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Imprinting and Recalling Cortical Ensembles

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Abstract

Neuronal ensembles are coactive groups of neurons that may represent emergent building blocks of neural circuits. They could be formed by Hebbian plasticity, whereby synapses between coactive neurons are strengthened. Here we report that repetitive activation with two-photon optogenetics of neuronal populations in visual cortex of awake mice generates artificially induced ensembles which recur spontaneously after being imprinted and do not disrupt preexistent ones. Moreover, imprinted ensembles can be recalled by single cell stimulation and remain coactive on consecutive days. Our results demonstrate the persistent reconfiguration of cortical circuits by two-photon optogenetics into neuronal ensembles that can perform pattern completion.

Neuronal ensembles are groups of coactive neurons evoked by sensory stimuli (1–3) or motor behaviors (4–6), and may represent emergent building blocks of cortical function (7, 8). In the absence of external inputs, ongoing cortical ensembles resemble sensory evoked ones (9–11), as if the cortex has an imprinted representation of the world, implemented by groups of neurons with strong synaptic connectivity. Ensembles could result from Hebbian plasticity, whereby connectivity between coactive neurons is strengthened due to overlapped activity (12). Indeed, optogenetic studies in which all expressing neurons and their axons are simultaneously photostimulated have demonstrated Hebbian plasticity (13). However, to artificially generate specific neuronal ensembles with single cell resolution has been experimentally difficult.

To do so, we used simultaneous two-photon calcium imaging and two-photon photostimulation (14, 15) in primary visual cortex of head-fixed mice running on a treadmill. GCaMP6s signals of layer 2/3 neurons were imaged through a reinforced thinned-skull window, while C1V1 expressing neurons were optogenetically stimulated with a second two-photon laser (16) (Fig. 1, A and B). Two-photon population photostimulation evoked calcium transients reliably in a specific subset of neurons (Fig. 1C). *In vivo* electrophysiological recordings demonstrated that population photostimulation evoked bursting activity with similar temporal features, independently of the spatial location of the neurons (fig. S1). Neurons responding to direct photostimulation were differentiated from

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photostimulation light artifacts and from other active neurons (Fig. 1, D and E) by their different temporal responses (fig. S1). This enabled us to distinguish the photostimulated cells from those that became active because of the effect of photostimulation on the circuit.

Repeated optogenetic stimulation reliably recruited specific groups of neurons, generating an artificial “photoensemble”, i.e., a group of neurons that were optically stimulated together. To measure ensembles, we analyzed population activity using multidimensional population vectors to quantitatively define clusters of coactive neurons (17–19) and found that photoensembles engaged a different population of neurons than visually evoked ensembles, with only 20.17 ± 9.4 % neurons in common (Fig. 2, A to D. and fig. S2). Although the number of ensembles was similar in both experimental conditions, photoensembles activated more neurons than visually evoked ones (Fig. 2E). Neurons belonging to photoensembles or visual ensembles had a widespread spatial distribution and were spatially intermingled (Fig. 2, F and G). Visual ensembles remained stable after population photostimulation (fig. S3) indicating that repetitive photostimulation did not disrupt preexistent cortical ensembles.

Interestingly, we noted that some photostimulated neurons became active spontaneously (see below). We named these spontaneously active photostimulated neurons “imprinted” ensembles, as if the artificial ensemble had been imprinted into the cortex. Moreover, the activation of a single cell was able to recall imprinted ensembles (Fig. 3A), demonstrating pattern completion. Pattern completion (20) is found in hippocampus (21–23) and is a property of attractor neural networks (22, 24). Single cell activation before population photoactivation did not produce alterations of overall network activity and was unable to consistently recall cortical ensembles (Fig. 3B–D, left; and fig. S4). Nevertheless, after population training, photoactivation of selected members (8 ± 2.5 %) of the imprinted ensemble (Fig. S5) activated a group of cells (Fig. 3, C and D, right). These recalled ensembles, evoked by single cell stimulation, did not disrupt the overall network activity and were interspersed in time with ongoing cortical ensembles (Fig. 3D; top). While the number of ensembles after population training remained stable (Fig. 3E), single cell photostimulation reliably recalled a specific group of neurons that was not coactive before (Fig. 3E; 64.5 ± 12.63 % recalling after population training). The spatial location of neurons in recalled ensembles had a broader distribution than the occasional neurons that were indirectly activated before population training (Fig. 3, F and G). On the other hand, the number of calcium transients during ongoing activity in non-photostimulated neurons remained constant after population training whereas it increased in photostimulated neurons of imprinted ensembles (Fig. 4, A and B), ruling out the possibility that population photostimulation changed the basal level of activity in the whole network. This modification of the functional connectivity between photostimulated neurons also required a minimal number of trials (Fig. 4C) indicating that the observed changes were driven by a change in the circuit triggered by repeated photostimulation of a specific population of neurons.

To investigate whether imprinted ensembles were persistently integrated in ongoing cortical activity, we imaged the same area on consecutive days. Indeed, single cell photostimulation was still able to recall previously imprinted ensembles on consecutive days (fig. S6). The analysis of ongoing activity from non-photostimulated (Fig. 4D, left) and photostimulated

neurons showed that imprinted ensembles recurred spontaneously even on consecutive days (Fig. 4D, right). While cross-correlations between non-responsive neurons were not altered (Fig. 4E, left), they were enhanced between photostimulated neurons and remained stable the next day even after further photostimulation (Fig. 4E, right). Thus, optogenetic activation of identified neurons enhanced their functional connections for at least one day (Fig. 4F).

Recalled ensembles shared similar characteristics to ongoing ones, such as number of neurons and spatial distribution (fig. S7), but the mean distance between active neurons was smaller (fig. S7D), demonstrating that the effect of the photostimulation is local. Recalled ensembles were also composed of combinations of neurons which may or may not belong to ongoing ensembles (fig. S7, D and E), demonstrating that recalled ensembles are indeed novel and not just dormant preexisting ensembles. However, given that cortical connections are not in a *tabula rasa* state, we expect that imprinted ensembles may recruit segments of physiologically relevant circuit motifs.

Previously, electrical or optogenetic stimulation (25) showed that co-activation of neuronal groups can produce physiologically relevant behaviors (13,26). Here, we show the possibility to train individual neurons to build artificial neuronal ensembles (13), which then become spontaneously active (Fig. 4D, right). Our results can explain the similarity between visually evoked and spontaneous ensembles (9) and the finding that neurons responding to similar visual stimuli have a higher interconnectivity (27). In both cases, recurrent co-activation of a neuronal group would enhance their functional connectivity, imprinting ensembles into the circuit.

More than sixty years ago, Hebb proposed that repeated co-activation of a group of neurons might create a memory trace through enhancement of synaptic connections (12). Because of technical limitations, this hypothesis has been difficult to test in awake animals with single cell resolution. Our work, combining novel imaging and photostimulation techniques (14, 15) and analytical tools (19), can be interpreted as a confirmation of the Hebbian postulate and as a demonstration that cortical microcircuits can perform pattern completion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and Notes

1. Kampa BM, Roth MM, Gobel W, Helmchen F. Representation of visual scenes by local neuronal populations in layer 2/3 of mouse visual cortex. Frontiers in neural circuits. 2011; 5:18. [PubMed: 22180739]
2. Ohki K, Chung S, Ch'ng YH, Kara P, Reid RC. Functional imaging with cellular resolution reveals precise micro-architecture in visual cortex. Nature. 2005; 433:597–603. [PubMed: 15660108]
3. Sawinski J, et al. Visually evoked activity in cortical cells imaged in freely moving animals. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:19557–19562. [PubMed: 19889973]
4. Churchland MM, et al. Neural population dynamics during reaching. Nature. 2012; 487:51–56. [PubMed: 22722855]
5. Peters AJ, Chen SX, Komiyama T. Emergence of reproducible spatiotemporal activity during motor learning. Nature. 2014; 510:263–267. [PubMed: 24805237]
6. Cao VY, et al. Motor Learning Consolidates Arc-Expressing Neuronal Ensembles in Secondary Motor Cortex. Neuron. 2015; 86:1385–1392. [PubMed: 26051420]
7. Mountcastle VB. The columnar organization of the neocortex. Brain. 1997; 120(Pt 4):701–722. [PubMed: 9153131]
8. Yuste R. From the neuron doctrine to neural networks. Nat Rev Neurosci. 2015; 16:487–497. [PubMed: 26152865]
9. Miller JE, Ayzenshtat I, Carrillo-Reid L, Yuste R. Visual stimuli recruit intrinsically generated cortical ensembles. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111:E4053–4061. [PubMed: 25201983]
10. Kenet T, Bibitchkov D, Tsodyks M, Grinvald A, Arieli A. Spontaneously emerging cortical representations of visual attributes. Nature. 2003; 425:954–956. [PubMed: 14586468]
11. Luczak A, Bartho P, Harris KD. Spontaneous events outline the realm of possible sensory responses in neocortical populations. Neuron. 2009; 62:413–425. [PubMed: 19447096]
12. Hebb, DO. A Wiley book in clinical psychology. Wiley; New York: 1949. The organization of behavior; a neuropsychological theory; p. xixp. 335
13. Johansen JP, et al. Optical activation of lateral amygdala pyramidal cells instructs associative fear learning. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:12692–12697. [PubMed: 20615999]
14. Packer AM, Russell LE, Dalgleish HW, Hausser M. Simultaneous all-optical manipulation and recording of neural circuit activity with cellular resolution in vivo. Nature methods. 2015; 12:140–146. [PubMed: 25532138]
15. Rickgauer JP, Deisseroth K, Tank DW. Simultaneous cellular-resolution optical perturbation and imaging of place cell firing fields. Nature neuroscience. 2014; 17:1816–1824. [PubMed: 25402854]
16. Drew PJ, et al. Chronic optical access through a polished and reinforced thinned skull. Nature methods. 2010; 7:981–984. [PubMed: 20966916]
17. Brown SL, Joseph J, Stopfer M. Encoding a temporally structured stimulus with a temporally structured neural representation. Nature neuroscience. 2005; 8:1568–1576. [PubMed: 16222230]
18. Carrillo-Reid L, et al. Encoding network states by striatal cell assemblies. Journal of neurophysiology. 2008; 99:1435–1450. [PubMed: 18184883]
19. Carrillo-Reid L, Miller JE, Hamm JP, Jackson J, Yuste R. Endogenous Sequential Cortical Activity Evoked by Visual Stimuli. J Neurosci. 2015; 35:8813–8828. [PubMed: 26063915]
20. Hunsaker MR, Kesner RP. The operation of pattern separation and pattern completion processes associated with different attributes or domains of memory. Neurosci Biobehav Rev. 2013; 37:36–58. [PubMed: 23043857]
21. O'Reilly RC, McClelland JL. Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off. Hippocampus. 1994; 4:661–682. [PubMed: 7704110]
22. Rolls ET, Treves A. Neural networks in the brain involved in memory and recall. Prog Brain Res. 1994; 102:335–341. [PubMed: 7800823]

23. Rolls ET, Kesner RP. A computational theory of hippocampal function, and empirical tests of the theory. Prog Neurobiol. 2006; 79:1–48. [PubMed: 16781044]
24. Hopfield JJ. Neural networks and physical systems with emergent collective computational abilities. Proceedings of the National Academy of Sciences of the United States of America. 1982; 79:2554–2558. [PubMed: 6953413]
25. Jackson A, Mavoori J, Fetz EE. Long-term motor cortex plasticity induced by an electronic neural implant. Nature. 2006; 444:56–60. [PubMed: 17057705]
26. Liu X, et al. Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature. 2012; 484:381–385. [PubMed: 22441246]
27. Ko H, et al. Functional specificity of local synaptic connections in neocortical networks. Nature. 2011; 473:87–91. [PubMed: 21478872]
28. Yuste R, Katz LC. Control of postsynaptic Ca2+ influx in developing neocortex by excitatory and inhibitory neurotransmitters. Neuron. 1991; 6:333–344. [PubMed: 1672071]
29. Packer AM, et al. Two-photon optogenetics of dendritic spines and neural circuits. Nature methods. 2012; 9:1202–1205. [PubMed: 23142873]
30. Mukamel EA, Nimmerjahn A, Schnitzer MJ. Automated analysis of cellular signals from large-scale calcium imaging data. Neuron. 2009; 63:747–760. [PubMed: 19778505]
31. Cossart R, Aronov D, Yuste R. Attractor dynamics of network UP states in neocortex. Nature. 2003; 423:283–289. [PubMed: 12748641]
32. Shmiel T, et al. Temporally precise cortical firing patterns are associated with distinct action segments. Journal of neurophysiology. 2006; 96:2645–2652. [PubMed: 16885517]
33. Sasaki T, Matsuki N, Ikegaya Y. Metastability of active CA3 networks. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2007; 27:517–528. [PubMed: 17234584]
34. Stopfer M, Jayaraman V, Laurent G. Intensity versus identity coding in an olfactory system. Neuron. 2003; 39:991–1004. [PubMed: 12971898]
35. Schreiber S, Fellous JM, Whitmer D, Tiesinga P, Sejnowski TJ. A new correlation-based measure of spike timing reliability. Neurocomputing. 2003; 52–4:925–931.
36. Garbarine E, DePasquale J, Gadia V, Polikar R, Rosen G. Information-theoretic approaches to SVM feature selection for metagenome read classification. Comput Biol Chem. 2011; 35:199–209. [PubMed: 21704267]
37. Islamaj Dogan R, Lu Z. Click-words: learning to predict document keywords from a user perspective. Bioinformatics. 2010; 26:2767–2775. [PubMed: 20810602]
38. Lu Z, Kim W, Wilbur WJ. Evaluating relevance ranking strategies for MEDLINE retrieval. J Am Med Inform Assoc. 2009; 16:32–36. [PubMed: 18952932]
39. Lan M, Tan CL, Su J, Lu Y. Supervised and traditional term weighting methods for automatic text categorization. IEEE Trans Pattern Anal Mach Intell. 2009; 31:721–735. [PubMed: 19229086]
40. Margrie TW, et al. Targeted whole-cell recordings in the mammalian brain in vivo. Neuron. 2003; 39:911–918. [PubMed: 12971892]

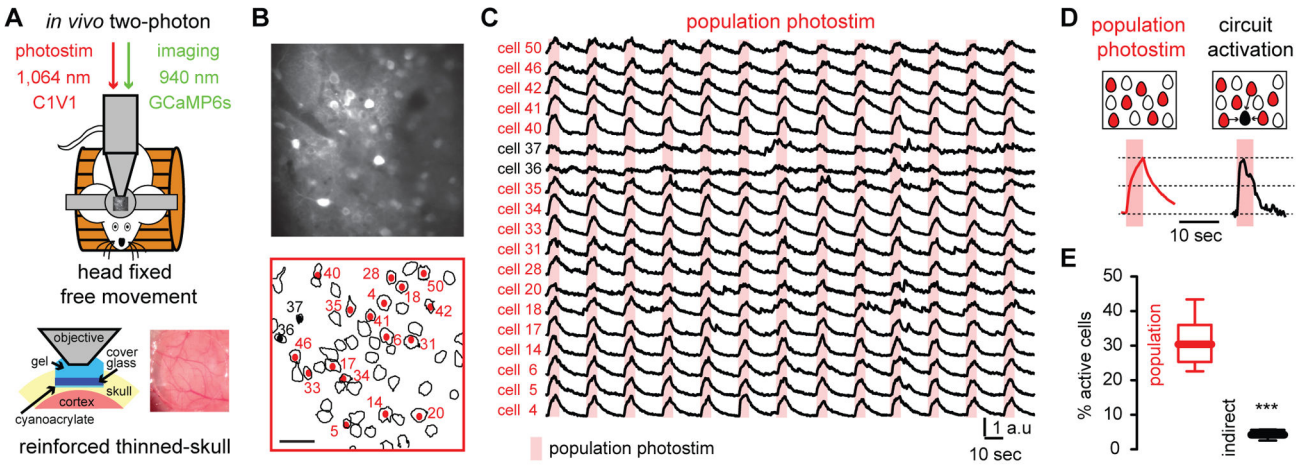

Fig. 1. Two-photon optogenetic photostimulation reliably activates specific neuronal populations

(**A**), Simultaneous two-photon imaging and two-photon optogenetic photostimulation was performed in layer 2/3 over left primary visual cortex (V1) in awake head fixed mice through a reinforced thinned skull window. (**B**) Automatic contour detection of cortical neurons. Red cells denote neurons that reliably respond to optogenetic population photostimulation. Scale bar 50 μm. (**C**) Calcium transients of neurons activated by population photostimulation (red) and neurons activated indirectly (black). (**D**) Calcium transients from directly photostimulated neurons differed from calcium transients evoked indirectly by circuit activation. (**E**) Indirectly activated neurons represent a small percentage of the population (n = 6 mice; ***P = 0.0006; Mann-Whitney test). Data presented as whisker box plots displaying median and interquartile ranges.

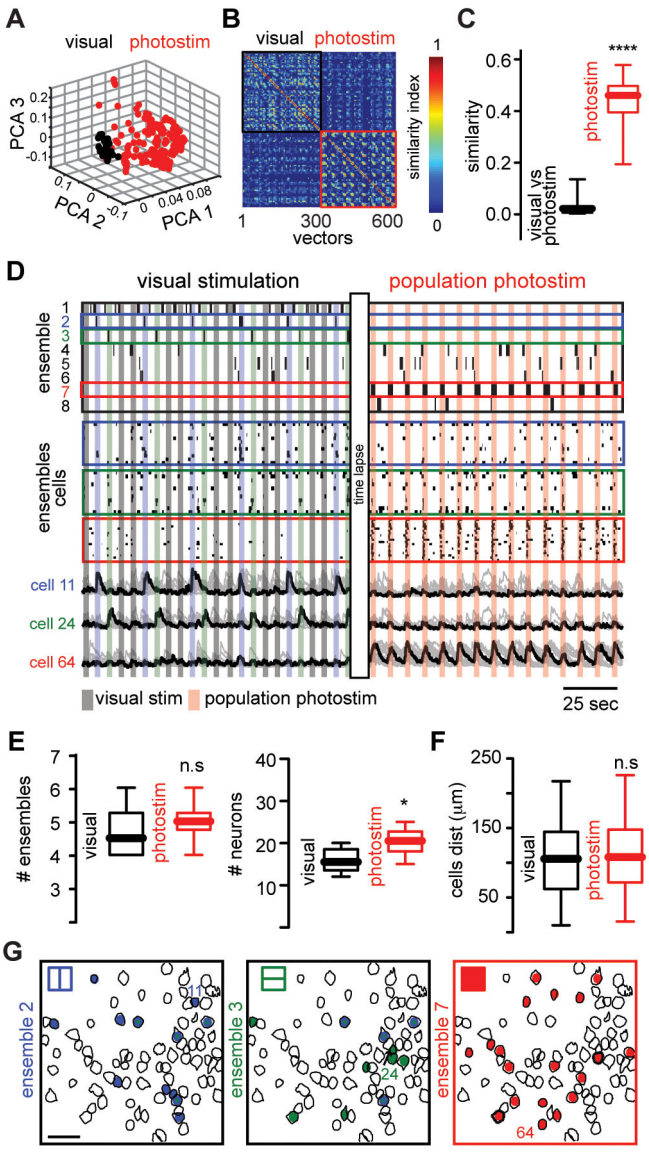

Fig. 2. Population photostimulation generates artificial cortical ensembles

(**A**) Principal component analysis (PCA) of population vectors evoked by visual stimuli (black) and optogenetic photostimulation (red). (**B**) Similarity map representing the angle between population vectors during visual stimuli (black) or population photostimuli (red). (**C**) Population similarity between visually and photostimulated evoked activity (n = 6 mice; ****P < 0.0001). (**D**) Time course activation of evoked cortical ensembles (top) aligned with raster plots representing the activity of visually evoked ensembles and photoensemble (middle) and calcium transients (bottom) of the most representative neurons of each ensemble. Colored boxes indicate ensemble label. (**E**) The total number of ensembles remained stable in both conditions (top; n = 6 mice; n.s P=0.4315). The number of cells defining photoensembles is significantly higher than neurons defining each visually evoked ensemble (bottom, n = 6 mice; *P = 0.0446). (**F**) Spatial maps of cortical ensembles in both experimental conditions. Scale bar 50 µm. (**G**) Distance between all neurons belonging to

each ensemble (n = 6 mice; n.s. P = 0.3720). Data presented as whisker box plots displaying median and interquartile ranges analyzed using Mann-Whitney test.

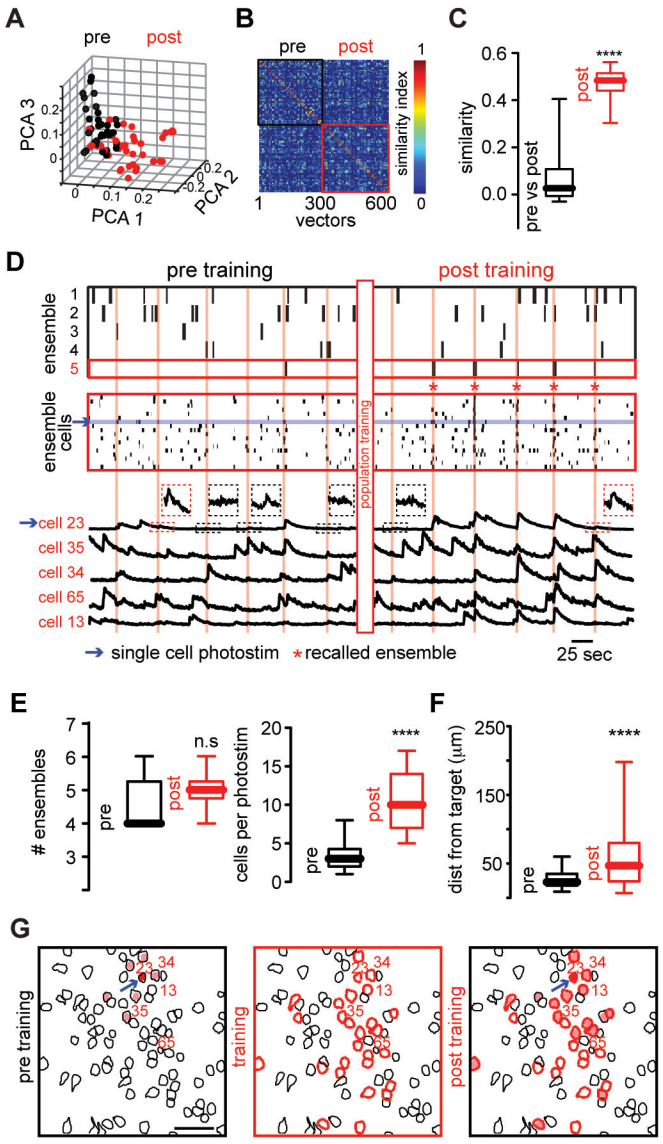

Fig. 3. Pattern completion of artificially imprinted ensembles

(**A**) PCA projection of population vectors during single cell photostimulation before and after population training. (**B**) Similarity map of population vectors from ongoing cortical activity. (**C**) Single cell photostimulation after population training recalled population vectors with high similarity (n = 6 mice; ****P < 0.0001). (**D**) Time course activation of cortical ensembles (top) aligned with raster plot of all the cells that belong to recalled ensemble (middle) and calcium transients (bottom) of representative neurons from recalled ensemble (red labels) before and after population training. (**E**) The number of ensembles before and after population training remains stable (top; n = 6 mice; n.s. P = 0.2259). After population training single cell photostimulation consistently recruits a group of neurons significantly larger than control conditions (bottom; n = 6 mice; ****P < 0.0001). (**F**) Spatial maps of neurons recruited by single cell photostimulation before (left) during (middle) and after population training (right). Arrow indicates stimulated neuron. Scale bar

50 μm. (**G**) After population training the distance from the target cell and activated neurons is increased (n = 6 mice; **** P < 0.0001). Data presented as whisker box plots displaying median and interquartile ranges analyzed using Mann-Whitney test.

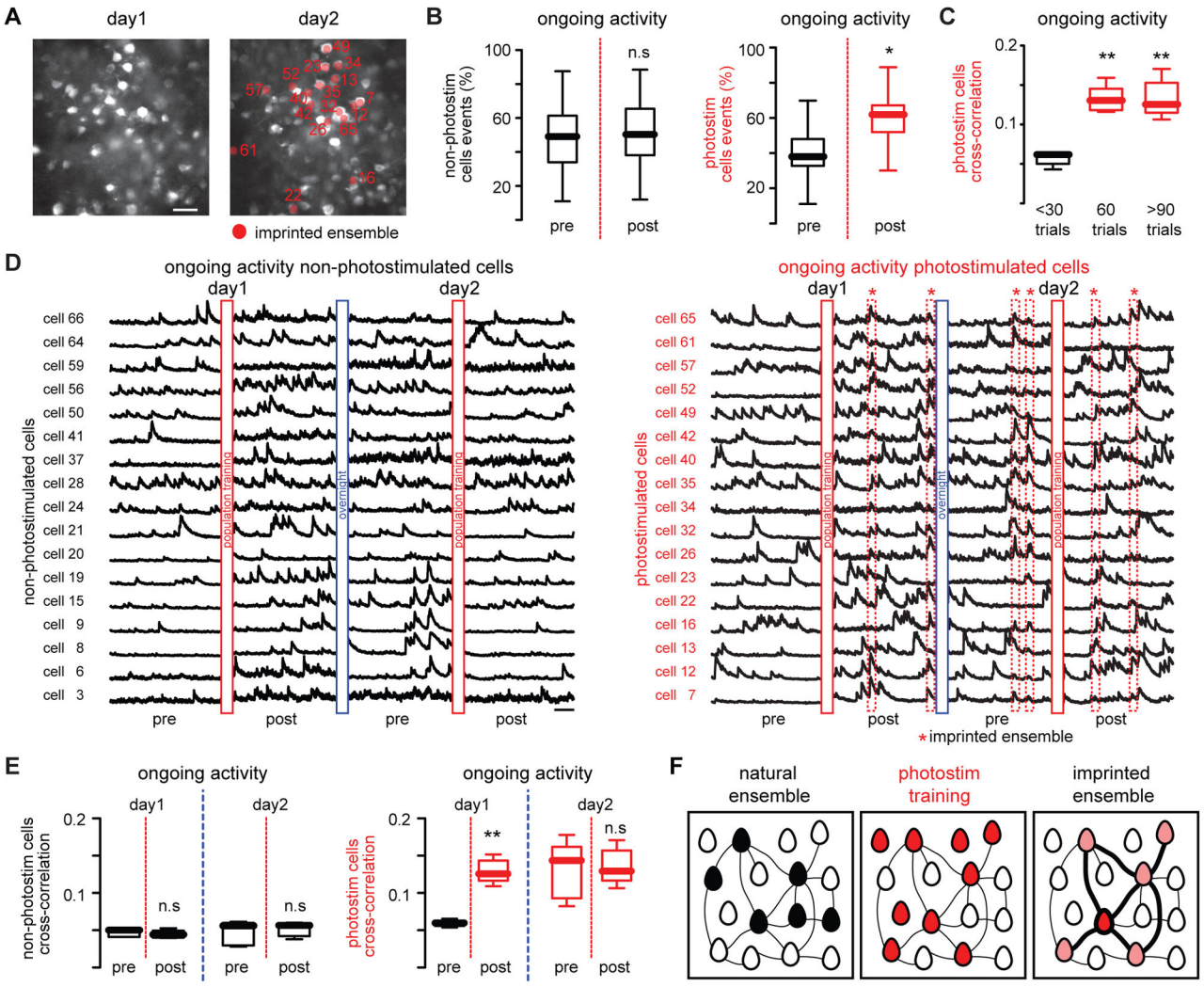

Fig. 4. Imprinted ensembles persist after consecutive days

(**A**) Images showing the same optical field at two different days. Scale bar 50 μm. (**B**) Percentage of events during ongoing activity of non-photostimulated cells remains stable (left; n = 5 mice; n.s P=0.5664; Wilcoxon matched-pairs signed rank test) whereas photostimulated cells increased their activity (right; n = 5 mice; *P = 0.0147; Wilcoxon matched-pairs signed rank test) after population training. Red line denotes population training. (**C**) The enhancement of cross-correlation between photostimulated cells depends on the number of training trials (n = 5 mice; **P = 0.0092; Kruskal-Wallis test). (**D**) Calcium transients of non-photostimulated neurons (left) and photostimulated neurons (right) during ongoing cortical activity at two different days before and after population training. Imprinted ensembles recur spontaneously at consecutive days (dotted red boxes). (**E**) Cross-correlation between non-photostimulated neurons (left; n = 5 mice; day 1: n.s. P = 0.5476; day 2: n.s. P = 0.8413; Mann-Whitney test) and photostimulated neurons (right; n = 5 mice; day 1: **P = 0.079; day 2: n.s. P = 1; Mann-Whitney test) during ongoing activity at consecutive days. (**F**) Population photostimulation enhances the functional connectivity between responsive neurons. Lines widths represent the strength of the functional

connectivity between neurons. Data presented as whisker box plots displaying median and interquartile ranges.