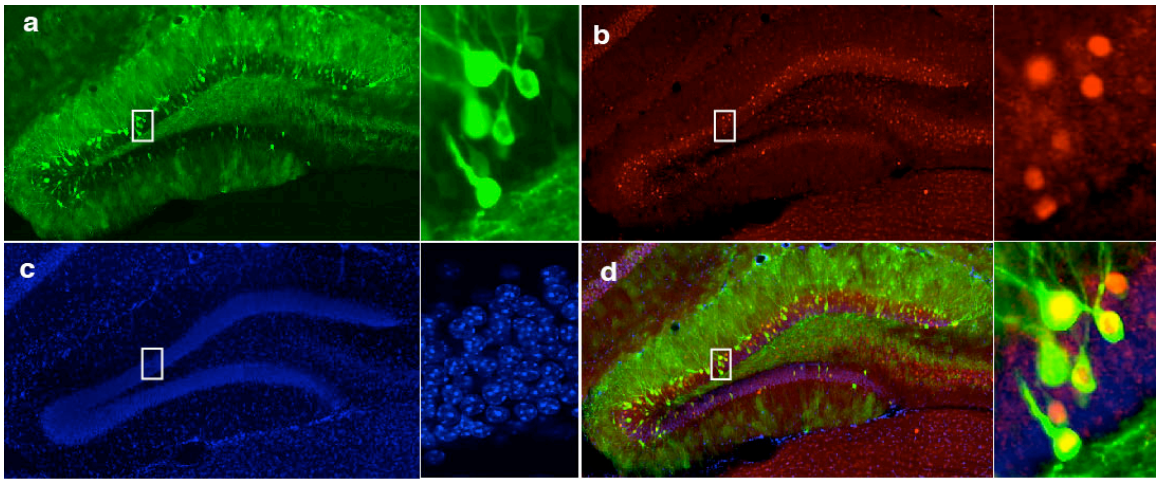
doi:10.1038/nature11028

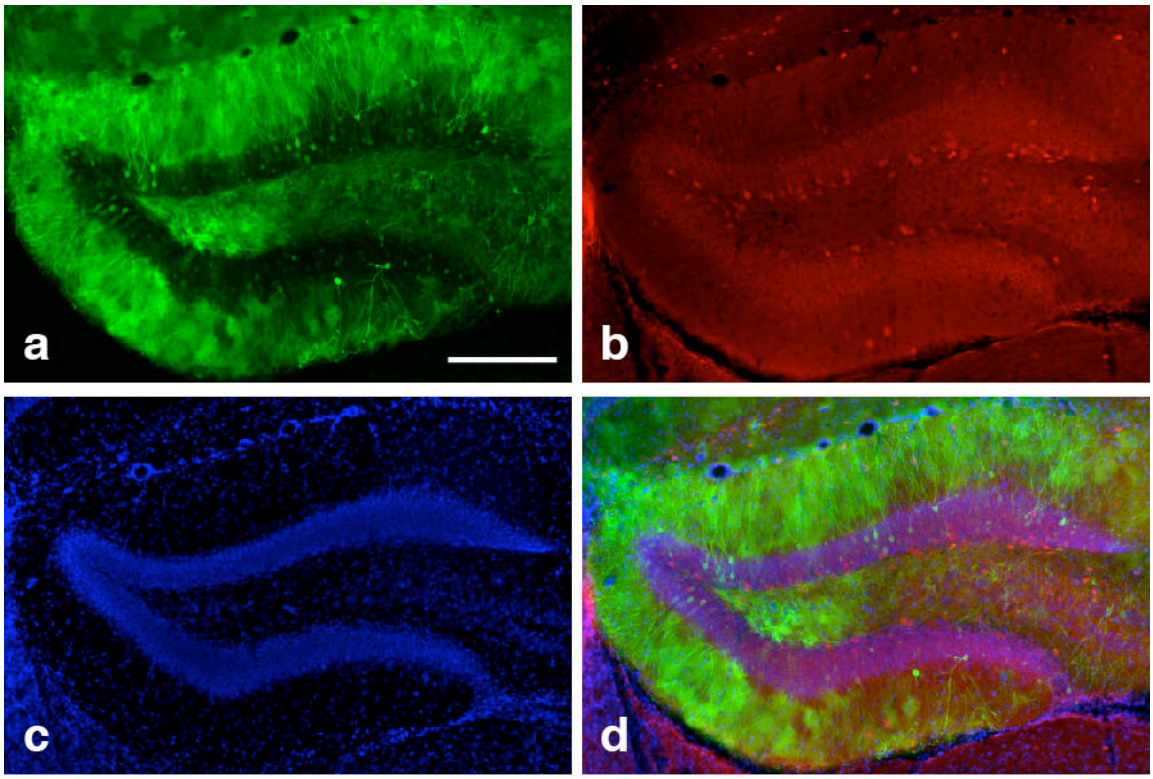


e

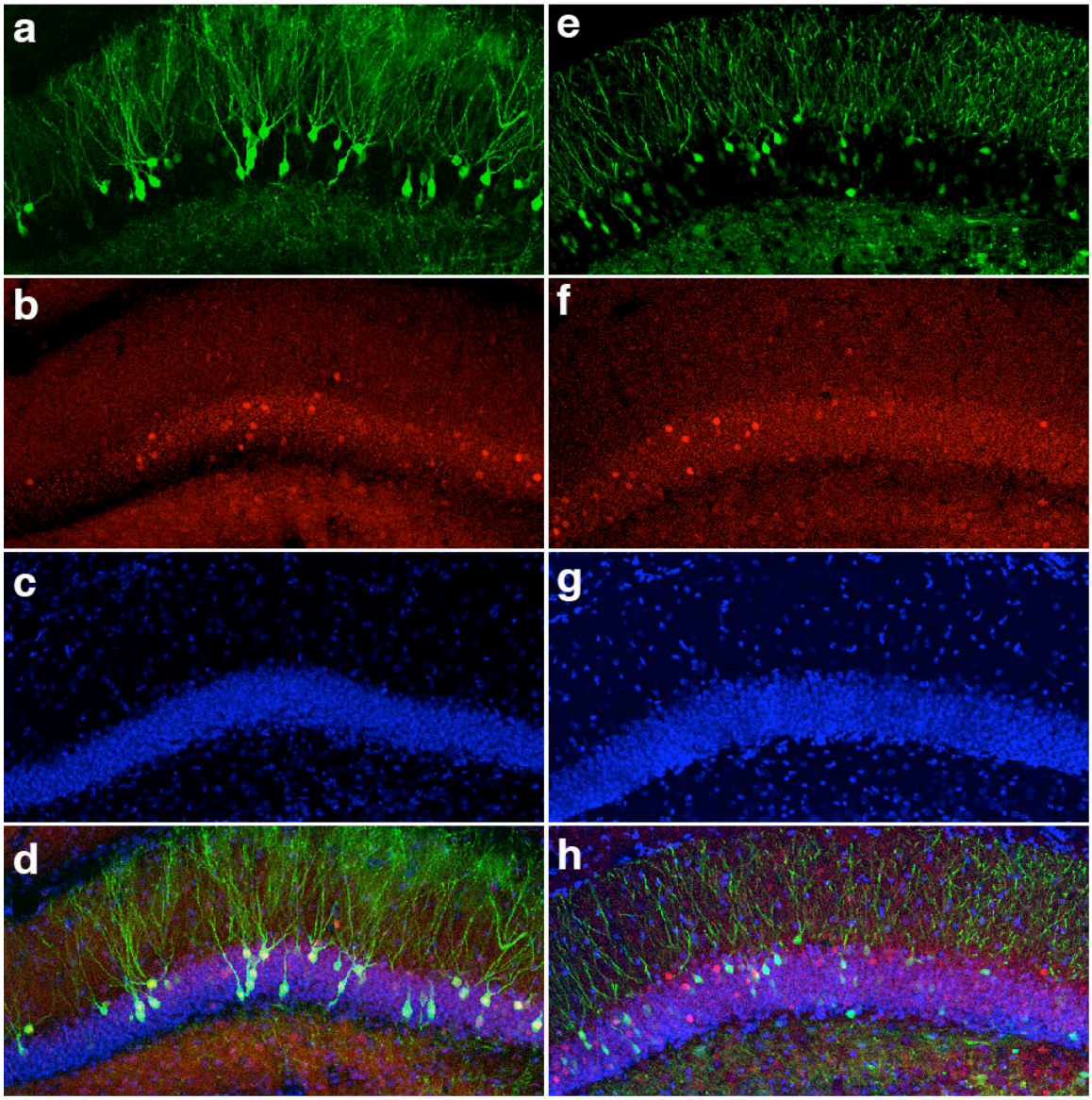
Subject	Cell marker	Cell Counts
Mouse 1	ChR2	360 ± 19.5
	c-fos	348 ± 18.1
	Overlap: ChR2 (%c-fos)	94.8 ± 0.02
Mouse 2	ChR2	426 ± 8.1
	c-fos	407 ± 6.1
	Overlap: ChR2 (%c-fos)	93.2 ± 0.01
Mouse 3	ChR2	406 ± 10.6
	c-fos	386 ± 11.1
	Overlap: ChR2 (%c-fos)	95.7 ± 0.02

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

The c-fos-tTA mice were injected with AAV_9-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).

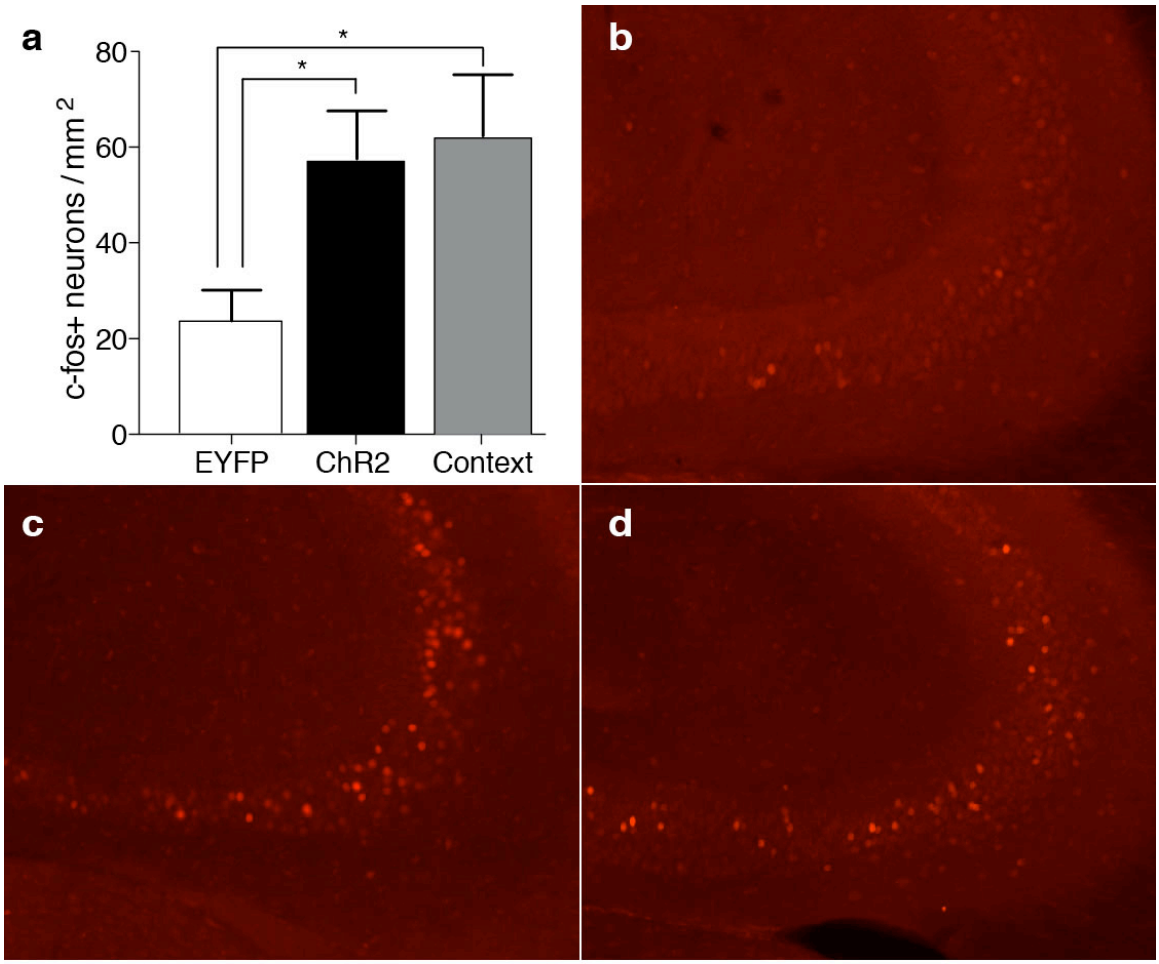


Supplementary Figure 2: GABA and ChR2-EYFP–positive cells do not overlap. (**a**) DG from experimental mice kept off Dox for two days and then subjected to fear conditioning expressed ChR2-EYFP in excitatory cells. (**b**) Anti-GABA. (**c**) DAPI. (**d**) Merged image. Scale bar in (**a**) 250 μm.

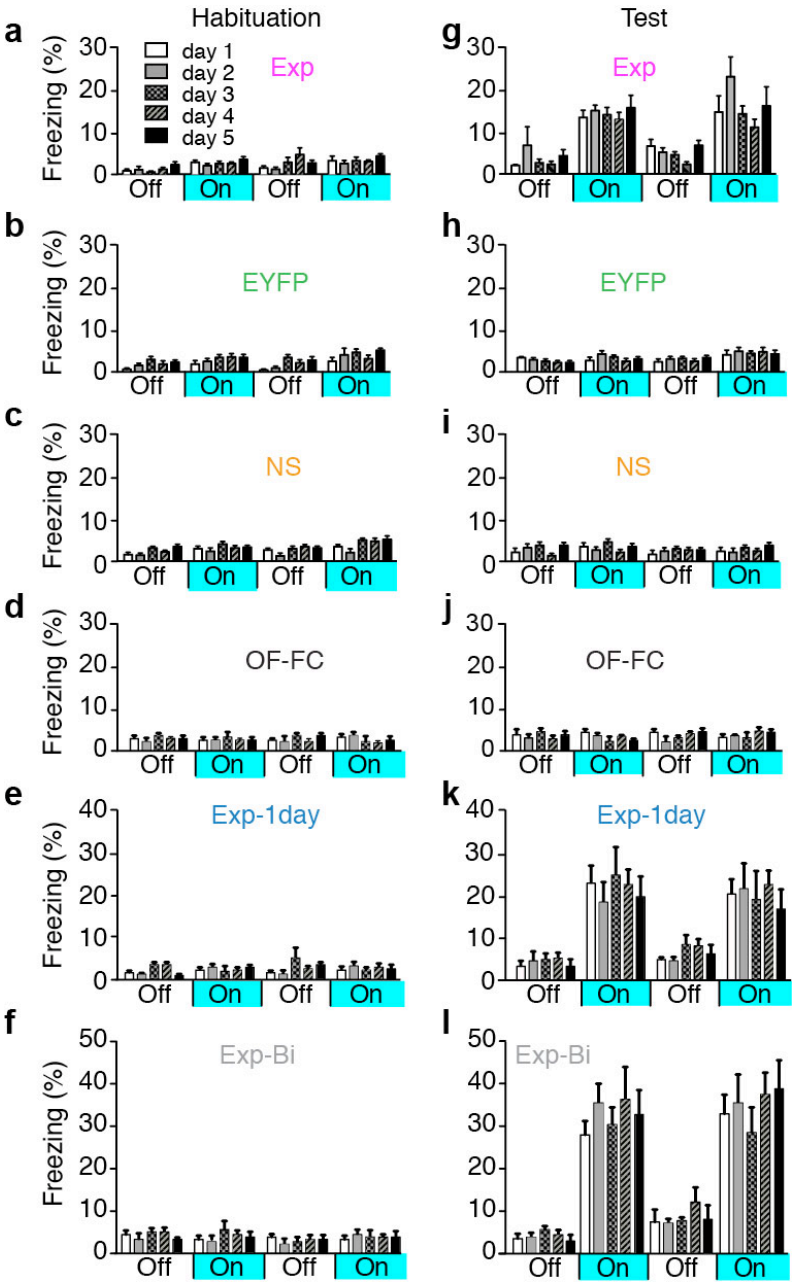


Supplementary Figure 3: Light stimulation induces c-fos expression in cells expressing ChR2-EYFP but not EYFP.

(**a**)–(**d**) Representative DG cells after light stimulation in c-fos-tTA mice injected with AAV_9-TRE-ChR2-EYFP. (**e**)–(**h**) Representative DG cells after light stimulation in c-fos-tTA mice injected with AAV_9-TRE-EYFP. (**a**) ChR2-EYFP. (**e**) EYFP. (**b**), (**f**) c-fos. (**c**), (**g**) DAPI. (**d**), (**h**) Merged images.

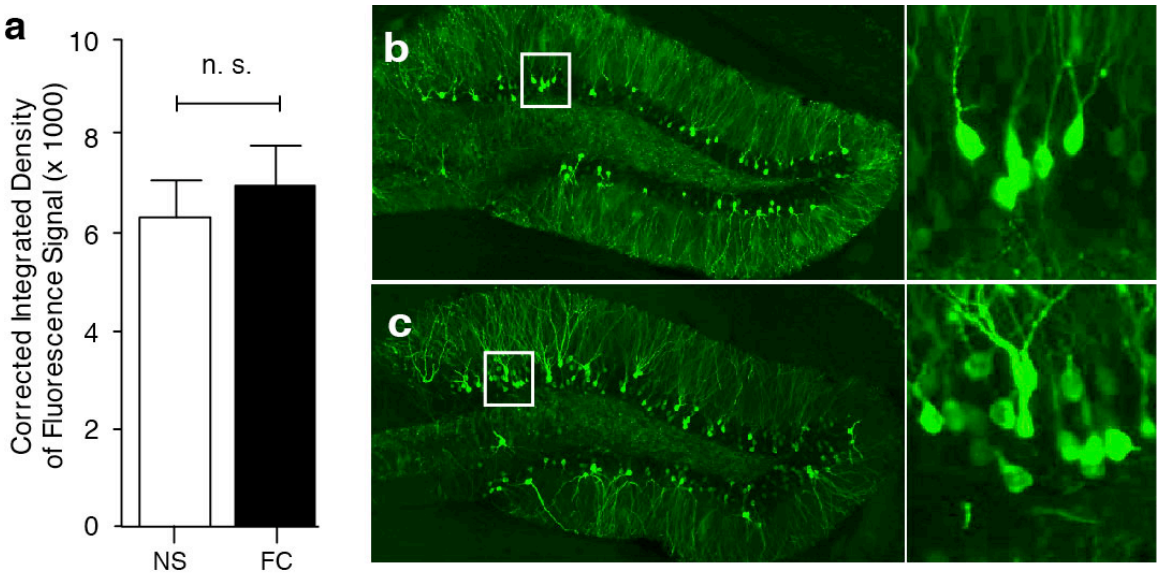


Supplementary Figure 4: *In vivo* optical stimulation of DG cells increases c-fos expression in CA3. (**a)** Quantifications of c-fos expression in the CA3 of mice expressing ChR2-EYFP or EYFP after light stimulation, or a control group of mice after physiological contextual fear memory recall in the original trained context (n = 3/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**).



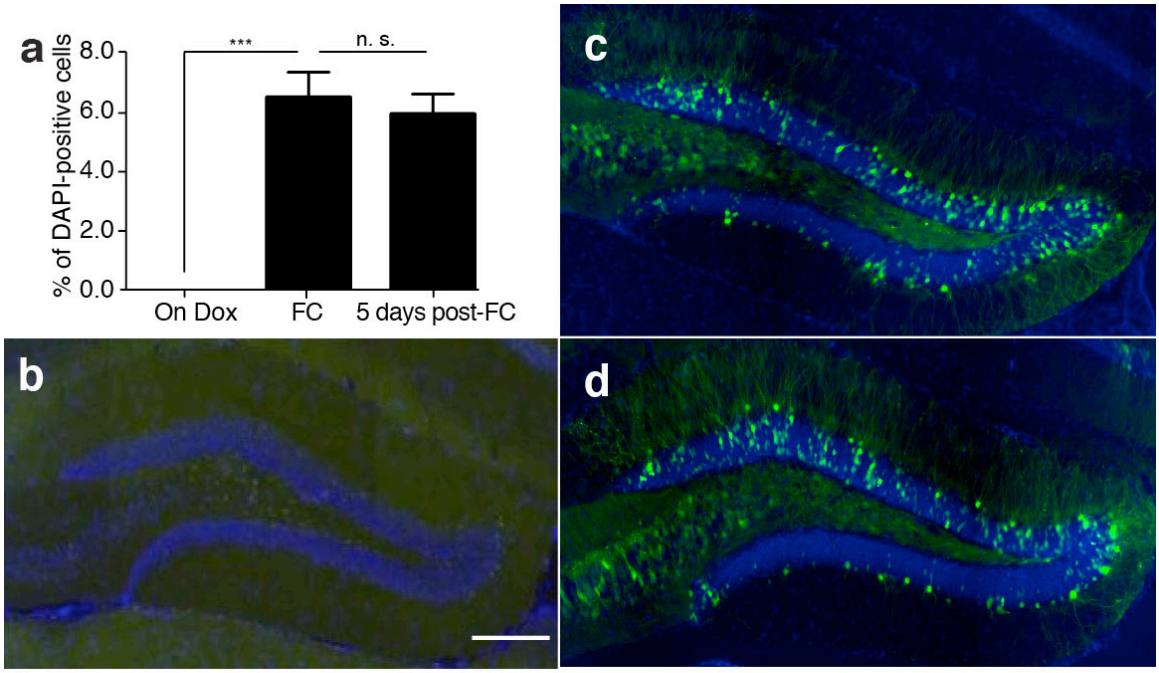
Supplementary Figure 5: Habituation and testing sessions across five days.

(**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.

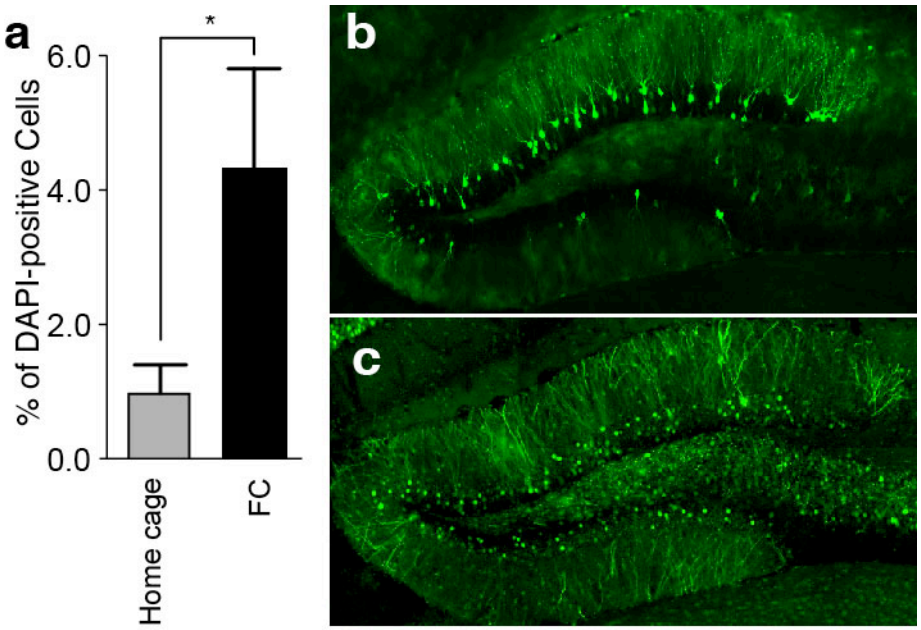


Supplementary Figure 6: Similar ChR2-EYFP expression levels within cells after fear conditioning and no shock exposure.

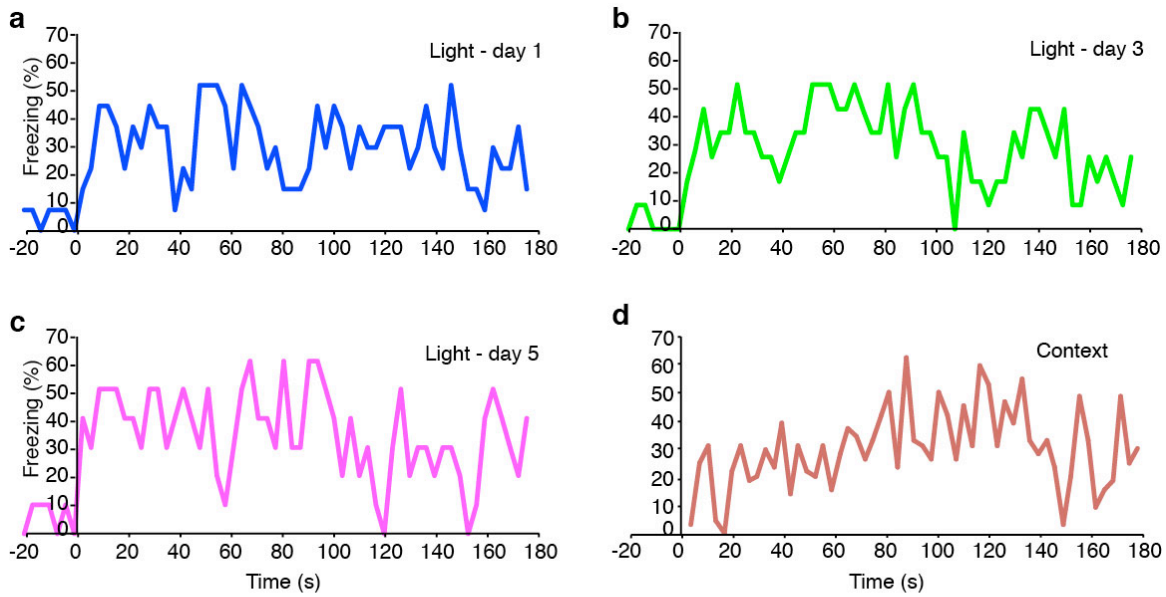
(**a**) ChR2-EYFP in no shock (NS) and fear conditioned (FC) groups show similar levels of fluorescence signal (n = 5 subjects each). Representative images of DG from the NS group (**b**) or the FC group (**c**). Each white rectangle is magnified to the right of the image.



Supplementary Figure 7: Inducible and activity-dependent EYFP expression.
(**a**) Quantifications of EYFP expression levels in c-fos-tTA mice injected with AAV_9-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.

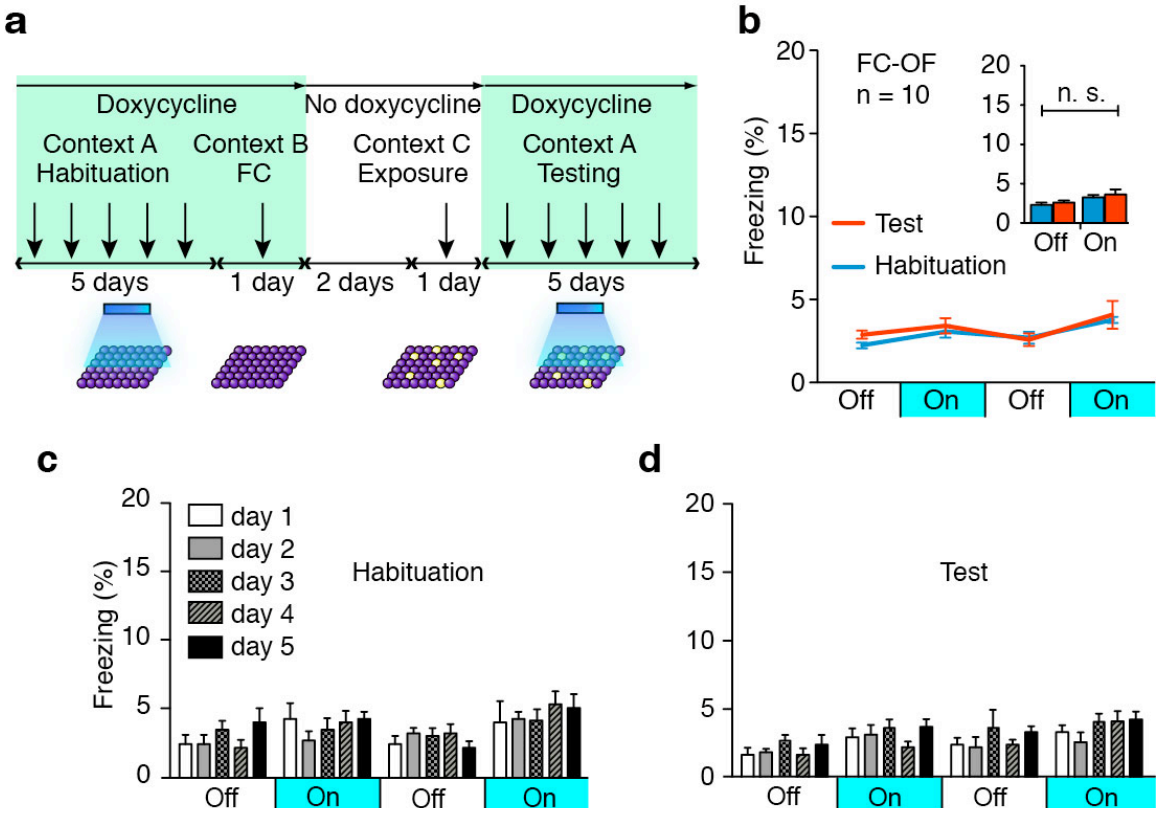


Supplementary Figure 8: Dox removal for one day is sufficient to induce ChR2-EYFP expression. (a) Quantifications revealed that mice kept off Dox for one day in home cage showed low basal levels of ChR2-EYFP and significantly higher levels of ChR2-EYFP after fear conditioning (n = 3 per condition, *P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

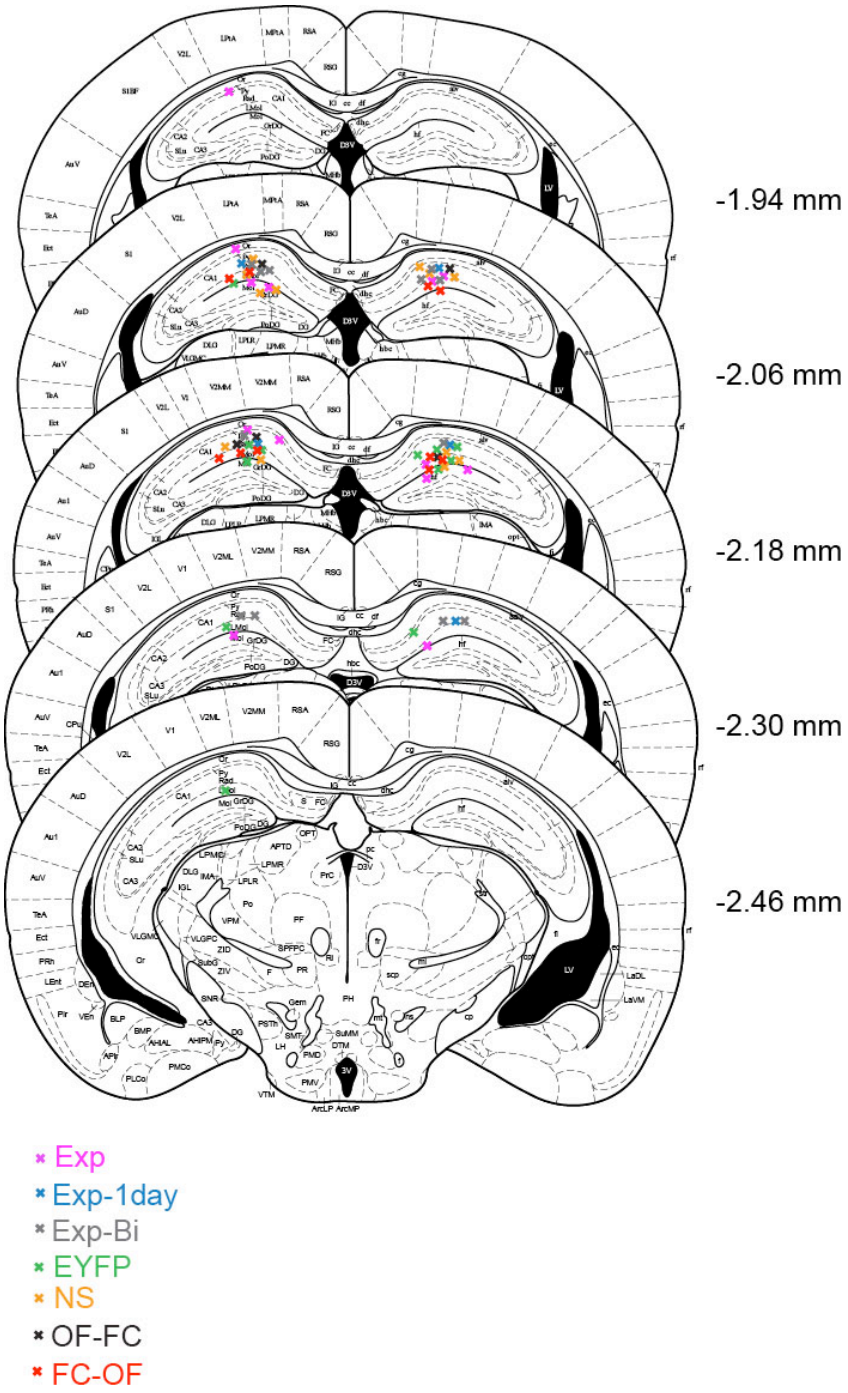


Supplementary Figure 9: Freezing time course during light stimulation and context probe trials.

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 AAV_9-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**).



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.