

BRAIN MICROCIRCUITS

Imprinting and recalling cortical ensembles

Luis Carrillo-Reid,* Weijian Yang, Yuki Bando, Darcy S. Peterka, Rafael Yuste

Neuronal ensembles are coactive groups of neurons that may represent building blocks of cortical circuits. These ensembles 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 from the visual cortex of awake mice builds neuronal ensembles that recur spontaneously after being imprinted and do not disrupt preexisting 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.

Cortical ensembles are groups of coactive neurons evoked by sensory stimuli (1–3) or motor behaviors (4–6) and likely constitute 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 the connectivity between coactive neurons becomes strengthened (12). Optogenetic studies in which all expressing neurons and their axons are simultaneously photostimulated have dem-

onstrated Hebbian plasticity (13). However, the artificial generation of cortical ensembles with single-cell resolution has so far been experimentally difficult.

To do so, we used simultaneous two-photon calcium imaging and two-photon photostimulation (14, 15) in the 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, whereas CIV1-expressing neurons were optogenetically stimulated with a second two-photon laser (16) (Fig. 1, A and B).

Two-photon photostimulation of a neuronal population (fig. S1) evoked calcium transients reliably in a specific subset of neurons (Fig. 1, C to E). In vivo electrophysiological recordings demonstrated that population photostimulation evoked action potential bursts independently of the spatial

location of the neurons (fig. S1). Neurons responding to direct photostimulation were differentiated from other active neurons and from photostimulation light artifacts by their different temporal responses (fig. S1). This enabled us to distinguish photostimulated cells from those that became active because of the effects of photostimulation on the circuit.

Repeated optogenetic stimulation reliably recruited specific groups of neurons, generating an artificial “photoensemble” (i.e., a group of optically activated neurons). To quantify this, we used multidimensional population vectors to analyze population activity (17, 18) and found that photoensembles activate different populations of neurons than visual stimuli do (19), with only $20.17 \pm 9.4\%$ 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), which indicates that repetitive photostimulation did not disrupt pre-existing cortical ensembles.

We noted that some photostimulated cells became coactive spontaneously (see below), as if the artificial photoensemble had been imprinted into the cortex. Moreover, the activation of a single cell was able to recall these imprinted ensembles (Fig. 3A), demonstrating pattern completion. Pattern completion (20) has been described in the hippocampus (21–23) and is a property of attractor neural networks (22, 24). Single-cell activation before population photostimulation did not produce considerable alterations of overall network activity (Fig. 3, B, C, and D, left, and fig.

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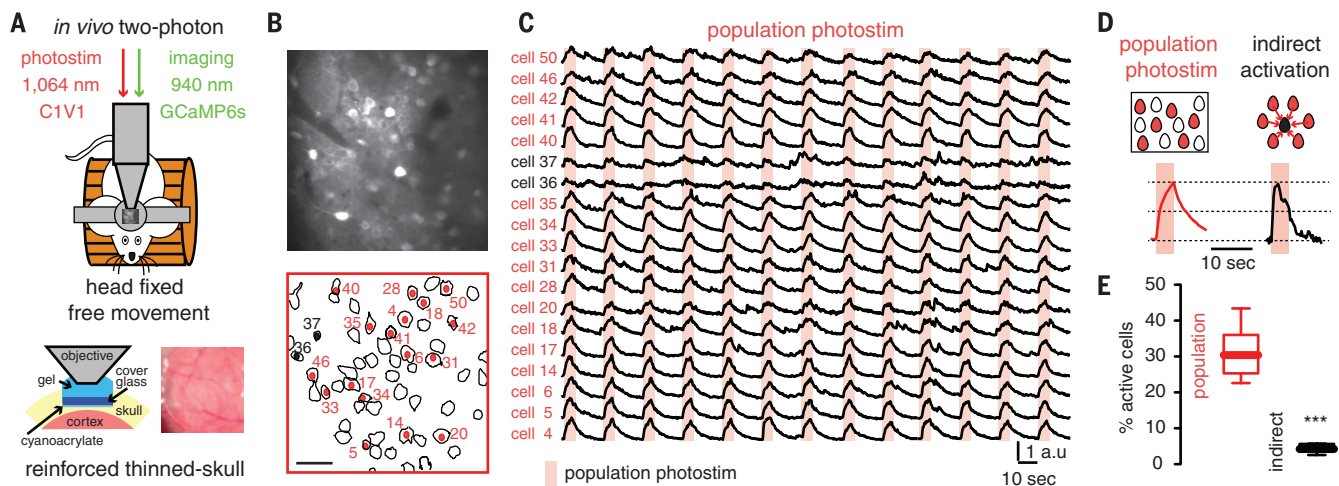


Fig. 1. Two-photon optogenetic photostimulation reliably activates specific neuronal populations. (A) Simultaneous two-photon imaging and two-photon optogenetic photostimulation were performed in layer 2/3 over the left primary visual cortex in awake head-fixed mice through a reinforced thinned-skull 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 ac-

tivated by population photostimulation (red) and neurons indirectly activated (black). a.u., arbitrary units. (D) Calcium transients from directly photostimulated neurons (left) differed from calcium transients evoked indirectly (right) by circuit activation. (E) Indirectly activated neurons represent a small percentage of the population ($n = 6$ mice; $***P = 0.0006$; Mann-Whitney test). Data in (E) are presented as box-and-whisker plots displaying median and interquartile ranges.

S4). However, after population photostimulation, photoactivation of selected members ($8 \pm 2.5\%$) of the imprinted ensemble (fig. S5) consistently

recalled an associated 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). Though the number of ensembles during

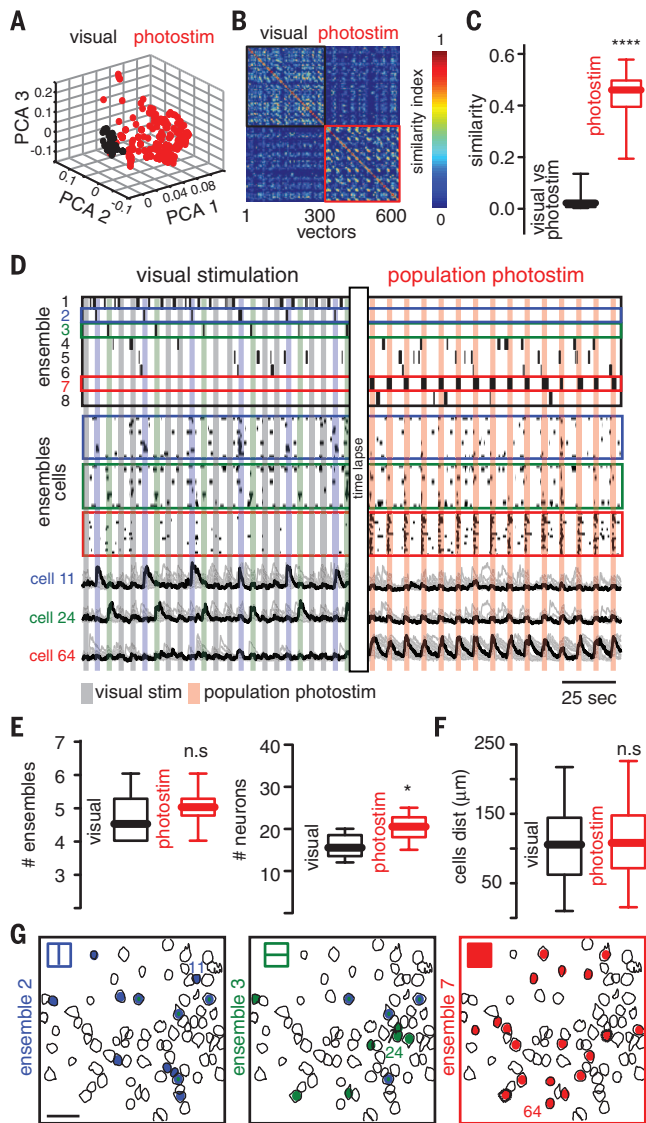


Fig. 2. Population photostimulation generates artificial cortical ensembles. (A) Principal component analysis (PCA) of population vectors evoked by visual stimuli (black) and optogenetic photostimulation (red). (B) Similarity map representing the angle between population vectors during visual stimuli (black) or population photostimuli (red). (C) Population similarity between visually and photostimulated evoked activity ($n = 6$ mice; $****P < 0.0001$). (D) Time-course 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 correspond to ensemble labels (blue, vertical stimulus; green, horizontal stimulus; red, photoensemble). (E) The total number of ensembles remained stable in both conditions [left; $n = 6$ mice; n.s. (not significant) $P = 0.4315$]. The number of cells defining photoensembles is significantly higher than the number of neurons defining each visually evoked ensemble (right; $n = 6$ mice; $*P = 0.0446$). (F) Distance between all neurons belonging to each ensemble ($n = 6$ mice; n.s. $P = 0.3720$). (G) Spatial maps of cortical ensembles in both experimental conditions. Scale bar, $50 \mu\text{m}$. Data in (C), (E), and (F) are presented as box-and-whisker plots displaying median and interquartile ranges analyzed using the Mann-Whitney test.

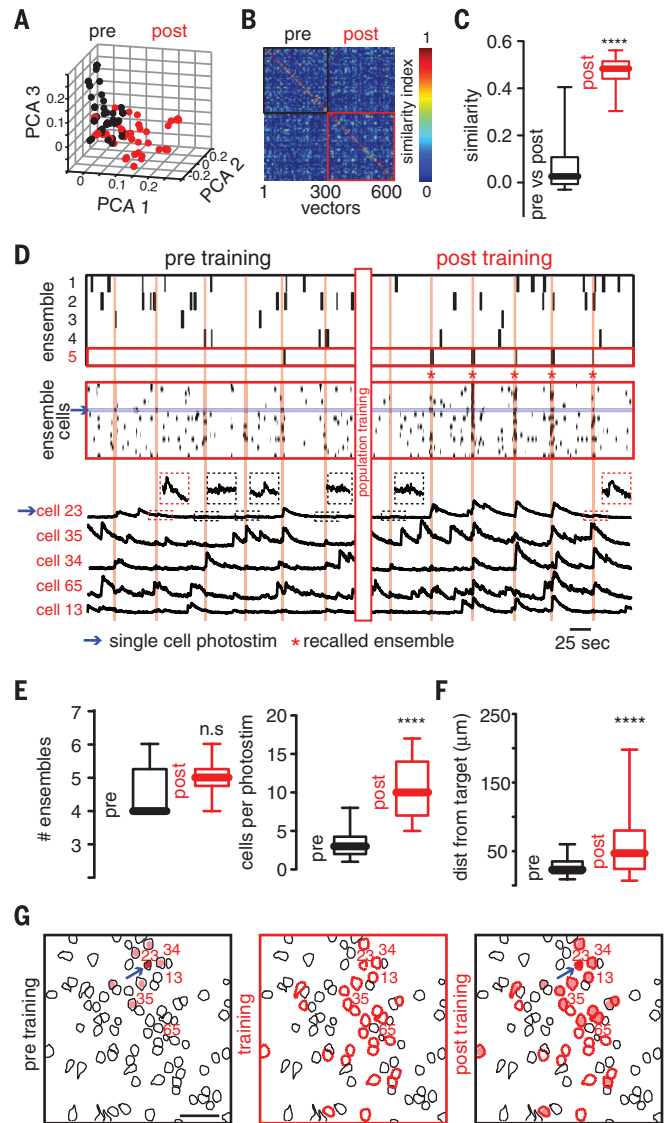


Fig. 3. Pattern completion of artificially imprinted ensembles. (A) PCA projection of population vectors during single-cell photostimulation before and after population training. (B) Similarity map of population vectors from ongoing cortical activity. (C) Single-cell photostimulation after population training enabled the recall of population vectors with high similarity ($n = 6$ mice; $****P < 0.0001$). (D) Time-course of cortical ensembles (top) aligned with a raster plot of all cells that belong to recalled ensembles (middle) and calcium transients (bottom) of representative neurons from recalled ensembles (red labels) before and after population training. Note how ensemble 5 (bottom, red) is reliably recalled by single-cell stimulation (cell 23) after training. (E) The number of ensembles before and after population training remains stable (left; $n = 6$ mice; n.s. $P = 0.2259$). After population training, single-cell photostimulation consistently recruits a group of neurons significantly larger than that existing under control conditions (right; $n = 6$ mice; $****P < 0.0001$). (F) After population training, the distance between the target cell and the activated neurons is increased ($n = 6$ mice; $****P < 0.0001$). (G) Spatial maps of neurons recruited by single-cell photostimulation before (left), during (middle), and after population training (right). The arrow indicates a stimulated neuron. Scale bar, $50 \mu\text{m}$. Data in (C), (E), and (F) are presented as box-and-whisker plots displaying median and interquartile ranges analyzed using the Mann-Whitney test.

single-cell photostimulation before and after population training remained stable (Fig. 3E), single-cell photostimulation after population training reliably enabled the recall of a specific group of neurons that was not coactive before; this occurred $64.5 \pm 12.63\%$ of the time (Fig. 3E). 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). Also, after population training, the number of calcium

transients in nonphotostimulated neurons remained constant whereas it increased in photostimulated neurons (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 required a minimal number of trials (Fig. 4C), indicating that the observed changes were driven by an alteration 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. Single-cell photostimulation still enabled the recall of previously imprinted ensembles on consecutive days (fig. S6). Our analysis of ongoing activity from nonphotostimulated (Fig. 4D, left) and photostimulated neurons showed that imprinted ensembles recurred spontaneously, even on consecutive days and after additional population photostimulation (Fig. 4D, right). Although

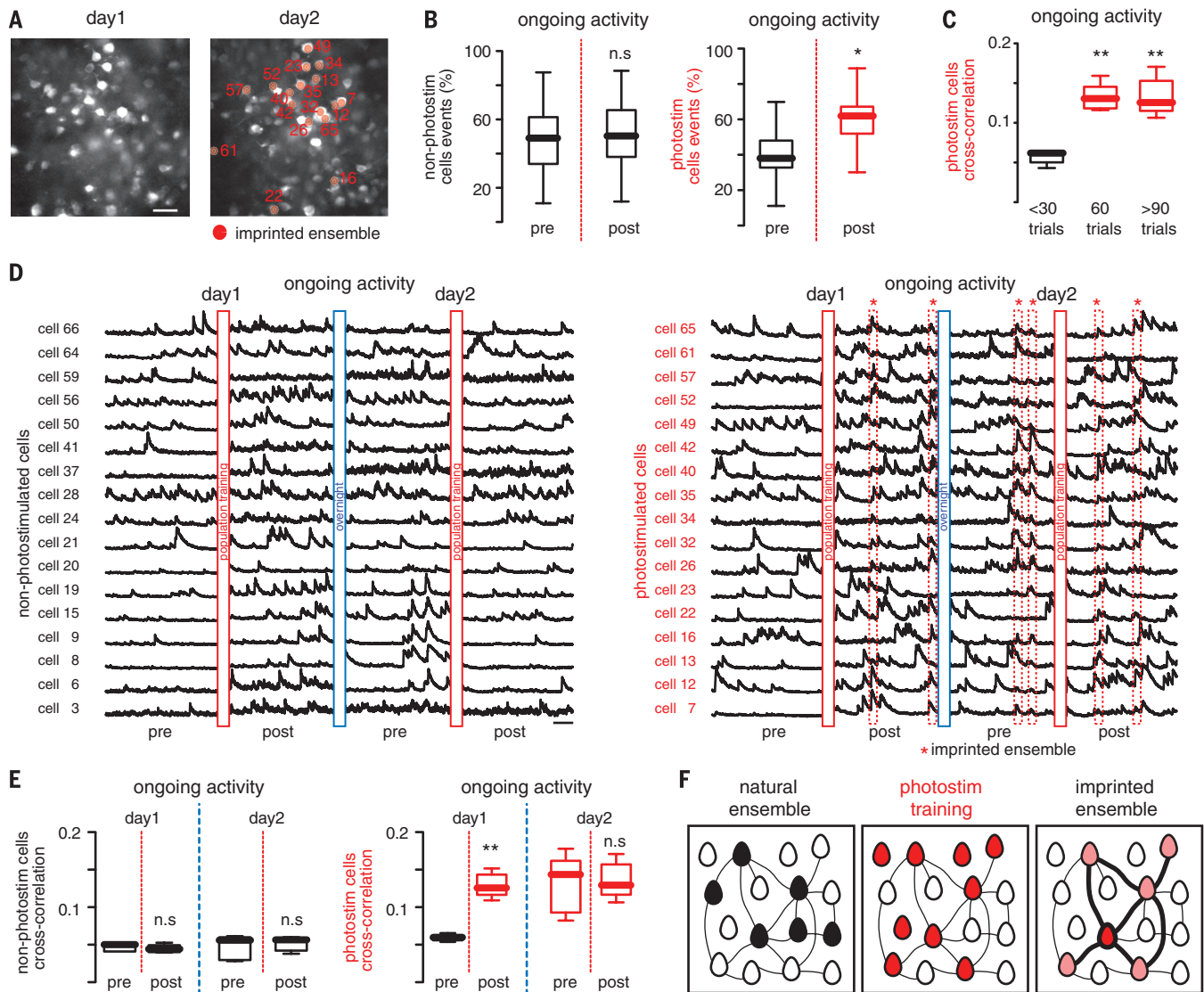


Fig. 4. Imprinted ensembles persist after consecutive days. (A) Images showing the same optical field on two different days. Scale bar, 50 μm . (B) The percentage of events during ongoing activity of nonphotostimulated cells remains stable (left; $n = 5$ mice; $n.s.$ $P = 0.5664$; Wilcoxon matched-pairs 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. The red dashed lines denote the occurrence of population training. (C) 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 nonphotostimulated neurons (left) and photostimulated neurons (right) during ongoing cortical activity on two different days before and after population training. Imprinted ensembles recur spontaneously

on consecutive days (indicated by the dotted red boxes), even after a second photostimulation training. (E) Cross-correlation between nonphotostimulated 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 on consecutive days. (F) Proposed model: Population photostimulation enhances the functional connectivity between responsive neurons. Line widths represent the strength of the functional connectivity between neurons. Black, neurons belonging to preexisting ensembles; red, photostimulated neurons; pink, recalled neurons during pattern completion. Data in (B), (C), and (E) are presented as box-and-whisker plots displaying median and interquartile ranges.

cross-correlations between nonresponsive neurons were not altered (Fig. 4E, left), they were increased between photostimulated neurons and remained stable the next day (Fig. 4E, right). Thus, optogenetic activation of identified neurons enhanced their local functional connections for at least 1 day (Fig. 4F).

Recalled ensembles shared similar characteristics—such as number of neurons and spatial distribution—with ongoing ensembles (fig. S7), but the mean distance between active neurons was shorter (fig. S7D), which indicates that the effect of the photostimulation is local. Recalled ensembles often had neurons that did 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 likely not in a tabula rasa state, we expect that imprinted ensembles may recruit segments of physiologically relevant circuit motifs (Fig. 4F).

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

More than 60 years ago, Hebb proposed that repeated coactivation 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 with single-cell resolution in awake animals. By combining novel imaging and photostimulation techniques (14, 15) and analytical tools (19), our work can be interpreted as a confirmation of the Hebbian postulate and as a demonstration that cortical microcircuits can perform pattern completion.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S7
References (28–40)

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ECONOMIC POLICY

The impact of homelessness prevention programs on homelessness

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Despite the prevalence of temporary financial assistance programs for those facing imminent homelessness, there is little evidence of their impact. Using data from Chicago from 2010 to 2012 ($n = 4448$), we demonstrate that the volatile nature of funding availability leads to good-as-random variation in the allocation of resources to individuals seeking assistance. To estimate impacts, we compare families that call when funds are available with those who call when they are not. We find that those calling when funding is available are 76% less likely to enter a homeless shelter. The per-person cost of averting homelessness through financial assistance is estimated as \$10,300 and would be much less with better targeting of benefits to lower-income callers. The estimated benefits, not including many health benefits, exceed \$20,000.

Over 2 million people experience homelessness each year in the United States (1). Historically, the primary approach to combating homelessness has been to provide emergency shelters or transitional housing services to those who are already homeless. More recently, policy-makers have increased their focus on homelessness prevention efforts. One of the most common prevention strategies is to provide temporary financial assistance to

people facing eviction in order to keep them in their residences. In the United States, 93% of households live in an area that has such a program, and these programs receive over 15 million calls a year (2). Despite the prevalence of these efforts, there is little evidence about the extent to which they actually prevent homelessness (3, 4).

Here we examine the effectiveness of temporary financial assistance by using data from the Homelessness Prevention Call Center (HPCC) in Chicago, which processes about 75,000 calls annually. Chicago residents at risk of becoming homeless can call 311 to request temporary financial assistance for rent, security deposits, or utility bills. These callers are routed to the HPCC, which is a centralized processing center that screens callers for eligibility and connects eligible callers with local funding agencies.

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Editor's Summary

Building new networks in the brain

Donald Hebb's hypothesis that coactivation of neurons leads to the formation of ensembles of neurons has inspired neuroscientists for decades. The experimental creation of such ensembles has been technically challenging. Using two-photon optogenetic stimulation with single-cell resolution, Carrillo-Reid *et al.* discovered that recurrent activation of a group of neurons creates an ensemble that is imprinted in the brain circuitry. Activation of a single neuron can lead to recall of the entire ensemble in a phenomenon called pattern completion. The artificial ensemble persists over days and can be reactivated at later time points without interfering with endogenous circuitry.

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