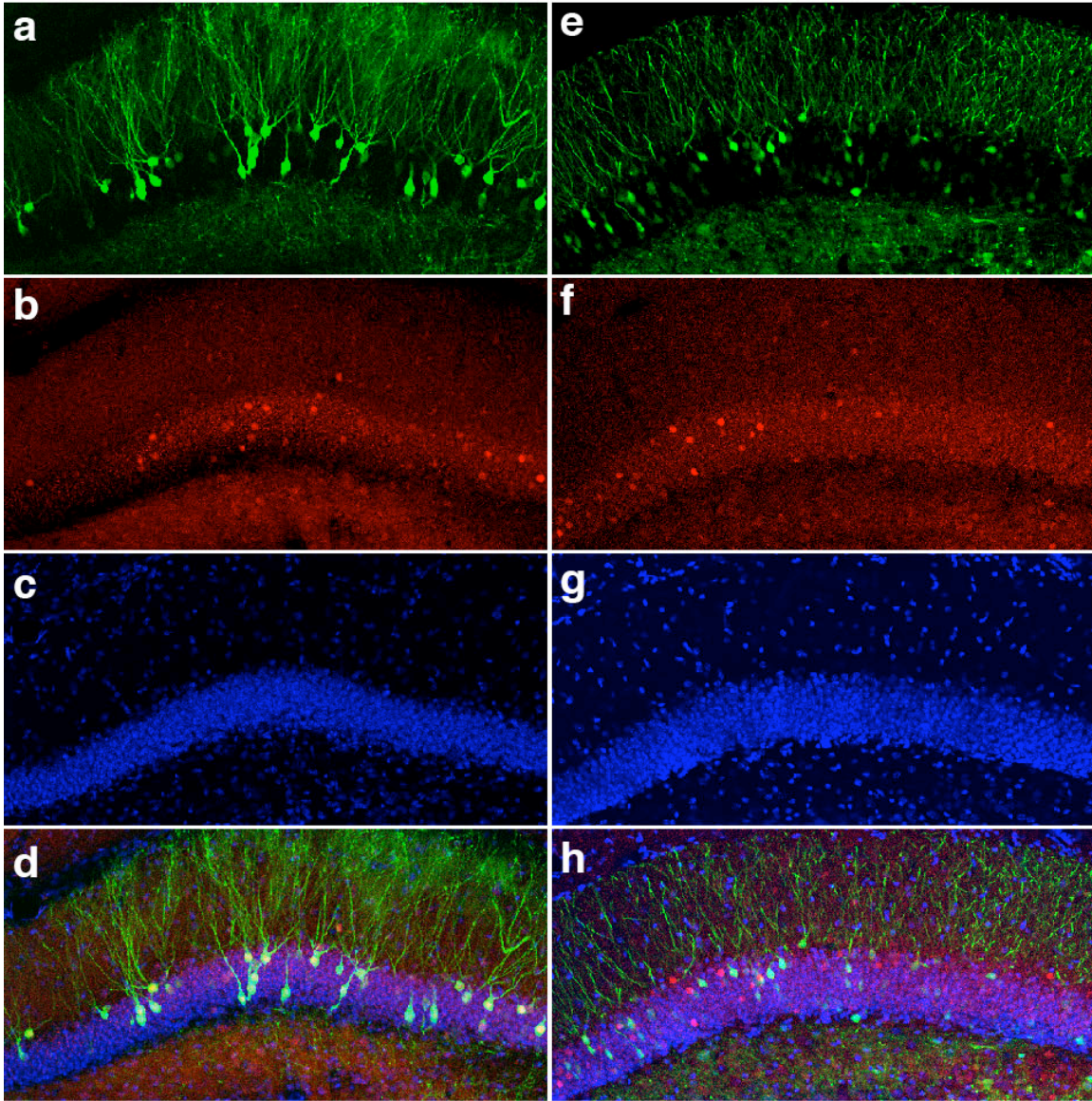
| Subject | Cell marker | Cell Counts |
|---------|---------------------------|-------------|
| Mouse 1 | ChR2 | 360 ± 19.5 |
| | c-fos | 348 ± 18.1 |
| | Overlap: ChR2 (%c-fos) | 94.8 ± 0.02 |
| Mouse 2 | ChR2 | 426 ± 8.1 |
| | c-fos | 407 ± 6.1 |
| | Overlap: ChR2 (%c-fos) | 93.2 ± 0.01 |
| Mouse 3 | ChR2 | 406 ± 10.6 |
| | c-fos | 386 ± 11.1 |
| | Overlap: ChR2 (%c-fos) | 95.7 ± 0.02 |

Supplementary Figure 1: ChR2-EYFP expression after fear conditioning recapitulates endogenous c-fos expression.

The c-fos-tTA mice were injected with AAV₉-TRE-ChR2-EYFP targeting the DG and kept on Dox for a month prior to training. Then, they were taken off Dox for two days to open a window of activity-dependent labeling by ChR2-EYFP. The mice were next fear conditioned and sacrificed 1.5 hours after training to measure ChR2-EYFP and c-fos expression. (a) ChR2-EYFP, (b) c-fos, (c) DAPI, and (d) merged images. Each white rectangle is magnified to the right of the image. (e) Quantifications revealed > 93% of c-fos-positive cells also expressed ChR2-EYFP after training (*n* = 4–6 slices of dorsal DG/subject).

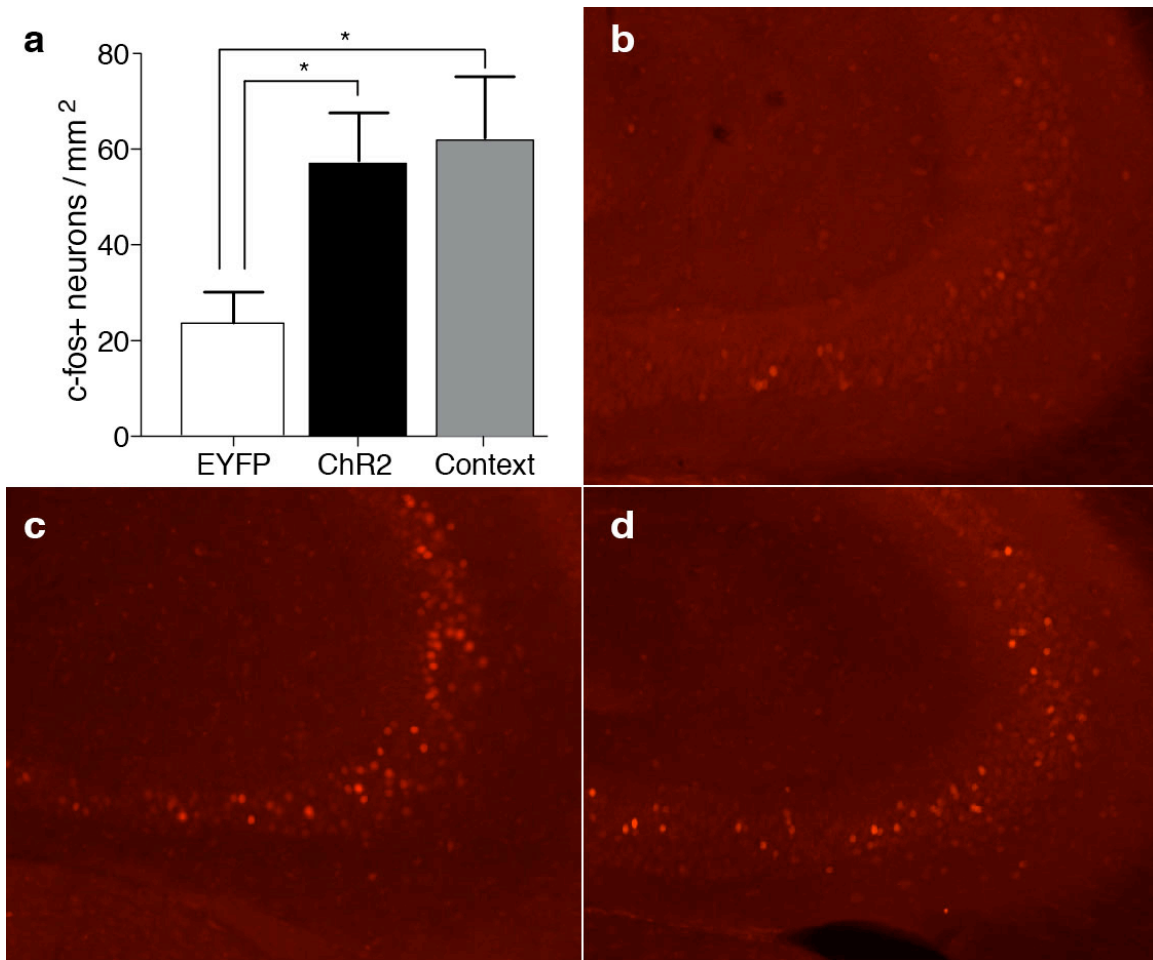


Supplementary Figure 2: GABA and ChR2-EYFP-positive cells do not overlap.
(a) DG from experimental mice kept off Dox for two days and then subjected to fear conditioning expressed ChR2-EYFP in excitatory cells. (b) Anti-GABA. (c) DAPI. (d) Merged image. Scale bar in (a) 250 μm .

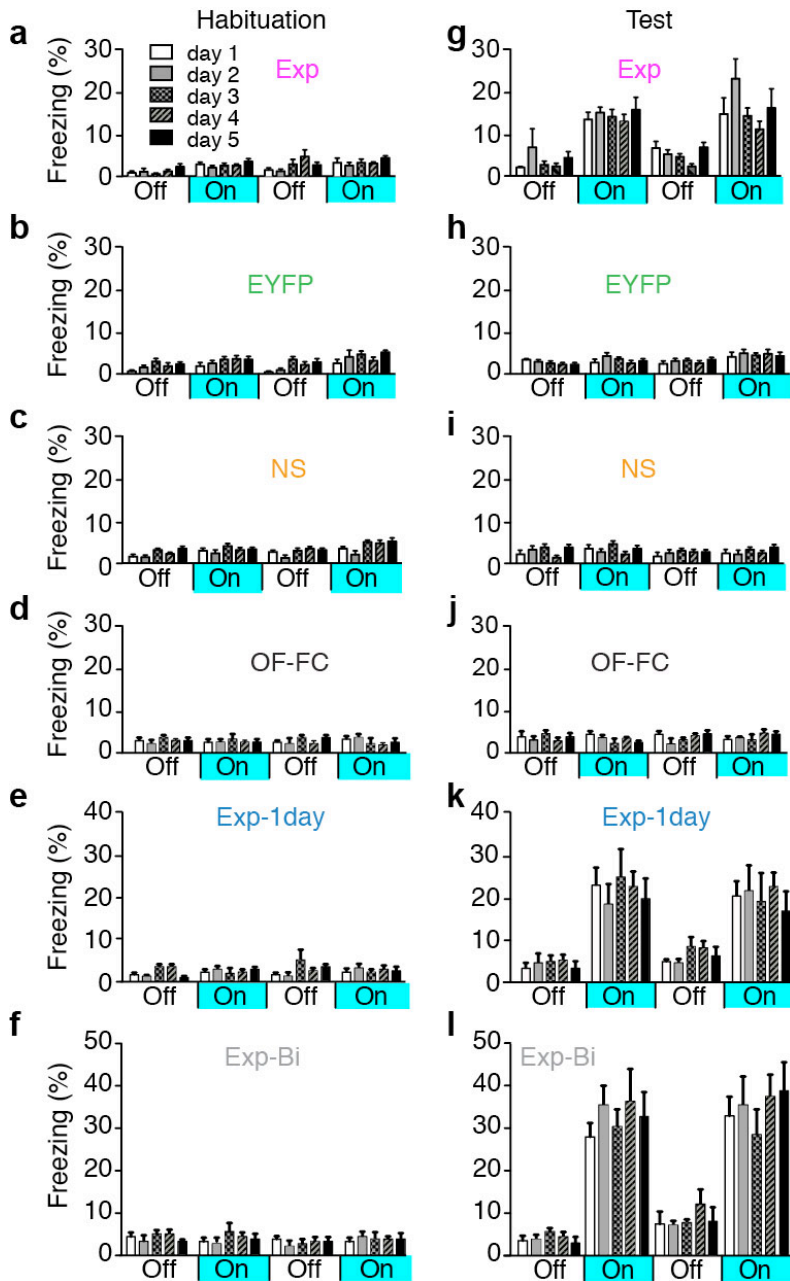


Supplementary Figure 3: Light stimulation induces c-fos expression in cells expressing ChR2-EYFP but not EYFP.

(a)–(d) Representative DG cells after light stimulation in c-fos-tTA mice injected with AAV₉-TRE-ChR2-EYFP. (e)–(h) Representative DG cells after light stimulation in c-fos-tTA mice injected with AAV₉-TRE-EYFP. (a) ChR2-EYFP. (e) EYFP. (b), (f) c-fos. (c), (g) DAPI. (d), (h) Merged images.

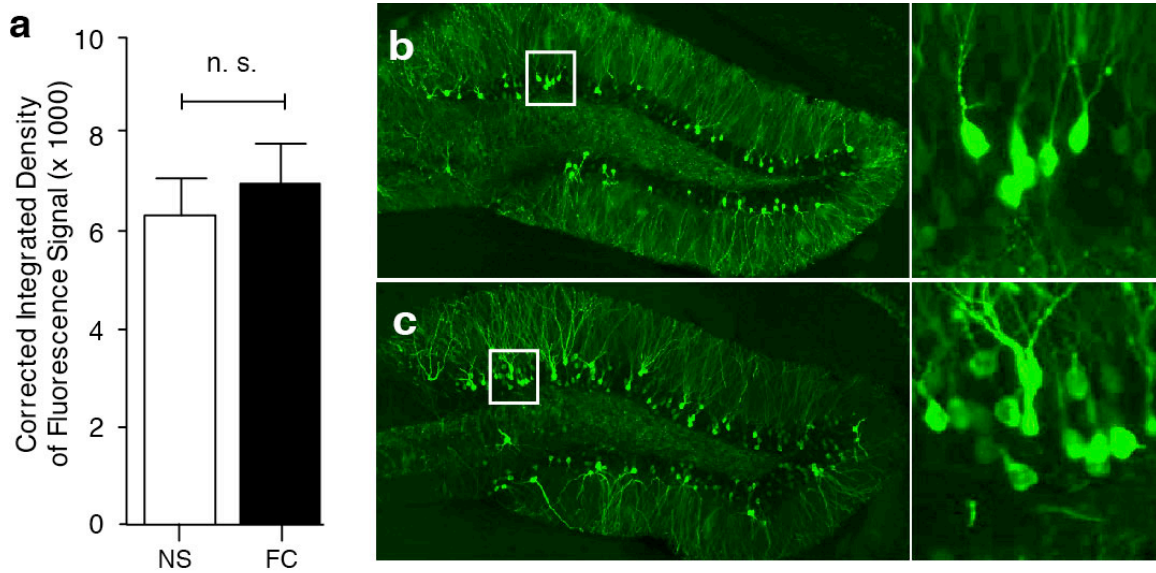


Supplementary Figure 4: *In vivo* optical stimulation of DG cells increases c-fos expression in CA3. (a) Quantifications of c-fos expression in the CA3 of mice expressing ChR2-EYFP or EYFP after light stimulation, or a control group of mice after physiological contextual fear memory recall in the original trained context ($n = 3/\text{group}$, $F_{2,6} = 4.898$, $*P < 0.05$). Representative c-fos positive CA3 cells in the EYFP-only group (b), ChR2-EYFP group (c), or physiological contextual fear memory recall group (d).



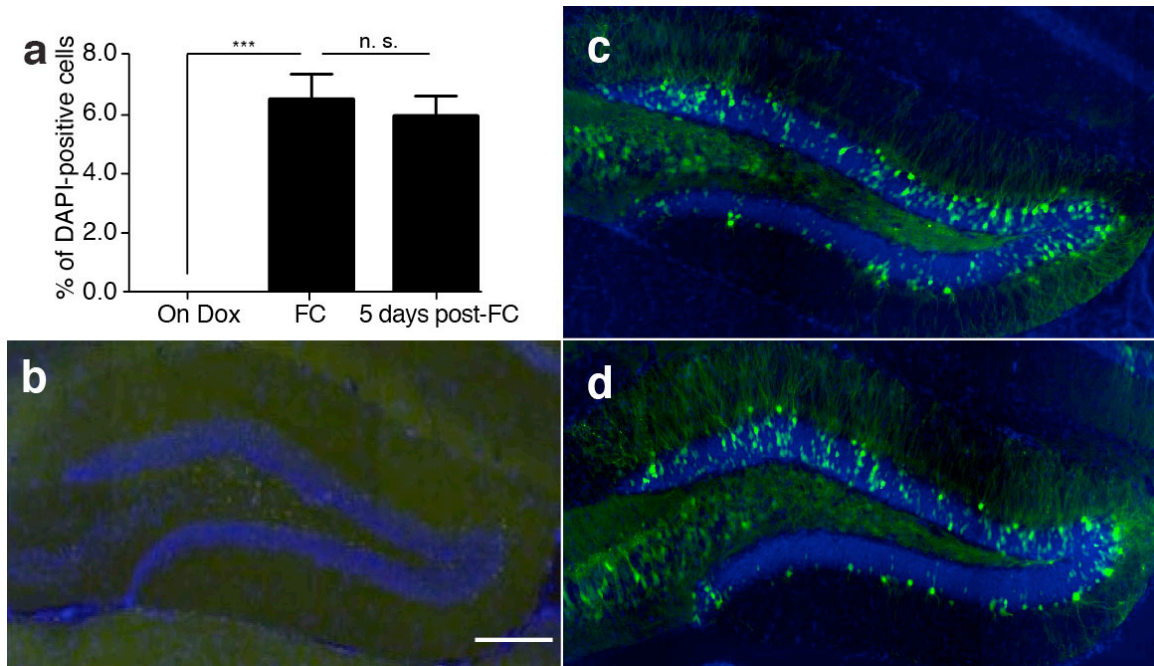
Supplementary Figure 5: Habituation and testing sessions across five days.

(a)–(f) Habituation sessions produced < 5% freezing in the Exp (a), EYFP (b), NS (c), OF-FC (d), Exp-1day (e), and Exp-Bi (f) groups. (g)–(i) Testing sessions produced significant increases in freezing during light-on epochs only in the Exp (g), Exp-1day (k), and Exp-Bi (l) groups, but not in the EYFP (h), NS (i), or OF-FC (j) groups throughout the five days. $n = 12$ for Exp, NS, and EYFP groups, $n = 5$ for Exp-1day and OF-FC groups, and $n = 6$ for Exp-Bi group.



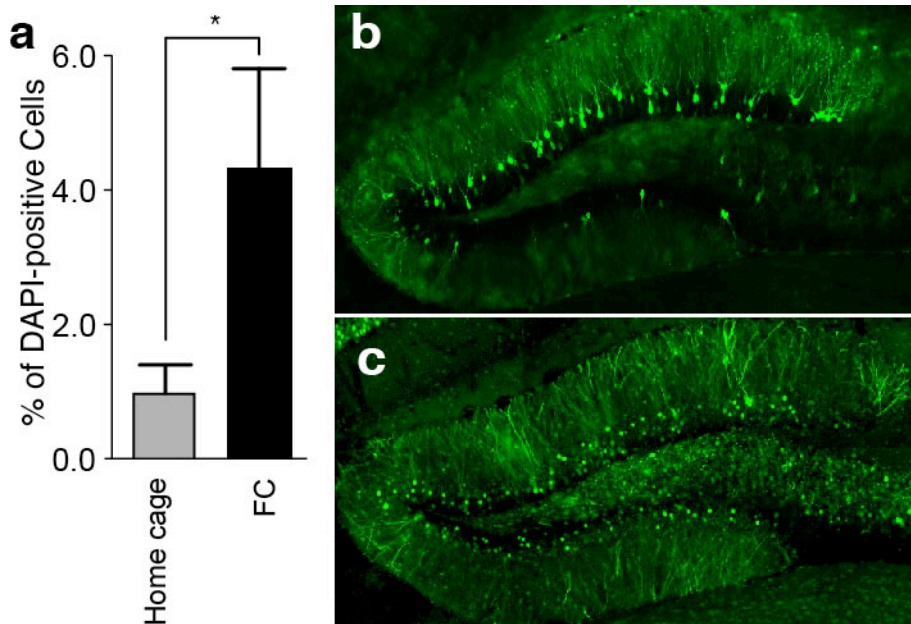
Supplementary Figure 6: Similar ChR2-EYFP expression levels within cells after fear conditioning and no shock exposure.

(a) ChR2-EYFP in no shock (NS) and fear conditioned (FC) groups show similar levels of fluorescence signal ($n = 5$ subjects each). Representative images of DG from the NS group (b) or the FC group (c). Each white rectangle is magnified to the right of the image.

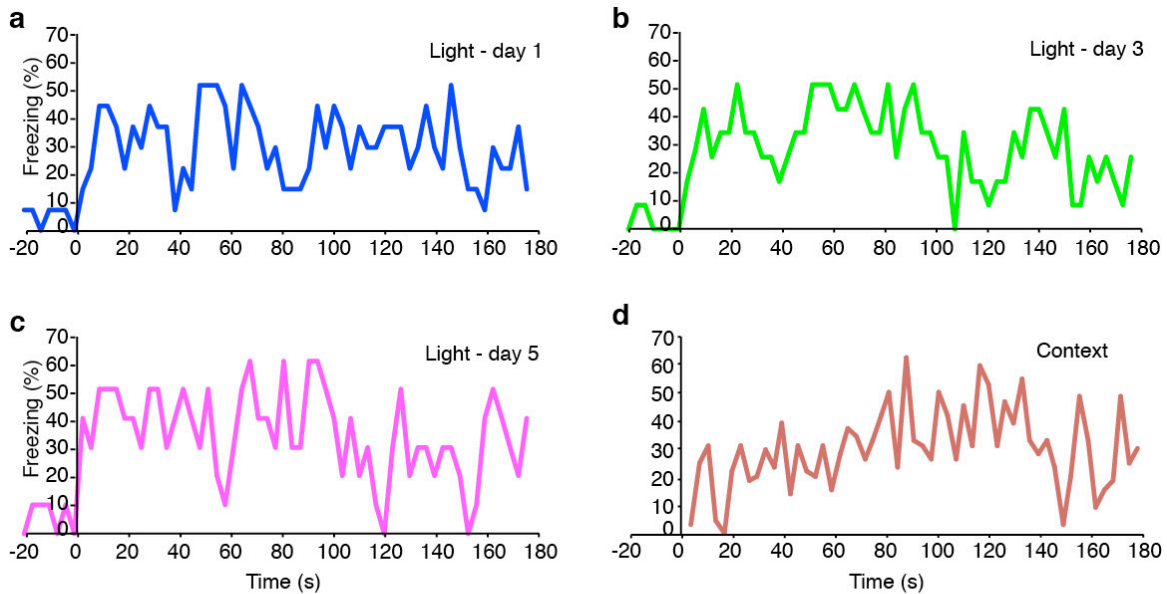


Supplementary Figure 7: Inducible and activity-dependent EYFP expression.

(a) Quantifications of EYFP expression levels in *c-fos-tTA* mice injected with AAV₉-TRE-EYFP and then underwent different treatments ($n = 3$ per group, $F_{2,6} = 32.52$, *** $P < 0.001$). **(b)** Minimal expression of EYFP in the presence of Dox. When subjects were taken off Dox for two days and fear conditioned, EYFP expression was robust throughout the dentate gyrus **(c)** and this expression was still present five days post-training **(d)**. DAPI (blue), EYFP (green). Scale bar in **(b)** 250 μm .

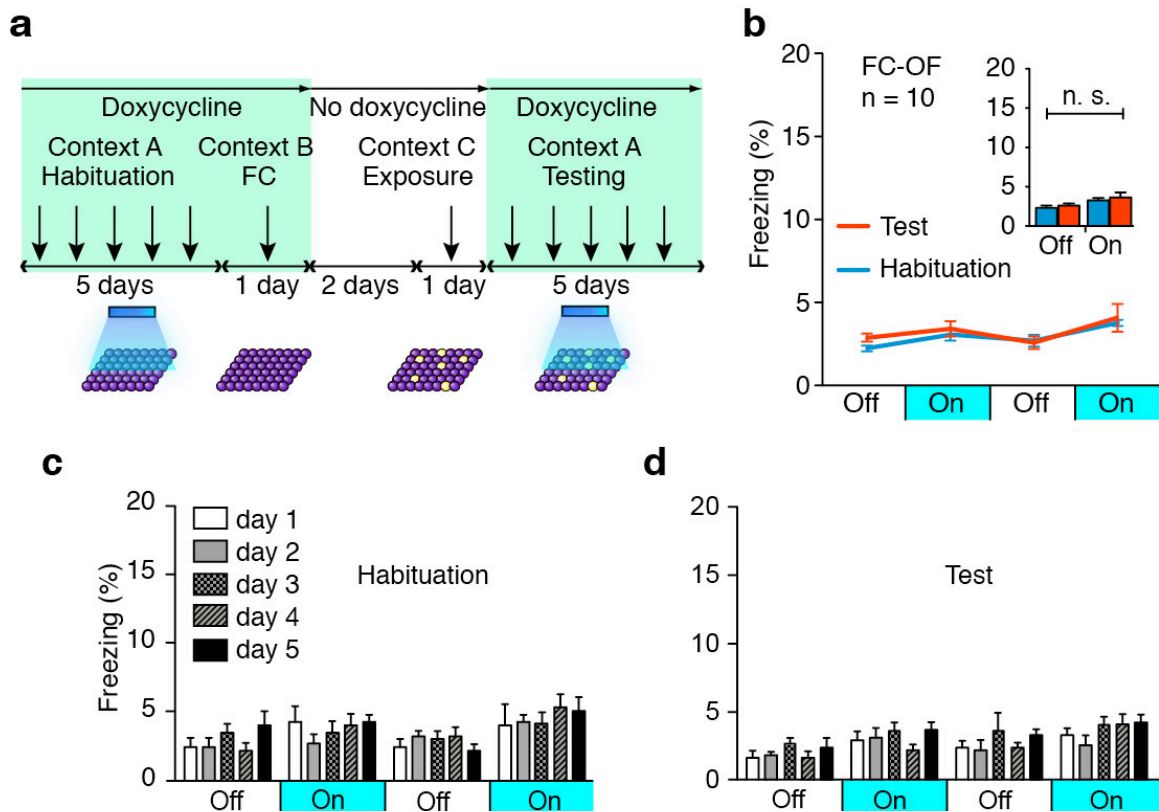


Supplementary Figure 8: Dox removal for one day is sufficient to induce ChR2-EYFP expression. (a) Quantifications revealed that mice kept off Dox for one day in home cage showed low basal levels of ChR2-EYFP and significantly higher levels of ChR2-EYFP after fear conditioning ($n = 3$ per condition, $*P = 0.0343$). Representative DG section from a mouse kept off Dox for one day in home cage (b) or after fear conditioning (c).

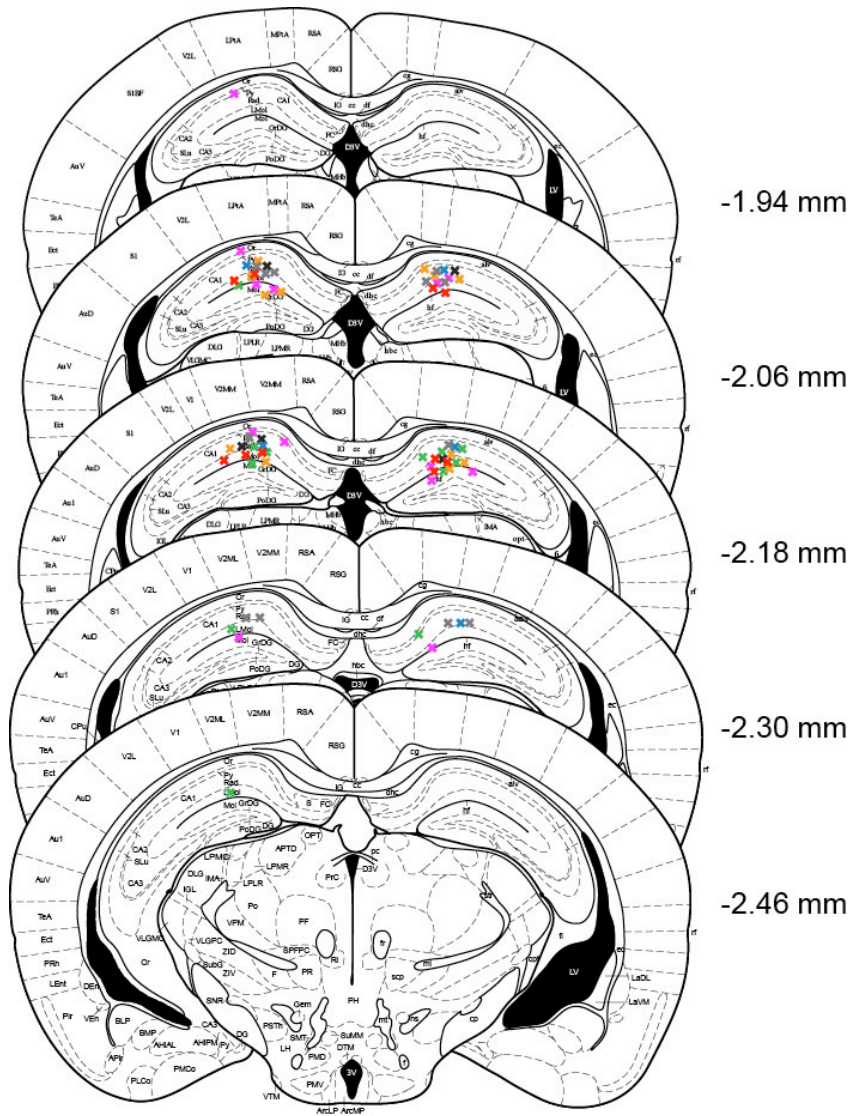


Supplementary Figure 9: Freezing time course during light stimulation and context probe trials.

Freezing time course for Exp-Bi group during light stimulation on day one (a), day three (b), and day five (c) of test sessions, and during a context probe trial (d). For light stimulation, time 0 indicates the beginning of light onset. For the context probe trial, time 0 indicates when the mouse entered the chamber ($n = 6$).



Supplementary Figure 10: Labeling and stimulation of independent DG cell populations. (a) Basic behavior setup for the Fear Conditioned-Open Field (FC-OF) group. The *c-fos-tTA* mice were injected with AAV₉-TRE-ChR2-EYFP and implanted with an optical fiber targeting the DG and kept on Dox food for two weeks. Then they were habituated to context A with light-on and light-off epochs for five days, and fear conditioned in context B while still on Dox. The next day the mice were taken off Dox for two days to open a window for activity-dependent labeling during which they were exposed to context C to label cells activated in this environment with ChR2-EYFP. Once placed back on Dox, the mice were tested in context A with light-on and light-off epochs for five days. (b) Averaged data reveal < 5% freezing across all light-off and light-on epochs throughout habituation and test sessions. Mice trained with FC-OF ($n = 10$) do not show increased light-induced freezing during five days of habituation sessions (c) or test sessions (d).



- * Exp
- * Exp-1day
- * Exp-Bi
- * EYFP
- * NS
- * OF-FC
- * FC-OF

Supplementary Figure 11: Optical fiber placements among different mice used in behavior tests.

The histologically verified optical fiber tip locations are marked by ×. Each mouse was implanted unilaterally, except the Exp-Bi group, which received bilateral implants. Different colors represent different groups. Numbers indicate the anteroposterior coordinates from bregma.

Supplementary Movie Legends

Supplementary Movie 1: This movie shows one representative mouse from the Exp-Bi group during a test session post-training. The first 20 seconds of the movie show the light-off epoch and the subsequent 40 seconds show the light-on epoch. The movie is played at 4× normal speed. Note that freezing levels increase dramatically only during the light-on epoch.

Supplementary Movie 2: This movie shows one representative mouse from the Exp-Bi group pre-training (on Dox, habituation session). The first 20 seconds of the movie show the light-off epoch and the subsequent 40 seconds show the light-on epoch. The movie is played at 4× normal speed. Note that freezing levels do not increase throughout the session.