Image sequence reactivation in awake V4 networks

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Edited* by Ranulfo Romo, Universidad Nacional Autónoma de México, Mexico City, Mexico, and approved September 28, 2012 (received for review July 13, 2012)

In the absence of sensory input, neuronal networks are far from being silent. Whether spontaneous changes in ongoing activity reflect previous sensory experience or stochastic fluctuations in brain activity is not well understood. Here we describe reactivation of stimulus-evoked activity in awake visual cortical networks. We found that continuous exposure to randomly flashed image sequences induces reactivation in macaque V4 cortical networks in the absence of visual stimulation. This reactivation of previously evoked activity is stimulus-specific, occurs only in the same temporal order as the original response, and strengthens with increased stimulus exposures. Importantly, cells exhibiting significant reactivation carry more information about the stimulus than cells that do not reactivate. These results demonstrate a surprising degree of experience-dependent plasticity in visual cortical networks as a result of repeated exposure to unattended information. We suggest that awake reactivation in visual cortex may underlie perceptual learning by passive stimulus exposure.

electrophysiology | monkey

In natural environments, the visual system is often exposed to successive, random image patches that are briefly inspected during periods of fixation. Although the temporal coding of image sequences has been investigated during active vision by examining responses to sensory stimulation (1–6), whether and how cortical neurons and networks encode temporal image sequences in the absence of sensory stimulation is largely unknown. Here, we examined the possibility that, during brief periods of quiescence, stimulus-evoked responses could be "rehearsed," or reactivated, by visual cortical networks previously activated during stimulus presentation.

Reactivation of stimulus-induced neuronal activity is the phenomenon by which neurons in selected brain regions exhibit specific spiking patterns during periods of sleep and quiescent awake states resembling previously evoked responses. For instance, hippocampal cells firing together during a task period have been shown to exhibit increased correlations during subsequent sleep (7) compared with the period preceding the task. Subsequent studies have not only supported the fact that task-coactivated hippocampal neurons are reactivated together during posttask slow-wave (8) and REM (9) sleep, but have also shown that the temporal firing pattern of responses reoccurs in the same order as during the task (10, 11). Although reactivation had originally been reported in the hippocampus as a mechanism of memory consolidation (12–14), it may constitute a fundamental property of neural ensembles in many brain areas. Indeed, in addition to hippocampus, reactivation has been reported in rat prefrontal cortex (15–17), motor and somatosensory cortex during quiescent awake states (18), rat primary visual cortex (V1) during slow-wave sleep (19), and rat and cat V1 immediately after stimulus presentation during anesthesia (20–22).

An important issue is whether the reactivation of previously evoked neuronal activity can be demonstrated in the awake state, not only during sleep or anesthesia. Indeed, sleep or anesthetized states are characterized by high synchronous activity due to widespread oscillations in the same frequency band and a global decrease in brain activity (23). Conversely, awake reactivation has been recently demonstrated during quiescent periods in hippocampal cells (24–28), and it has been shown to be influenced by the animal's current location (25–27, 29), to occur with elevated precision in novel environments (25, 29), and to represent pathways not previously experienced by the animal (28). Furthermore, a more recent study (22) found reactivation in awake rat visual cortical cells in response to a moving dot stimulus swept across a linear path of adjacent receptive fields following a conditioning period. Nonetheless, the issue of whether neuronal populations can exhibit experience-dependent reactivation of evoked activity remains unclear. Specifically, reactivation of neuronal responses has been exclusively demonstrated when cells are activated sequentially in a temporal sequence. Although sequential firing may be representative of neuronal firing in areas such as the hippocampus—where place cells fire in a specific temporal order as the animal explores the environment—sequential firing is less common in sensory cortex, where neuronal responses represent incoming stimuli as a complex temporal spiking pattern. For instance, in visual cortex, neurons with nonoverlapping receptive fields respond sparsely to successive fixation patches during natural viewing such that spikes from multiple neurons often occur coincidentally or at different times during the same viewing episode (2, 3). Whether neuronal networks can exhibit reactivation of complex, random patterns, such as those encountered in natural viewing conditions, is unknown.

We examined here the capacity of neuronal populations to exhibit reactivation in visual cortical area V4, where neurons respond to complex image features (30–33) and play a key role in perceptual learning (34, 35). Response reactivation was investigated by using a random presentation of image patches reminiscent of stimuli encountered during successive fixation episodes during natural viewing. We describe a unique form of rapid cortical reactivation at the network level induced in the awake state precisely at the time when a stimulus is expected to occur. Specifically, we found that repeated, brief stimulation with random image sequences causes a significant "memory trace" in a subsequent blank fixation trial and an increased similarity between the stimulus-evoked response and the network ongoing spiking pattern.

Results

Response Reactivation in V4 Populations. We performed extracellular recordings using multiple electrodes while monkeys performed a passive fixation task. The stimulus consisted of a 10×10-degree (deg) image encompassing multiple receptive field locations (Fig. S1). The image was divided into 2×2-deg image patches, and each patch was presented serially in a random spatiotemporal sequence (Fig. S2 and Fig. 1*A*). Each receptive field was stimulated at least once during sequence presentation, and 25 image patches were presented throughout the 3-s movie (each image patch was flashed for 120 ms). Each 3-s stimulus trial was followed by a 3-s blank trial (Fig. 1*A*) triggered by the onset of a fixation point (the duration of the blank trial was equal to that of the stimulus trial). Each session comprised 150 stimulus and 150 blank trials. Baseline ongoing activity was assessed over 30 blank fixation trials before stimulus presentation (prestimulus), and this condition was repeated following the alternating stimulus–blank

Author contributions: V.D. designed research; S.L.E. performed research; S.L.E. analyzed data; and S.L.E. and V.D. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1212059109/-/DCSupplemental.

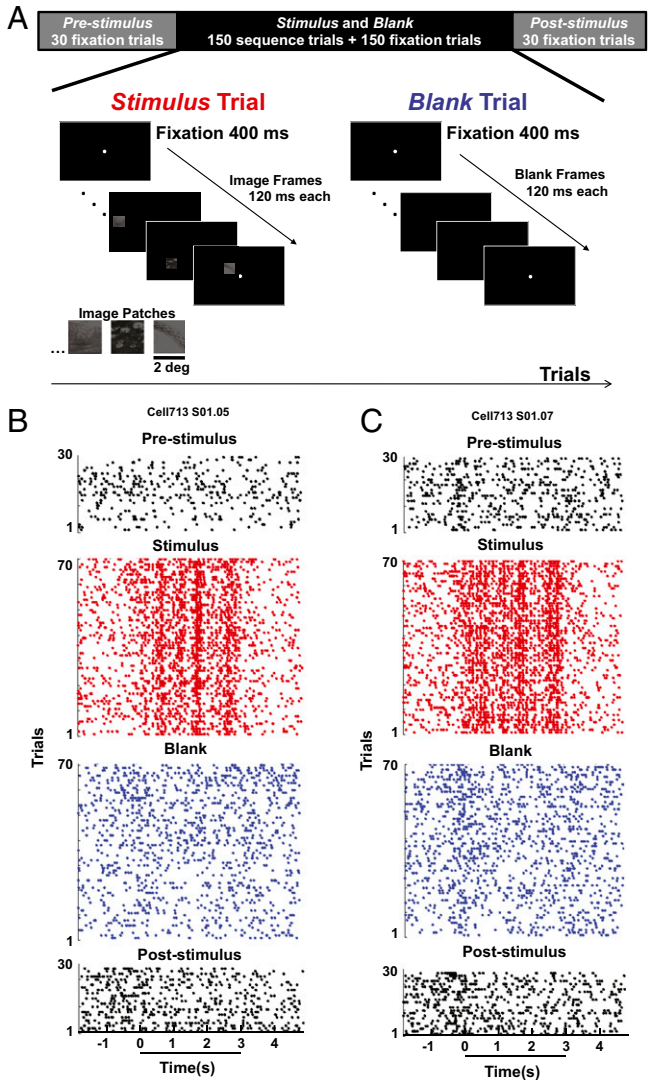

Fig. 1. Experimental paradigm. (*A*) Stimulus protocol: Two monkeys performed a passive fixation task. The sequence presentation (stimulus) consisted of 2 × 2-deg patches of a natural scene presented serially in a random spatiotemporal sequence for 3 s. Each 3-s stimulus trial was followed by a blank fixation trial of similar duration and was triggered by the onset of the fixation point. Baseline spontaneous activity was determined over 30 blank fixation trials before stimulus presentation (prestimulus). This condition was mirrored by 30 blank fixation trials following the alternating stimulus–blank presentations (poststimulus; these trials were identical to the interleaved blank fixation trials). (*B*) Raster plots depicting the responses of one neuron in one session composed of successive blocks of prestimulus/stimulus/blank/poststimulus trials. (*C*) Same as *B* for a different neuron.

presentations at the end of the session (poststimulus; these trials were identical to blank fixation trials). Sessions in which the monkeys did not achieve and maintain fixation for at least 70% of the trials were excluded. A total of 149 visually responsive cells were isolated across 19 sessions in two monkeys.

Contrary to expectation, the firing rates of the neurons activated by the stimulus were increased not only when the movie was presented, but also during the alternating blank trials (Fig. 1 *B* and *C* and Fig. S3). Indeed, we analyzed the responses of the cells in our population throughout an extended time window starting with stimulus offset and ending with the subsequent blank presentation. By collapsing this time window analysis across trials for all of the recorded cells (Fig. 2), we found an increase in neuronal responses to the stimulus followed by a decrease in the intertrial interval, and then a pronounced increase immediately after the onset of the fixation point in the subsequent blank condition ($P < 0.001$, Wilcoxon signed rank test; comparing the mean firing rates in the 3-s window before fixation onset in the blank condition and the 3-s window after the blank trial onset). The increase in firing rate in the blank condition raised the possibility that neuronal responses may exhibit reactivation of the previously evoked spiking activity during the stimulus trial.

Thus, we tested the hypothesis that repeated stimulus exposure causes a reactivation (at the same time scale) of the temporal pattern of stimulus-evoked neuronal responses across the population of cells in the absence of sensory stimulation. To quantify reactivation across the population of cells, we measured the degree of similarity between the temporal pattern of neuronal firing in the stimulus and blank conditions. This measurement was done by using the 2D Pearson correlation coefficient (CC) after time binning and z-scoring the neurons' average firing rates (firing rates were computed for the entire 3 s of stimulus presentation by using 10-ms bins and then averaged across trials for each condition; pre, stim, blank, and post were all averaged and z-scored separately). Because cells with high average firing rates may impact our correlation measure more than those with low firing rates (36), the responses of each cell were normalized across trials for each condition (using z scores; Fig. 3*A*; we found that the mean firing rates were uncorrelated with our Pearson correlation of z-scored response-time matrices; Fig. S4). Because the increase in firing rates in the blank condition occurred at the same time as during stimulus presentation, we measured the correlation between the two response-time matrices using the same time scale. To determine the statistical significance of the correlation, we created a pseudoblank matrix by shuffling the blank z-scored neuronal responses across time bins and cells, which allowed us to compare the correlation between the stimulus and pseudoblank to the correlation between stimulus and blank (bootstrap method)—we found that 75% of sessions (24 out of 32) exhibited significant reactivation [Fig. S5*A*; $P < 0.05$; this result was calculated from a total of 32 sequence presentations (sequences); 13 sessions contained blocks of two unique sequences; we also tested significance using a greater number of shuffles and found similar results; Fig. S5*B*].

We next assessed the magnitude of reactivation by comparing the correlation between the temporal responses across the network of cells during the stimulus and blank periods to that between stimulus and prestimulus. Our expectation was that the evoked response pattern would be more similar to the blank

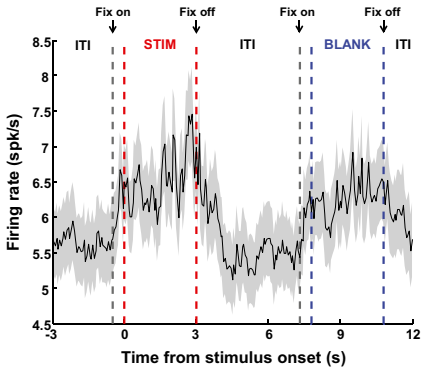

Fig. 2. Neuronal response following stimulus presentation increases during subsequent blank. The peristimulus time histogram of the average firing rate across all trials and neurons in our population is shown relative to stimulus onset. Red lines indicate the onset and offset of the stimulus sequence; blue lines indicate the onset and offset of the following blank period. Gray lines indicate the onset of the fixation point in both stimulus and blank conditions (400 ms before stimulus or blank onset). Shaded envelopes represent SEM of all visually responsive cells (n = 149) in all sessions (19 sessions, 32 sequences).

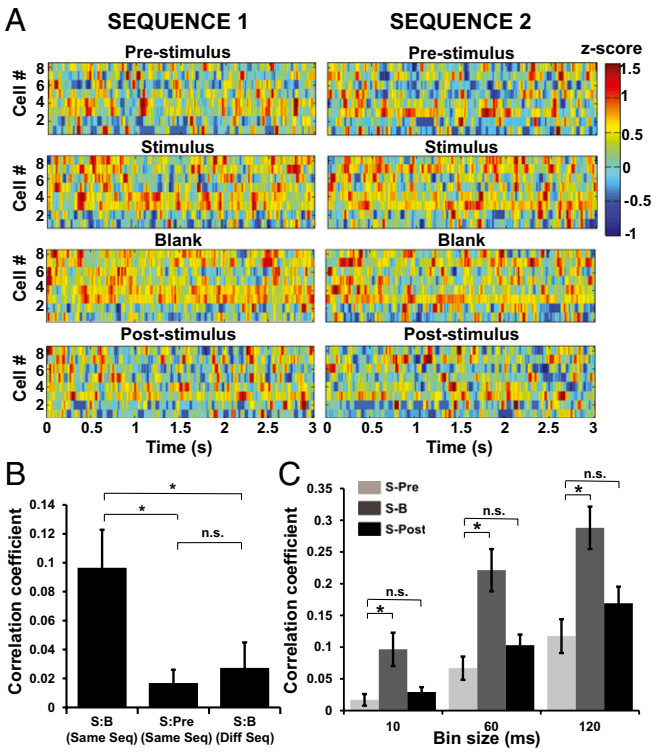

Fig. 3. Response reactivation in visual cortical networks. (*A*) Averaged and z-scored response-time matrices for one population of cells in each condition for two different stimulus presentations (sequences 1 and 2) are depicted. (*B*) Stimulus specificity of response reactivation. We exposed the network of cells to two successive stimulus sequences and computed correlations between the stimulus–blank response-time matrices within the same sequence and between sequences. (*C*) The effect of bin size on stimulus–blank correlations using 10-, 60-, and 120-ms bins. Correlations between stimulus and prestimulus (S-Pre), stimulus and blank (S-B), and stimulus and poststimulus (S-Post) were compared for all bin sizes. *$P < 0.05$; n.s., $P > 0.05$. Error bars represent SEM.

response than to the prestimulus response. Indeed, we found that the average response correlation between the stimulus and blank conditions was greater than that between stimulus and prestimulus (Fig. 3*B*; $CC_{S\text{-}B} = 0.10$, $CC_{S\text{-}Pre} = 0.02$, $P = 0.004$, Wilcoxon rank sum test; results from 32 sequences). In addition, we compared the correlation between the stimulus and prestimulus conditions with that between stimulus and poststimulus, but failed to find a significant difference between the two ($CC_{S\text{-}Pre} = 0.02$, $CC_{S\text{-}Post} = 0.03$, $P = 0.28$, Wilcoxon signed rank test; results from 32 sequences). We confirmed that these differences were not due to differences in eye movements between the different conditions (Table S1). Furthermore, we assessed whether reactivation was larger in the first half of stimulus presentation (i.e., the first 1.5 s) than in the second half of sequences that elicited significant reactivation (24 sequences, $CC_{S\text{-}B}$ first half = 0.15, $CC_{S\text{-}B}$ second half = 0.14, $P = 0.10$, Wilcoxon signed rank; both values were significantly different from the correlation between stimulus and prestimulus conditions $P = 0.00001$ [first], $P = 0.003$ [last], Wilcoxon rank sum).

In principle, our results might have been influenced by the size of the time bin (10 ms) used to measure neuronal activity. We found that the increase in bin size causes a significant increase in stimulus–blank correlation [Fig. 3*C*; 10-ms bins, $CC_{S\text{-}B} = 0.10$; 60-ms bins, $CC_{S\text{-}B} = 0.22$; 120-ms bins, $CC_{S\text{-}B} = 0.29$; $F(2, 32) = 3.24$; $P = 0.04$, one-way ANOVA]. Specifically, the 60- and 120-ms binned stimulus–blank correlation coefficients were significantly greater than the 10-ms binned correlation coefficient, but not significantly different from each other. However, despite the fact that correlations increased with bin size, the difference between the stimulus/blank and stimulus/prestimulus correlations remained statistically significant for all bin sizes [$P = 0.00001$ (60 ms), $P = 0.0003$ (120 ms), Wilcoxon rank sum; correlation values between the stimulus and poststimulus responses were not significantly higher than those between stimulus and prestimulus, $P = 0.22$ (60 ms), $P = 0.27$ (120 ms), Wilcoxon rank sum]. In addition, we found that 72% (23 out of 32) of the 60-ms binned and 69% (22 out of the 32) of the 120-ms binned sessions showed significant reactivation (using the pseudoblank and bootstrap method; results from 32 sequences).

To rule out the fact that reactivation in the blank condition could be due to a general increase in firing rates of neurons—possibly caused by a stimulus-independent increase in arousal or attention—we examined whether the effects described above exhibited stimulus specificity. We addressed this issue by exposing the network of cells to a second stimulus following initial stimulation; that is, after the initial completion of a prestim1/stim1/blank1/poststim1 block of trials, we exposed the same network to a new block, prestim2/stim2/blank2/poststim2, by presenting a new stimulus sequence (stim2) consisting of identical image patches presented in a new temporal order. Using the same correlation analysis as described above, we compared the correlation between stimulus and blank periods within the same sequence (stim1–blank1 and stim2–blank2) and between sequences (stim1–blank2 and stim2–blank1). We found a clear signature of stimulus specificity —the stimulus–blank correlations within each sequence were significantly greater than those between sequences (Fig. 3*B*; mean $CC_{Within} = 0.10$; mean $CC_{Between} = 0.03$, $P = 0.001$, Wilcoxon signed rank test; results from 13 sessions with two unique sequences). Importantly, this effect was not due to differences in recording stability because firing rates remained stable between sessions (Fig. S6).

Previous reports of response reactivation have shown that this phenomenon can occur in the forward or reverse direction (9). To determine the direction of response reactivation in our study, we reversed the blank period along the time axis (Fig. 4*A*) and performed the same correlation analysis using only the sessions with statistically significant reactivation (24 sequences). We found that the average correlation between the stimulus and "forward" blank responses was significantly higher than that between the stimulus and "reverse" blank responses (Fig. 4*B*; $CC_{Forward} = 0.14$, $CC_{Reverse} = -0.02$, $P = 0.0000002$, Wilcoxon signed rank test).

Our results so far depend critically on the temporal correlation between the population responses in the stimulus and blank conditions measured in a fixed 3-s window. Even though firing rates in the blank condition clearly increased immediately after the onset of the fixation spot (Fig. 2), it may be possible that correlations reached statistical significance even before the 3-s period following blank onset. To control for this possibility, we computed the correlation between the 3-s stimulus-evoked response and a 3-s moving window response sliding between stimulus offset and the end of the subsequent blank trial only in those sessions showing significant reactivation (24 sequences). We used 60-ms time-binned, averaged, z-scored responses, and the time window was shifted in 60-ms increments until 4.2 s after blank onset (Fig. 5). Interestingly, the correlation coefficient reached a maximum exactly at the starting point of the 3-s window corresponding to blank onset (Fig. 5*A*; correlation values were normalized within each session). We further computed the statistical significance of the correlation as a function of time (using the pseudoblank and bootstrap method) and found that the only time window in which the correlation was significant (i.e., $P < 0.05$) was the 3-s blank window signaled by blank onset (Fig. 5*B*). All together, these results further confirm the significance of the temporal correlation between the stimulus and blank trial responses.

Temporal Dynamics of Reactivation. We examined the temporal dynamics of stimulus-specific reactivation. To this end, correlations

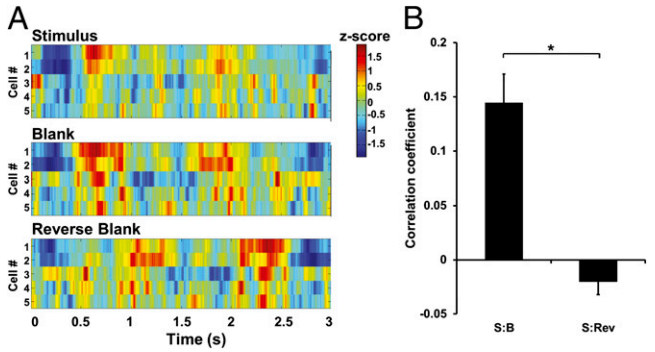

Fig. 4. Reactivation only occurs in the forward direction. (*A*) Averaged and z-scored response-time matrices of stimulus, blank, and reverse-blank conditions for one session. The "reverse blank" condition is the blank response reversed along the time axis. (*B*) Correlation analysis comparing the forward and reverse reactivations. Only the sessions with a statistically significant effect were included in this analysis; $*P < 0.05$. Error bars represent SEM.

were calculated for blocks of two trials and then normalized by the SD of correlations for all conditions in each session. We performed this analysis on the 24 sequences (from 16 recording sessions) that showed significant reactivation. Fig. 6*A* shows average normalized correlation values across sessions—stimulus–blank correlation increased with the number of stimulus exposures ($r = 0.42$, $P = 0.002$). In addition, we found a significant correlation between stimulus and blank trials (assessed using the bootstrap method, $P < 0.05$) even after few stimulus presentations—73% of sessions were associated with a significant reactivation after 6 stimulus presentations; 94% of all sessions had a significant reactivation after 12 stimulus presentations. We also found that, on average, ~42% of sessions were associated with significant reactivation for each block of two trials (Fig. S7). Furthermore, the probability of significant reactivation was increased as the neuronal population was exposed to more stimulus presentations ($r = 0.37$, $P = 0.006$).

Reactivation Depends on Population Size. Does the strength of reactivation change when the number of cells in the network varies? To examine this issue, we used a cell-dropping procedure to calculate the percentage of populations showing significant reactivation when the number of cells in the network is gradually decreased (by using all possible combinations of simultaneously recorded cells). Specifically, after determining whether the stimulus–blank correlation was significant (for the entire network of n cells recorded within a session), we removed one cell from the population and recalculated the stimulus–blank correlation for the population of $(n - 1)$ cells, and then assessed the statistical significance of the correlation. This procedure was repeated until we were left with only one cell. The cell-dropping procedure was repeated multiple times such that all possible combinations of cells were analyzed. We found that whereas 47% of single cells showed significant reactivation, the percentage of populations with statistically significant reactivation increased with the number of cells included in the population ($r = 0.94$, $P = 0.00001$; Fig. 6*B*; we only used neuronal populations that exhibited significant reactivation in the first sequence presentation, if multiple sequences were presented, when all neurons were considered). Furthermore, we extended our correlation analysis to local field potentials (LFPs). We observed significant reactivation as well as significant differences between the stimulus/prestimulus correlations and stimulus/blank correlations (Fig. S8 *A* and *B* and *SI Methods*).

Stronger Reactivation in Highly Informative Cells. We further examined whether the populations of cells exhibiting significant reactivation are those that are most informative about the stimulus. We tested this hypothesis by determining how much information about the stimulus is carried by the population response by computing mutual information between the population responses and image patches (37). We found that all information values were statistically significant regardless of whether the population responses exhibited reactivation or not. Interestingly, we found that populations exhibiting statistically significant reactivation carried more information about the stimulus (Fig. 6*C*). That is, the populations of cells (24 sequences) showing statistically significant reactivation had a statistically higher average mutual information—0.99 bits—whereas the populations of cells not showing reactivation (8 sequences) had an average mutual information of 0.39 bits ($P = 0.02$, Wilcoxon rank sum test; Fig. 6*C*).

Discussion

We have demonstrated that populations of neurons in awake macaque visual cortex exhibit stimulus-specific, cue-triggered reactivation of previous evoked responses at the timescale of visual fixation. We found that the network reactivation of evoked activity is more robust in larger populations of cells and is observed in both multiple neuron responses and LFP activity. Additionally, we have demonstrated that the presence of reactivation is related to the capacity of neuronal populations to carry information about the stimulus.

One might argue that our results may be due to stimulus expectation, arousal, or attention because neurons in visual cortex are known to increase their responses when a stimulus is expected or when attention is directed toward it (38, 39). However, although we did observe a firing rate increase in blank trials, the fact that response reactivation is stimulus specific (i.e., stimulus–blank within-sequence correlation is greater than that between sequences) and occurs exclusively in the forward direction argues against a general modulatory effect due to expectation, arousal, or attention. In addition, the fact that firing rates were normalized (using z scores) before calculating correlation coefficients argues against a general modulatory effect on temporal correlations between stimulus and blank neuronal responses.

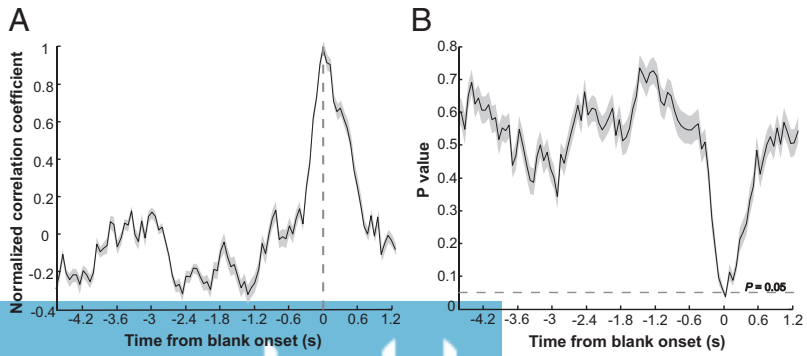

Fig. 5. Correlation between the stimulus-evoked response and a 3-s moving window. (*A*) Average normalized correlation between the 3-s stimulus-evoked response and a 3-s window (shifted every 60 ms) moving between stimulus offset and 1.2 s after blank onset (indicated by the gray dashed line). (*B*) Statistical significance of the correlation coefficient as a function of time. The only time window in which correlation is significant ($P \leq 0.05$) corresponds to the starting point of the 3-s blank window associated with blank onset. Represented is the average P value corresponding to each 3-s moving window (shifted every 60 ms). Shaded envelopes represent SEM.

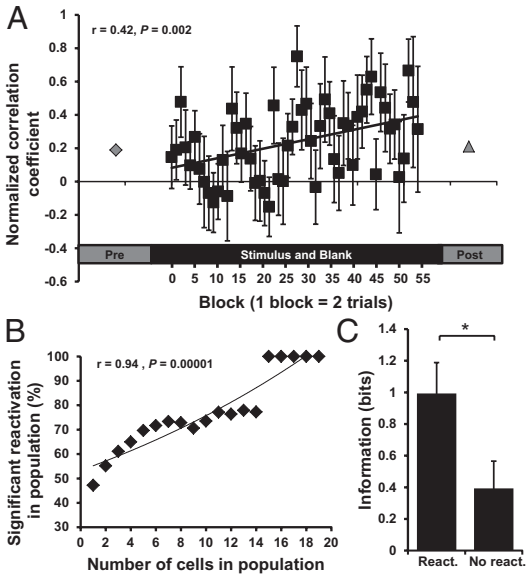

Fig. 6. Temporal dynamics of response reactivation, effect of population size, and mutual information analysis. (*A*) Stimulus–blank correlation strength increases with the number of stimulus exposures. Each point represents the stimulus–blank correlation computed by averaging the z-scored network responses of two successive trials normalized by SD of correlations within each session (normalized correlations were averaged across sessions). To eliminate variability in the total number of trials across sessions, only the first 110 pairs of stimulus–blank trials were included in this analysis. The first and last points represent the mean correlations for stimulus–prestimulus and stimulus–poststimulus conditions computed for the 30 trials at the beginning and end of each session. (*B*) The probability of a significant reactivation event increases with the number of cells in the population. The percentage of combinations of cells showing significant reactivation was determined by comparing the $CC_{Stimulus,\ Blank}$ with $CC_{Stimulus,\ Pseudoblank}$. This analysis was exclusively performed on populations that showed significant reactivation. (*C*) Neuronal populations exhibiting significant reactivation carry more information about stimuli. $*P < 0.05$. Error bars represent SEM.

Our study differs from previous stimulus entrainment reports involving repetitive stimulus exposure to induce firing at the same frequency because our stimulus presentation did not occur at a fixed receptive field location (40, 41). Indeed, our stimulus presentation is significantly different from that during entrainment—the presentation of image patches occurs at random locations within a 10×10-deg window, thus making it impossible for image patches to stimulate V4 receptive fields at a fixed frequency. Furthermore, whereas entrainment studies describe how neuronal responses are modified immediately following stimulus exposure, our results demonstrate response reactivation exactly at the time when stimuli are expected to occur in the subsequent trial. One could also argue that the refresh rate of the monitor may be entraining neurons to exhibit reactivation. However, if this were the case, we would not find a significant difference between temporal correlations of responses occurring in the prestimulus/stimulus and blank/stimulus conditions, nor would we find stimulus specificity in our reactivation events (the same refresh rate is used throughout the experiments).

Previous studies using voltage-sensitive dye imaging (21, 42) and single-cell electrophysiology in cat V1 (20) have shown that ongoing activity resembles orientation map responses to grating stimuli (42) and natural movies (20), and it can exhibit a memory trace response immediately after the stimulus is extinguished (20). Furthermore, "recall" responses were recently found in visual cortical networks following conditioning with a moving dot stimulus upon presentation of the first dot in the sequence (22). However, most previous visual cortex reactivation studies have been performed in anesthetized V1 or during sleep, where the spatial similarity between ongoing activity and stimulus-evoked response was either independent of stimulus history (42) or was observed immediately after stimulus offset (20). In contrast, we found clear evidence for reactivation in the awake state in V4 networks at the expected time of stimulus onset while monkeys performed a fixation task. In agreement with the Xu et al. study (22), we were able to elicit response reactivation by using the fixation spot as a trigger stimulus. This is supported by our finding that neuronal activity in the blank condition increases at the same time relative to the onset of the fixation spot as in the stimulus condition and that the correlation between the evoked and ongoing activity reaches the maximum at exactly this time point.

The relationship between the stimulus-induced and ongoing cortical activity revealed in our study has certain similarities with the "replay" of neuronal activity in neural circuits mediating episodic (9, 24, 43) and sensorimotor (44) learning. In those studies, the temporal firing patterns of multiple neurons during learning are repeated either during sleep (9, 43, 44) or in the awake state (25). However, there are major differences between classical replay and the effects shown here. For instance, in hippocampal circuits, replay occurs at irregular intervals, and the replayed sequences are often compressed (25) or expanded (9) in time. In contrast, our study reveals reactivation patterns occurring at the same rate as stimulus presentation that can be externally controlled by a trigger cue. Finally, an important departure from previous work is our demonstration that neuronal networks in sensory cortex exhibit reactivation after exposure to a complex, random temporal stimulation that is representative of stimuli encountered during natural visual experience.

Altogether, our results are consistent with Hebb's hypothesis (45) that simultaneously activated neurons that share a common experience may form a "cell assembly" that exhibits cue-triggered recall. Because our stimulus sequence activates the receptive fields of neurons at different times, spike-timing–dependent plasticity (STDP) could alter the strength of intracortical synapses between successively activated neurons to increase their probability of spontaneous cofiring. Indeed, previous models and experimental work have suggested that STDP could be a mechanism by which recurrent excitatory connections could be altered to allow the learning of temporal sequences (45, 46). Consistent with this hypothesis is the fact that the strength of network reactivation increases with the number of stimulus exposures. Finally, our results raise the possibility that the capacity of neuronal networks to reverberate may explain how the brain is able to learn and store events that occur in time following passive stimulus exposure during sensory experience (47–51).

Methods

Behavioral Paradigm. All experiments were performed in accordance with protocols approved by National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (52). Two male rhesus monkeys (*Macaca mulatta*) were trained to fixate on a centrally located fixation point (0.4 deg in size) within a 2-deg fixation window. To ensure fixation, eye position was continuously monitored by using an eye tracker system operating at 1 kHz (EyeLink II; SR Research).

Visual Stimuli. Stimulus trials consisted of 2×2 deg image patches randomly presented in a spatiotemporal sequence. The image patches were clipped from a larger image (10×10 deg) that covered the multiple receptive fields recorded within a session (we ensured that each receptive field was stimulated at least once during sequence presentation). A total of 25 image patches were presented for 120 ms each for a total of 3 s. The same sequence of image patches was presented throughout a given session. To determine whether response reactivation is stimulus specific in a subset of the sessions (13 out of 19), we added another block of trials in which the same image patches were displayed in a new temporal order. Each block of trials contained prestimulus, stimulus, blank, and poststimulus conditions.

Electrophysiological Recordings. We used two recording techniques for multiple single-unit extracellular recordings in visual cortex area V4. First, in eight of the sessions, we used a custom Crist grid recording technique (51). Microelectrodes (tungsten, 1–2 MΩ at 1 kHz; FHC) were advanced transdurally

through stainless steel guide tubes into V4. We recorded up to 10 units simultaneously in each session at depths between 200 and 400 μm. Recording sites were located between 1 and 2 mm of each other. Second, in 11 of the sessions, we used laminar electrodes (U-probe; Plexon Inc.) consisting of a linear array of 16 equally spaced contacts. We recorded up to 19 units simultaneously in each session. Laminar electrodes were used either along with single contact electrodes or with multiple laminar electrodes. Real-time neuronal signals recorded from both electrode types (simultaneous 40-kHz A/D conversion on each channel) were analyzed by using the Multichannel Acquisition Processor (MAP) system (Plexon Inc.). Individual neurons were isolated through spike waveform sorting by using Plexon's offline sorter program. Recording sites were selected on the basis of the quality of the signal (signal-to-noise ratio) and responsiveness to visual stimuli.

Neuronal Reactivation Analysis. We used z-scored response-time matrices in all analyses (using 10-ms time bins). We separately assessed reactivation in neuronal populations and individual cells. We calculated the 2D Pearson correlation coefficient between two matrices containing the averaged z-scored firing rates of all of the cells in the recorded population as a function of time (in different stimulus conditions; see *SI Methods*). The correlation measures the degree of association between observed values. Correlation values range between −1 and +1. Negative values indicate that the firing rates are anticorrelated; i.e., high values in *S* are associated with low values in *B*. Positive correlation coefficients indicate that firing rates are positively correlated; i.e., high values in *S* are associated with high values in *B*. A correlation of 0 means there is no relationship between the firing rates in *S* and *B*. The correlation calculation can be saturated when neurons within the same population have largely different firing rates, such as when one neuron has very low firing rates and another one has very high firing rates; hence, responses were normalized using z scores. However, we did not find a significant relationship between firing rate and correlations (Fig. S4).

Mutual Information Analysis. We binned neuronal responses from each cell individually in 120-ms bins (the duration of each image frame). We calculated mutual information using the information breakdown toolbox (37). We compared the mutual information values between populations showing statistically significant reactivation (using shuffled responses and bootstrapping) and those that did not. Additionally, we validated our information values by shuffling the average firing rates and then performing the same analysis on the shuffled responses (*SI Methods*).

ACKNOWLEDGMENTS. This work was supported by the National Institutes of Health EUREKA Program, the National Eye Institute, the Pew Scholars Program, the James S. McDonnell Foundation, and the Norman Hackerman Advanced Research Program (to V.D.).

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