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Formation and Reverberation of Sequential Neural Activity Patterns Evoked by Sensory Stimulation Are Enhanced during Cortical Desynchronization

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SUMMARY

Memory formation is hypothesized to involve the generation of event-specific neural activity patterns during learning and the subsequent spontaneous reactivation of these patterns. Here, we present evidence that these processes can also be observed in urethane-anesthetized rats and are enhanced by desynchronized brain state evoked by tail pinch, subcortical carbachol infusion, or systemic amphetamine administration. During desynchronization, we found that repeated tactile or auditory stimulation evoked unique sequential patterns of neural firing in somatosensory and auditory cortex and that these patterns then reoccurred during subsequent spontaneous activity, similar to what we have observed in awake animals. Furthermore, the formation of these patterns was blocked by an NMDA receptor antagonist, suggesting that the phenomenon depends on synaptic plasticity. These results suggest that anesthetized animals with a desynchronized brain state could serve as a convenient model for studying stimulus-induced plasticity to improve our understanding of memory formation and replay in the brain.

INTRODUCTION

Memory formation is a fundamental process needed for adaptive behavior. A growing body of evidence suggests that learning and memory processes involve the modification of ongoing spontaneous activity in an experience-dependent fashion (Wilson and McNaughton, 1994). As an animal's exposure to an environment increases, the similarity between spontaneous activity and activity evoked by natural stimuli also increases (Berkes et al., 2011). This suggests that, during learning, spontaneous activity progressively adapts to the statistics of encountered stimuli (Fiser et al., 2010). In support of this idea, an imaging study of visual cortex in rats using voltage-sensitive dyes revealed that repetitive presentation of a visual stimulus modified global patterns of subsequent spontaneous activity such that these patterns more closely resembled the evoked responses (Han et al., 2008). Another compelling example suggesting adaptation of spontaneous activity was provided by a study using voltage-sensitive dyes, which showed that ongoing activity in cat visual cortex corresponded closely to functional orientation maps (Kenet et al., 2003). The similarity between spontaneous and evoked patterns is not restricted only to global activity patterns but has also been found in spike-timing relations among neurons. At the microcircuit level, the precise temporal sequence of spiking evoked by external stimuli is more similar to spontaneously occurring patterns than predicted by chance. This has been demonstrated both in vitro (MacLean et al., 2005) and in vivo (Luczak et al., 2009). These data suggest that the adaptation of ongoing activity to the statistical nature of experienced stimuli can also involve sculpting the corresponding microcircuit architecture (Luczak and Maclean, 2012). Other data from freely moving animals suggest that such changes in sequential spiking are related to behaviorally relevant learning and memory processes. Population recordings in hippocampus or neocortex have revealed that spiking sequences observed during behavior were subsequently replayed in similar temporal order during following resting periods (Euston et al., 2007; Ji and Wilson, 2007; Skaggs and McNaughton, 1996). Despite the likely importance of understanding the mechanisms by which stimulus-evoked sequences are "imprinted" in spontaneous activity, advances have been limited by the technological difficulty of recording neuronal population activity and manipulating neural processes in behaving animals.

The hallmark of memory formation in the brain activity of freely moving animals is the emergence of stimulus-induced (or behavior-induced) sequential activity patterns that are later spontaneously replayed (Euston et al., 2007; Ji and Wilson, 2007; Skaggs and McNaughton, 1996). Although many previous studies have emphasized replay during slow-wave sleep, there is abundant evidence that it can occur during periods of wakeful quiescence, even relatively brief ones, when the hippocampus exhibits large irregular activity containing sharp wave ripple

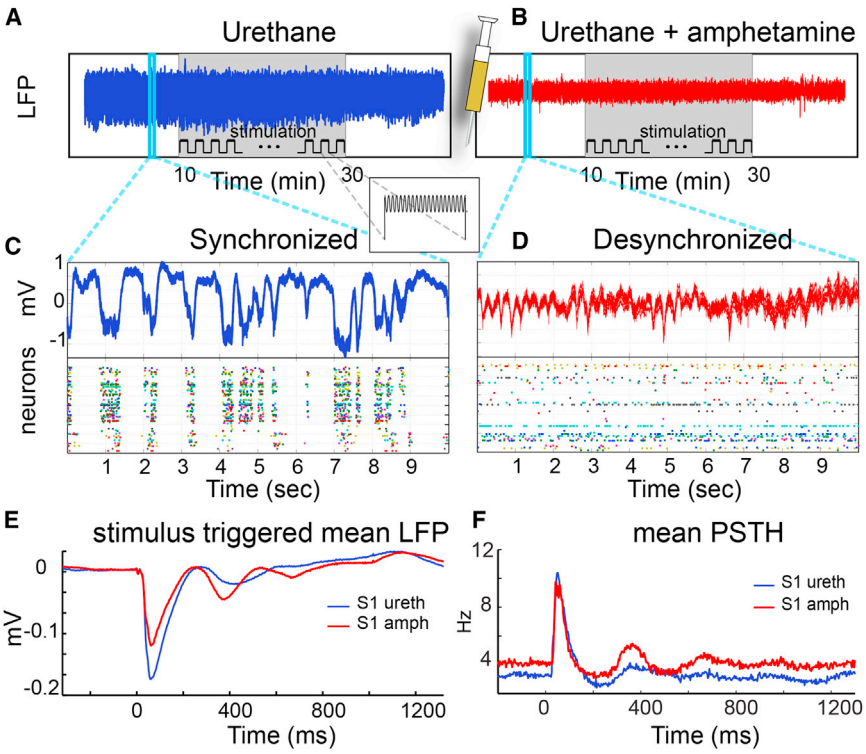

Figure 1. Experimental Protocol for Somatosensory Stimulation, and Synchronized and Desynchronized Brain States

(A) Example LFP in S1 under urethane anesthesia. The gray shaded area indicates the period of tactile stimulation consisting of 1 s long periods of vibration at 20 Hz (inset).

(B) Example LFP in S1 under urethane anesthesia after injection of amphetamine.

(C) Example LFP and unit activity under urethane anesthesia shown at a higher temporal resolution. Note prominent UP and DOWN states characteristic of the synchronized brain state.

(D) Example LFP and unit activity after amphetamine injection. Note that, in the desynchronized state, fluctuations of LFP and unit activity are of smaller amplitude.

(E) Mean stimulus-triggered LFP across animals in S1 in urethane only and urethane plus amphetamine conditions.

(F) The same as (E) for average spiking activity. PSTH, peristimulus time histogram.

(SPWR) events and the cortex is in a relatively synchronized state, exhibiting up-down state transitions. Moreover, the actual reactivation events occur during the up states, which can be considered as brief episodes of cortical desynchronization. Finally, there is also evidence that long-term potentiation (LTP) is suppressed during slow-wave sleep in general (Leonard et al., 1987) but is transiently re-enabled during SPWR events that are associated with neocortical up-state transitions (Buzsáki, 1984). To investigate if a similar phenomenon could be also studied in simpler (anesthetized) preparations and to study how the formation of sequential patterns depends on the brain state, we used population recordings in urethane-anesthetized rats. We found that spontaneous sequences of spiking activity become more similar to preceding stimulus-evoked sequences, particularly in desynchronized brain states. This effect lasted up to several minutes, was N-methyl-D-aspartate (NMDA) receptor-dependent, and was observed in both somatosensory and auditory cortices. The phenomenon was similar to what we observed in auditory cortex of awake, passively listening animals. These data suggest that the formation and reverberation of sensory-evoked patterns may partake in learning-related phenomena in multiple neocortical regions of anesthetized animals, which may provide a convenient model for the study of memory mechanisms in the brain.

RESULTS

We first investigated changes in spontaneous activity patterns induced by sensory stimulation by recording activity from neuronal populations in primary somatosensory cortex (S1). Under urethane anesthesia (Figure 1A), brain activity showed a synchronized state with characteristic slow wave oscillations (Steriade et al., 1993), in which generalized bursts of population activity (UP states) were interspersed with periods of neuronal silence (DOWN states) (Figure 1C, bottom). UP states were accompanied by negative deflections of the local field potential (LFP) (Figure 1C, top), indicative of synchronized synaptic inputs. Urethane promotes a condition of behavioral unconsciousness that closely mimics the full spectrum of natural sleep (Clement et al., 2008), although the duration of DOWN states is reported to be shorter in natural sleep (Johnson et al., 2010) as compared to anesthetized conditions. Injection of amphetamine rapidly changed the brain state; within a few minutes after injection, cortical activity transitioned to a strongly desynchronized state, which lasted for at least 30 min (Figures 1B and 1D). Tactile stimulation did not change either synchronized or desynchronized brain states (Figures 1A and 1B, shaded area). Surprisingly, the average stimulus-triggered responses in S1 were very similar in synchronized and desynchronized states, despite large differences in spontaneous neuronal activity among these states (Figures 1E and 1F).

Spontaneous Sequential Activity Patterns Are Modified by Tactile Stimulation

To investigate fine-scale temporal changes in spontaneous neuronal activity induced by sensory stimulation, we first calculated the relative latency of each neuron. This reflects its timing in relation to other neurons based on cross-correlogram analysis (see Experimental Procedures; Figure 2A). Figure 2B shows cross-correlograms of 32 neurons from a representative experiment, sorted by latency during the stimulation period after amphetamine injection (middle panel). Consistent with previous results from auditory and visual cortex (Jermakowicz et al., 2009; Luczak et al., 2009), neurons showed similar temporal patterns during spontaneous and stimulus-evoked conditions.

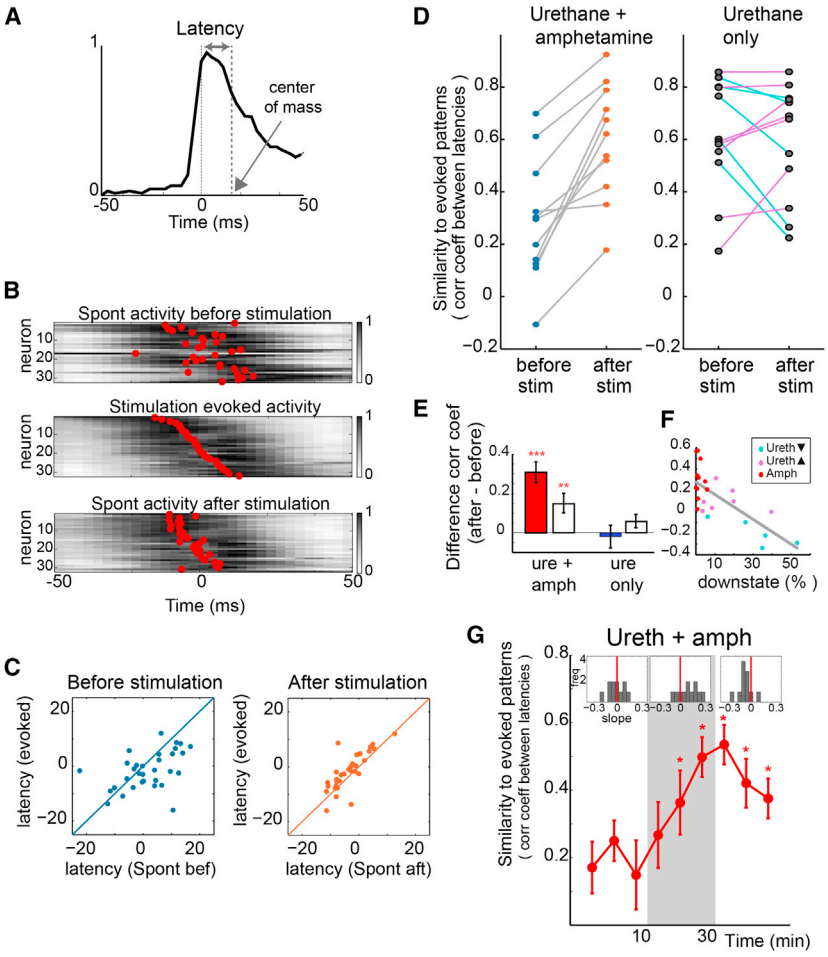

Figure 2. Similarity between Spontaneous and Evoked Temporal Patterns Is Enhanced by Amphetamine

(A) The latency of a neuron is defined as the center of mass of the cross-correlogram of the neuron with the summed activity of all other simultaneously recorded cells (MUA).

(B) Example of temporal patterns before, during, and after tactile stimulation in amphetamine condition from a representative experiment. Each row represents a cross-correlogram of a neuron with the summed activity of all other neurons normalized between zero and one. Neurons are ordered according to their latency (red dot) during stimulation (middle panel). The same order was used to plot the latency of the neurons before stimulation (top panel) and after stimulation (bottom).

(C) Left: Scatter plot of the latencies of neurons for evoked activity and spontaneous activity before stimulation plotted for the same neurons as in (B). Right: Scatter plot of the latencies for the same neurons for evoked and spontaneous activity after stimulation. Note that the distribution of points is closer to the identity line, indicating higher similarity between latencies for evoked and spontaneous period after stimulation.

(D) Similarity of spontaneous activity patterns to evoked activity patterns (quantified by latency correlation) before (blue) and after (orange) tactile stimulation for amphetamine (left) and urethane-only (right) conditions. Connected dots represent one animal. Note that, in the amphetamine condition, the similarity of spontaneous sequences to evoked sequences increases for all rats after stimulation. Rats that show latency correlation increase under urethane are shown in magenta, and rats that show latency correlation decrease under urethane are depicted with light blue lines.

(E) Average change in similarity to evoked patterns based on amphetamine and urethane data from (D); red and blue bar, respectively. White bars show average change in similarity to evoked patterns calculated from pair-wise cross-correlograms. Error bars represent the SEM.

(F) Synchronized brain state reduces formation of reverbatory activity in somatosensory cortex. *x* axis shows the percentage of time that the population activity spent in DOWN states; y axis units are the same as in (E). Rats that show latency correlation increase under urethane (Ureth ▲) are marked with magenta color, and rats that show latency correlation decrease under urethane (Ureth ▼) are shown in light blue. Red color denotes rats after amphetamine injection.

(G) Latency correlation evolution in time in S1 before, during, and after stimulation (each dot represents the average from all rats; error bars denote SEM). The shaded area corresponds to the stimulation period. The insets at the top show the slope distribution of latency correlations in the corresponding period of the experiment for each rat. Note how the spontaneous activity becomes gradually more similar to evoked patterns during stimulation and how the similarity slowly decreases after stimulation. Stars denote points significantly different from spontaneous activity before stimulation ($p < 0.05$; t test).

See also Figures S2, S3, and S4.

For example, neurons that were firing earlier than other neurons during stimulation also tended to fire earlier than other neurons during spontaneous activity before or after tactile stimulation (Figure 2B, top and bottom panel, respectively). This is explicitly shown in Figure 2C, where latencies from stimulation periods are compared to latencies from spontaneous periods for the same neurons. Note that latencies *after* stimulation are more similar to latencies during the stimulation period than to spontaneous latencies before stimulation (right and left panel in Figure 2C, respectively). We quantified this effect by comparing the correlation coefficient of latencies from stimulated and spontaneous periods. Figure 2D shows such correlation coefficient values for all rats. Consistent with data presented in Figures 2B and 2C, the latency correlation increased significantly after stimulation for all animals under amphetamine (Figure 2D, left panel and Figure 2E, red bar; mean correlation coefficient [corr. coef.] increase = 0.31 ± 0.062 SEM, $p = 0.0001$; t test). For the animals without amphetamine injection (urethane only), the increase in latency correlation after tactile stimulation was not significant (Figure 2D, right panel and Figure 2E, blue bar; mean corr. coef. change = −0.03 ± 0.06 SEM, $p = 0.35$; t test; see Figures S4C and S4D available online, ruling out ceiling effect). Similar results were obtained by computing latency from pairwise correlograms (Figure 2E, white bars; mean corr. coef. change: amphetamine (amph) = 0.098 ± 0.023 SEM; urethane (ureth) = 0.049 ± 0.025 SEM; see Experimental Procedures). However, the rats in the urethane-only condition that do show an increase in latency correlation tended to have a

more desynchronized brain state (Figure 2F; corr. coef. = −0.66, p = 0.01; see Supplemental Experimental Procedures for definition of brain state measure). This indicates that, in the desynchronized state induced by amphetamine or occurring spontaneously under urethane, the brain may be more plastic, such that the repeated tactile stimulation induced more extensive reorganization of spontaneous fine-scale temporal activity patterns. The increased similarity of evoked patterns and post-stimulation spontaneous patterns in this preparation could reflect similar processes as that underlying memory formation (Wang and Morris, 2010).

In order to investigate how spontaneous temporal patterns change over time, we divided each experimental condition into nine periods: three periods during the spontaneous activity before stimulation, three periods of the spontaneous activity occurring between the delivery of stimuli (e.g., the 1 s spontaneous activity intervals between the 1 s intervals of stimulation), and three periods for the spontaneous activity after stimulation (Figure 2G). For each period, the latency correlation between spontaneous and evoked activity was calculated (during the 20 min stimulation period, the stimulus was presented 600 times, and latency for evoked activity was calculated from all those 600 intervals of 1 s; to calculate, for example, latencies from the first spontaneous period during stimulation, we included data from the first 200 1 s intervals between stimulation presentations). In the amphetamine condition in S1, we observed that, as expected, the latency correlation between prestimulus spontaneous and evoked activity did not change significantly (first three points in Figure 2G, left inset; mean slope = −0.01 ± 0.1 SD, p = 0.73, t test). Following this, the latency correlation between spontaneous and evoked activity increases with time during stimulation (three points in the shaded area in Figure 2G, middle inset; mean slope = 0.11 ± 0.12 SD, p = 0.01). Once stimulation ceased, latency correlations decayed gradually (Figure 2G, right inset; mean slope = −0.07 ± 0.08 SD, p = 0.01; see Figure S4A for the same analyses with higher temporal resolution). Interestingly, this slow decrease in reactivation after stimulation is consistent with data from behaving animals, in which most reactivation is observed only within a few minutes after tasks (Euston et al., 2007). To quantify the significance of sequence reverberation, we compared averaged values of latency correlations before and after stimulation. The values of latency correlation were significantly higher after stimulation only for S1 in the amphetamine condition ($p < 0.0001$; t test) but were not significantly different for the urethane-only condition ($p > 0.1$). Thus, in anesthetized rats injected with amphetamine that induced brain state desynchronization, sensory stimulation caused a gradual reorganization of spontaneous activity patterns in S1, and the "memory" of that stimulation persisted in the following spontaneous activity patterns.

As an additional test that stimulus-evoked patterns in S1 are replayed during the following spontaneous activity, we used template-matching analysis as described in studies with behaving animals (Euston et al., 2007; Tatsuno et al., 2006; see Supplemental Experimental Procedures). Templates for each data set consisted of average stimulus-triggered activity from 0 to 200 ms after stimulus onset. Figures 3A–3C show template, sample raster plots, and template-matching scores for spontaneous activity before and after stimulation for a representative rat. We found that, in the amphetamine condition (but not the urethane only condition), the number of spontaneous patterns that closely matched the template was higher in the period following tactile stimulation (Figures 3D and 3E; $p_{ampth} = 0.02$, $p_{ureth} = 0.52$; t test). As compared to the results obtained using the latency measure, reverberation disappeared faster after stimulation when analyzed with template matching (Figure S4B). Although it is difficult to pinpoint the exact reason for this discrepancy, tests on simulated data suggest that latency measure could be more robust in small signal-to-noise regimes and less affected by any time compression of replayed patterns, thus giving better estimation of weak and varying reverbatory activity (Figure S2). Nevertheless, both analysis methods are otherwise consistent in revealing increased reverberation following stimulation in the desynchronized brain state (but not in the urethane-only condition).

Reactivation of Firing Rate Correlations

The foregoing analysis revealed that the timing relations among neurons during spontaneous activity have memory of previous stimulus-evoked temporal patterns. However, given that the number of spikes fired by a particular neuron can be significantly affected by stimulus presentation, we also investigated if firing rate correlations induced by tactile stimulation can be observed in subsequent spontaneous activity. To address this question, we smoothed spike trains with a Gaussian kernel (SD = 130 ms) and calculated the correlation coefficient between all pairs of neurons. The resulting firing rate correlation matrices for units recorded in S1 for evoked and spontaneous periods during amphetamine are shown in Figure 4A. The matrices for the spontaneous period after stimulation are more similar to the matrices for the stimulation period than the matrices for the spontaneous activity before stimulation (Figure 4A). In order to quantify similarities, we calculated the Euclidian distance between the firing rate correlation matrices. For the amphetamine case, the distance between correlation matrices for evoked periods and the following spontaneous periods was smaller than the distance between correlation matrices for evoked and the preceding spontaneous periods for all rats (Figure 4B; p = 0.003; paired t test). However, in the urethane-only condition, we found a nonsignificant increase in similarity between correlation matrices for evoked and following spontaneous periods (S1: p = 0.09; paired t test). Using the correlation coefficient as an alternative measure of similarity between matrices resulted in similar findings (data not shown). Our findings were preserved when the size of the smoothing kernel was varied from 30 to 180 ms. Thus, in the amphetamine case, the firing rate correlations induced by stimuli persist in subsequent spontaneous activity, which is consistent with memory reactivation studies in awake animals (Wilson and McNaughton, 1994).

In order to quantify the temporal profile of firing rate replay, we used the explained variance (EV) measure, which is a standard method applied to detect memory reactivation in behaving animal studies (Euston et al., 2007; Hoffman and McNaughton, 2002; Kudrimoti et al., 1999; Pennartz et al., 2004). EV is defined as the square of the partial correlation between firing rate correlation matrices during stimulation and subsequent activity,

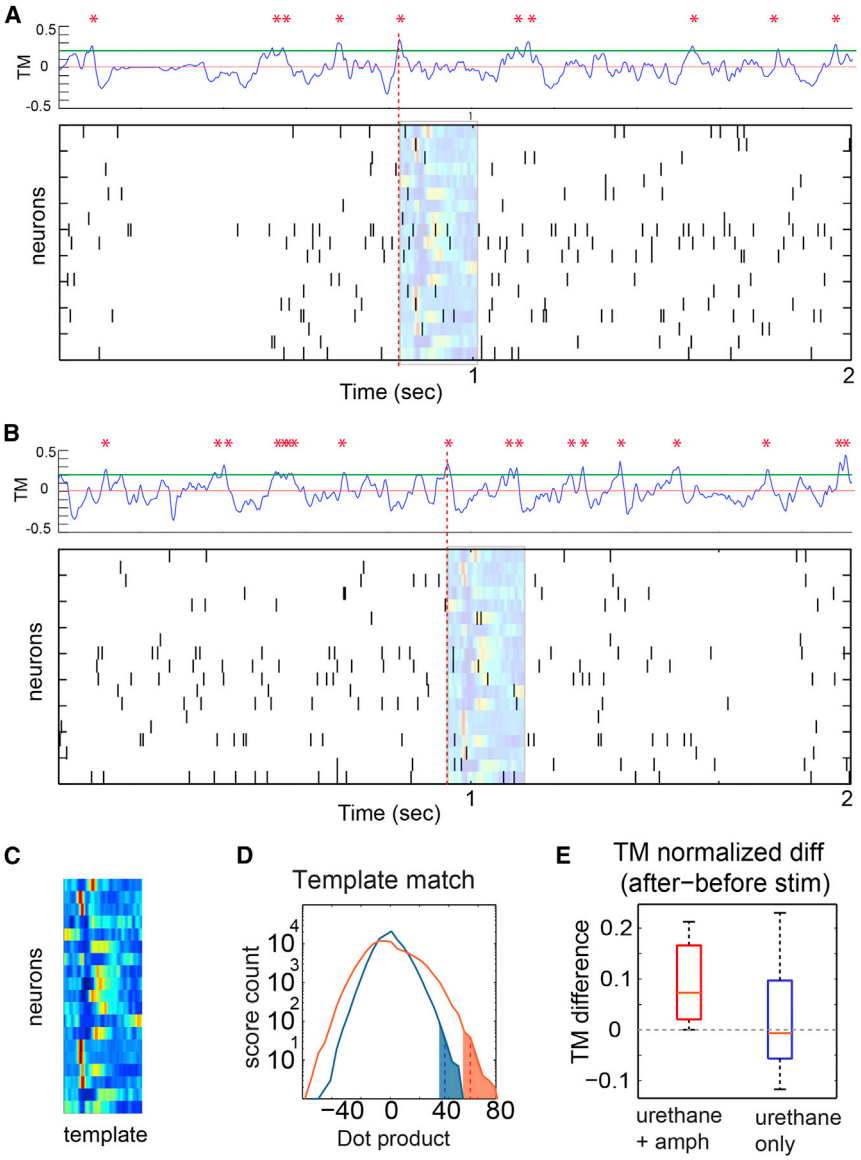

Figure 3. Template Matching

(A) Raster plot of representative 2 s of spontaneous activity before tactile stimulation. Blue traces at the top of rasters show matching score, and stars indicate "good matches", defined as above 95% of matching score. TM, template matching.

(B) Raster plot of 2 s of spontaneous activity after tactile stimulation. Note the greater number of good matches (denoted by stars) after stimulation than before.

(C) Stimulus-triggered activity used as a template. In (A) and (B), template is superimposed on sample activity windows, showing good match.

(D) Example of template-matching histograms for representative data from one rat injected with amphetamine. The solid blue (orange) line shows the frequency of the matching values before (after) stimulation. The shaded regions denote the 0.1% of the highest matching values. The dotted lines represent the mean of such values.

(E) Box plot of differences between the highest matching values for spontaneous activity after and before stimulation, which corresponds to the differences between the dotted lines in (D) for all rats. On each box, the central mark denotes the median, the edges of the box are the 25th and 75th percentiles, and the whiskers extend to the most extreme data points. In the amphetamine condition, the template matching between spontaneous and evoked patterns increases after stimulation, which is consistent with latency analyses shown in Figure 2. See also Figure S6.

taking into account the correlations that existed prior to the stimulation. (See the Supplemental Experimental Procedures, Tatsuno et al., 2006, and Kruskal et al., 2007 for more details.) Similar to our analyses using latency correlations, evoked and spontaneous periods were subdivided into three smaller time subperiods: the first spontaneous subperiods were used as reference (PRE) for calculating EV on the following subperiods (Figure 4C; Supplemental Experimental Procedures). In the amphetamine condition, significant firing rate reactivation was observed during the stimulation period in 1 s intervals of spontaneous activity in S1 (Figure 4C; $p < 0.05$; paired Kolmogorov-Smirnov test). The reactivation slowly decreased after stimulation, similar to the decrease observed in the latency correlation analysis (compare Figure 4C with Figure 2G). Under urethane anesthesia alone, we also observed significant firing rate reactivation during stimulation periods, but these did not remain significant after stimulation (data not shown).

Reverberation in Auditory Cortex

We next sought to test whether the reactivation described above generalizes to other cortical systems and other mechanisms of desynchronization. We therefore recorded in auditory cortex before, during, and after presentation of tone stimuli and induced desynchronization with amphetamine, tail pinch, or infusion of carbachol in the posterior hypothalamic nucleus (see Experimental Procedures). The sequence of experimental conditions used to record population activity in A1 in urethane anesthetized rats is illustrated in Figures 5A–5D. In every experimental condition, we recorded 10 min of spontaneous activity followed by 20 min of auditory stimulation with pure tones followed by 10 min of spontaneous activity (see Experimental Procedures). Under urethane anesthesia, auditory cortex showed similar activity as in S1: large fluctuation of LFP associated with alternation between UP and DOWN states characteristic of the synchronized brain state (although short periods of spontaneously occurring desynchronized periods were also observed, as reported before in Clement et al., 2008; Figure 5A). Tail pinch or infusion of carbachol resulted in desynchronization of the brain state (Figure 5B). Injection of amphetamine also induced desynchronization, but in this case, desynchronization was more stable in time (Figure 5C). In the last part of the experiment, each rat was injected with an NMDA receptor antagonist

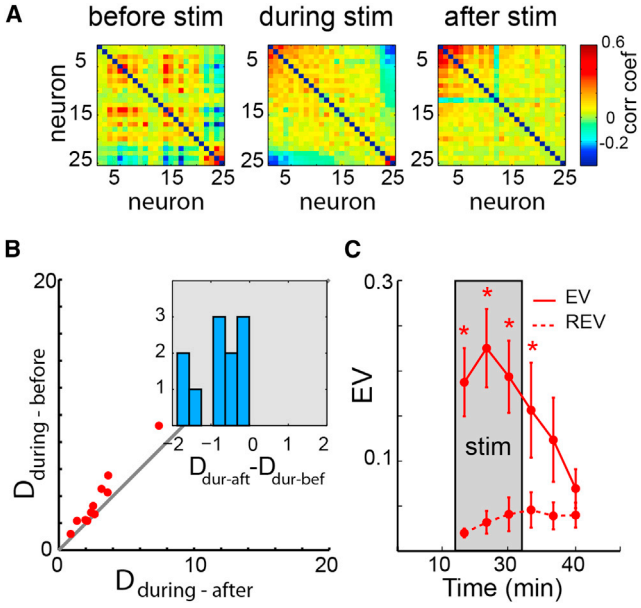

Figure 4. Persistence of Firing Rate Correlation in S1

(A) Pair-wise firing rate correlation matrices for the neurons in S1 before, during, and after tactile stimulation for a representative animal injected with amphetamine. To facilitate visual comparison of the matrices, the elements were sorted to group together neurons with similar correlations (for sorting, we used values of the first principal component calculated for the matrix from the stimulation period). The same order was used for the other matrices.

(B) Scatter plot of the similarities (measured as the Euclidean distance) between firing rate correlation matrices for each animal. Distribution of points above the identity line indicates that spontaneous firing rate correlations after stimulation become more similar to stimulus-evoked correlations. Inset shows the distribution of differences between distances in the corresponding scatter plots. D, Euclidian distance

(C) Changes in firing rate correlations over time analyzed with EV in the amphetamine condition. The gray shaded area corresponds to the stimulation period. The error bars correspond to the SEM. Solid lines represent the explained variance and dotted lines represent the reversed explained variance (REV; see Supplemental Experimental Procedures). Stars indicate points significantly different from REV (control) values ($p < 0.05$; t test).

(MK801). After MK801 injection, the auditory cortex persisted in a desynchronized state, although more short periods of neuronal silence resembling DOWN states tended to occur toward the end of the experiment (Figure 5D). To directly compare results obtained in desynchronized brain state in anesthetized animals with processes occurring in awake rats, we also analyzed population activity recorded in auditory cortex in three awake, head-restrained rats (Figure 5E). We did not find significant differences between desynchronized brain states in awake and anesthetized animals based on analysis using the brain state index (Figure 5F; the brain state index is defined as the percent of time that the neuronal activity spent in DOWN states, as previously described in Luczak et al., 2013; see Supplemental Experimental Procedures for details). Furthermore, stimulus-triggered LFPs were similar for awake and anesthetized animals (Figure 5G; see Figures S5A and S5B for significance tests). For spiking activity, stimulus-triggered onset and offset responses in anesthetized animals showed similar sharp increase and duration as in awake rats, although the amplitude of response was higher in awake animals (Figure 5H; see Figures S5C and S5D for significance tests and Figures S5E–S5G examples of single neuron responses). Altogether, these results suggest that cortical activity in the desynchronized state in anesthetized rats shows similar properties as in awake animals.

We next sought to investigate replay of stimulus-evoked patterns across experimental conditions. To do so, we used analyses based on cross-correlograms, as illustrated in Figure 2. Figures 6A–6D show the similarity of stimulus-evoked temporal patterns to spontaneous patterns preceding the epoch of stimulus presentations and following the epoch of stimulus presentations. Consistent with results in S1, the similarity between spontaneous and evoked patterns increased for a majority of animals following the auditory stimulation period in all desynchronized states, regardless of the induction method (tail pinch, carbachol, or amphetamine; Figures 6B and 6C), but this was not observed in the synchronized state (Figure 6A). Injection of the NMDA antagonist MK801 blocked formation of persistent activity patterns, despite desynchronization (Figure 6D). Summary statistics for difference in similarity after-before stimulation are shown by colored bars in Figures 6F–6I (mean corr. coef.$_{after}$ − corr. coef.$_{before}$ ± SEM: $\Delta cc_{ure} = -0.07 \pm 0.04$; $\Delta cc_{carb+tail} = 0.08 \pm 0.03$; $\Delta cc_{amph} = 0.14 \pm 0.7$; $\Delta cc_{MK} = -0.01 \pm 0.04$; $p_{ure\text{-}carb} = 0.019$; $p_{ure\text{-}ampth} = 0.027$; $p_{ure\text{-}MK} = 0.27$; paired t test). We also verified this effect using a different analysis based on cross-correlograms between all pairs of neurons, which had significant peaks in cross-correlogram. Results were consistent with previous analyses and are summarized by white bars in Figures 6F–6I (mean corr. coef.$_{after}$ − corr. coef.$_{before}$ ± SEM: $\Delta cc_{ure} = -0.02 \pm 0.02$; $\Delta cc_{carb+tail} = 0.07 \pm 0.02$; $\Delta cc_{amph} = 0.11 \pm 0.04$; $\Delta cc_{MK} = -0.02 \pm 0.06$; $p_{ure} = 0.43$; $p_{carb+tail} = 0.018$; $p_{amph} = 0.037$; $p_{MK} = 0.7$; t test). One possible confound here is that neuronal patterns could become more stereotyped with time, regardless of stimulation. As a control for this possibility, we assessed if the increase in similarity between evoked and spontaneous activity was specific for the unique stimulus used in each experimental condition (during each experimental condition, a different tone was repetitively presented). We did this by recomputing correlation coefficients between spontaneous and evoked latencies, where we used evoked latencies from a different experimental condition. After this substitution, changes in similarity after-before stimulation were not different from a chance level (mean corr. coef.$_{after}$ − corr. coef.$_{before}$: $\Delta cc_{ure} = 0.026 \pm 0.32$; $\Delta cc_{carb+tail} = 0.02 \pm 0.036$; $\Delta cc_{amph} = -0.01 \pm 0.02$; $\Delta cc_{MK} = 0.018 \pm 0.024$; $p > 0.4$ for all conditions; t test; Figures 6F–6I, gray bars), indicating that observed replay of patterned neural activity is stimulus-specific.

We next analyzed in more detail how the similarity between spontaneous and evoked patterns changed in time, using the same analyses as illustrated in Figure 2G. We divided each spontaneous and evoked period into three subperiods, in which we analyzed latency correlations. Figures 6K–6N shows how the similarity of spontaneous patterns to evoked patterns changed over time in different experimental conditions. Consistent with the above analyses and with S1 data, only in desynchronized state was there a significant increase in similarity between

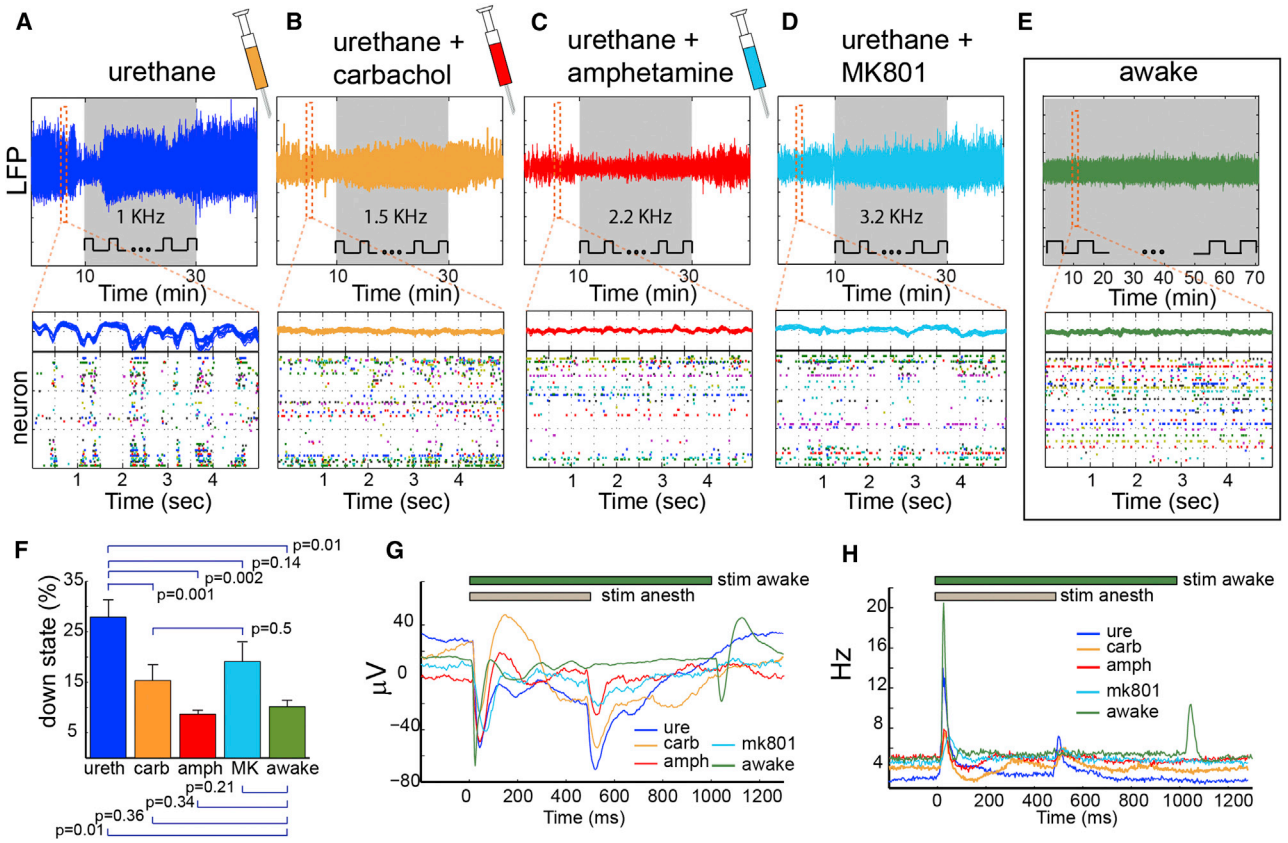

Figure 5. Experimental Protocol for Auditory Experiments

(A–D) Example LFP (top) and unit activity (bottom) in auditory cortex under urethane anesthesia alone (A) and after infusion of carbachol (B), amphetamine injection (C), and NMDA antagonist injection (D). Grey shaded area indicates the period of auditory stimulation consisting of 500 ms long tones interspersed with 1 s of silence (inset).

(E) Same type of plot as (A)–(D) for awake, head-restrained rat.

(F) Brain state in each condition measured as % of down states duration. Error bars denote the SEM.

(G) Stimulus-triggered LFP in A1 averaged across all animals for each experimental condition. The bars on top represent the stimulation duration for the awake and anaesthetized cases, respectively.

(H) The same as (G) for average spiking activity.

See also Figures S1 and S5.

spontaneous and evoked patterns, and this effect was not observed after injection of MK801 (mean slope ± SD: s_{ure} = −0.01 ± 0.04; $s_{carb+tail}$ = 0.04 ± 0.05; s_{amph} = 0.05 ± 0.04; s_{MK} = 0.01 ± 0.06; p_{ure} = 0.6; $p_{carb+tail}$ = 0.046; p_{amph} = 0.016; p_{MK} = 0.53; t test). Note that, in urethane and MK801 conditions, the higher baseline similarity may make it harder for the similarity to increase even further. To address this concern, we repeated analyses only on a subset of the data with intermediate values of prestimulation similarity, thus ensuring that values of similarity in all conditions are likewise (un)affected by any ceiling effects. Consistent with the previous results, the increase in similarity was significant only in amphetamine, carbachol, and awake conditions ($p < 0.001$; t test), thus showing that our results are not an artifact of ceiling effects (see Figures S4C and S4D for details). Altogether, these results show that, in the desynchronized brain state, repeated presentation of stimuli results in stimulus-specific reorganization of subsequent spontaneous activity and this process likely depends on NMDA-mediated plasticity.

To investigate if the persistent patterned activity observed under anesthesia in the desynchronized brain state also occurs in awake animals, we reanalyzed previously published data from head-restrained rats passively listening to tones (Experimental Procedures; Luczak et al., 2009). During stimulation, 1 s long tones were interspersed with 1 s periods of silence, and activity occurring during silent periods was regarded as spontaneous. Because we did not have a sufficiently long period of spontaneous activity before or after stimulation, we calculated correlations between spontaneous and evoked latencies for 10 min periods at the beginning and at the end of stimulation. We found a significant stimulation-induced increase of latency correlations in all animals (Δcc_{awake} = 1.74 ± 0.01 SEM; $p_{awake} < 0.01$; t test; Figures 6E and 6J). Consistent with these results, we observed a gradual increase in similarity when analyzing all consecutive periods during stimulation (mean slope: s = 0.009 ± 0.009 SD; p = 0.01; Figure 6O).

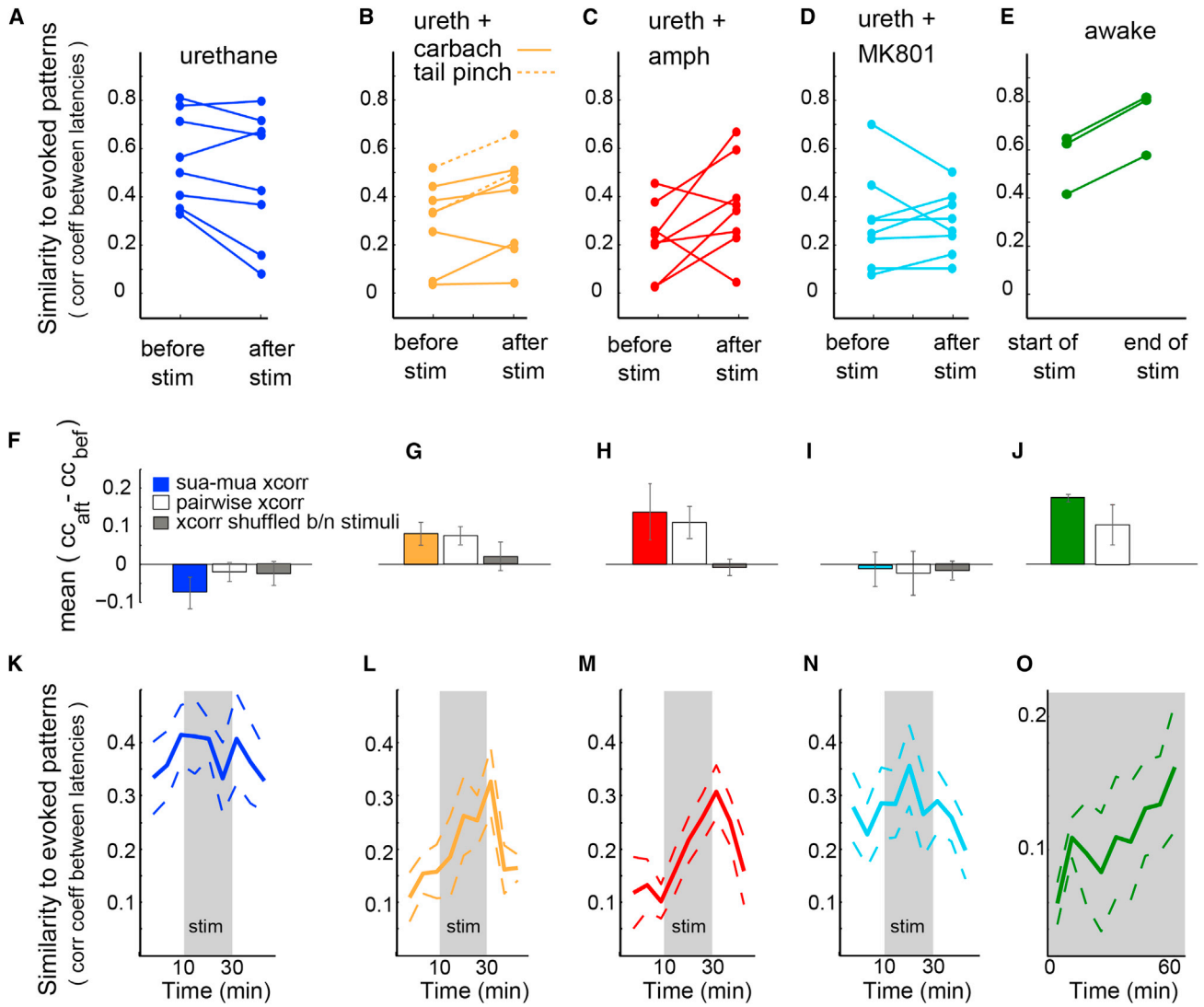

Figure 6. Changes in Similarity between Spontaneous and Evoked Temporal Patterns in Auditory Cortex for Different Experimental Conditions

(A–D) Similarity of spontaneous activity patterns to evoked activity patterns (quantified by latency correlation) before and after tactile stimulation during pharmacological treatments. Connected dots represent one animal.

(E) Similarity of spontaneous activity patterns to evoked activity patterns during first and last 10 min of tactile stimulation for awake rats.

(F–J) Color bars: average change in similarity of spontaneous and evoked patterns based on data from (A)–(E). White bars: average change in similarity of spontaneous and evoked patterns based on latencies calculated from pairwise cross-correlograms. Gray bars: average change in similarity of spontaneous and evoked patterns when latencies from stimulation periods were shuffled between experimental conditions. Values close to zero for gray bars in carbachol and amphetamine conditions indicate that reverberation was stimulus specific. Error bars denote SEM.

(K–O) Time course of changes in similarity between spontaneous and evoked patterns during experimental periods (each line represents the average from all rats; dashed lines denote SEM). Similarity is measured by correlation coefficient between latencies calculated from pairwise cross-correlograms. The shaded area corresponds to the stimulation period. Consistent with results from S1, in desynchronized brain state (except MK case), the spontaneous activity becomes gradually more similar to evoked patterns during stimulation, and this similarity slowly decreases after stimulation.

We further validated those findings by reanalyzing our data using template matching and EV analysis, which revealed consistent results. Template matching analysis revealed an increase in the number of close matches after stimulation in carbachol/tail pinch and in amphetamine conditions but not in urethane-only or MK801 conditions (Figure S6A; $V_{ureth} = -0.001 \pm 0.041$ SEM; $V_{carb/tail} = 0.096 \pm 0.031$; $V_{amph} = 0.031 \pm 0.019$; $V_{ureth} = -0.032 \pm 0.09$; $p_{ureth} = 0.9$; $p_{carb/tail} = 0.025$; $p_{amph} = 0.043$; $p_{MK} = 0.7$; t test). With EV, we observed significant replay after stimulation only in the amphetamine condition ($p < 0.05$; paired t test), although EV had a tendency to have higher values than the control data (reverse EV) for other experimental conditions (see Figures S6B–S6E). It should be noted that EV is insensitive to fine-scale temporal spiking patterns and thus provides

different information from that obtained with latency measures or template matching.

DISCUSSION

Memory formation is one of the most important processes in the brain, yet the neuronal dynamics underlying this process are only beginning to be understood, partly due to the technical difficulty of recording from large neuronal populations in behaving animals. Here, we report that the hallmarks of memory formation and memory replay—stimulus-induced sequential activity patterns that reactivate spontaneously—can also be observed in urethane-anesthetized rats. In this preparation, population recordings and other brain manipulations can be more easily performed, thus providing a convenient model for electrophysiological study of mechanisms, leading to formation of sequential patterns implicated in memory processes. Furthermore, we found similar replay in both somatosensory and auditory cortices, suggesting this may be a general mechanism in the cortex. Although previous studies using voltage-sensitive dye imaging in anesthetized animals have shown that ongoing spontaneous activity can reflect stimulus-evoked spatial patterns on a coarse spatial scale (Han et al., 2008; Kenet et al., 2003), our findings provide a major refinement of these results by demonstrating replay of fine-scale sequential spiking patterns (Figures 2 and 3) that is more analogous to sequential spiking patterns observed during memory replay in freely moving animals (Euston et al., 2007; Hoffman and McNaughton, 2002; Kudrimoti et al., 1999; Skaggs and McNaughton, 1996; Wilson and McNaughton, 1994).

In addition, our study indicates the importance of brain state during stimulus presentation. Although multiple studies show that most memory *replay* occurs during synchronized states (e.g., during slow wave sleep; Battaglia et al., 2004; Xu et al., 2012), the importance of the brain state during *encoding* is not clear. It is known that electrically evoked LTP is suppressed in this state (Leonard et al., 1987), so there is a precedent for our current finding that presentation of stimuli during a desynchronized state as compared to the synchronized state is significantly more effective in inducing lasting reorganization of temporal patterns (Figures 2 and 6), which subsequently results in stronger spontaneous replay of stimulus-induced patterns.

Why would the induction of desynchronized states in anesthetized animals facilitate the formation of tactile memories? A comprehensive explanation is lacking, but multiple lines of evidence suggest that desynchronization may be associated with increased brain plasticity. For example, amphetamine-induced desynchronization is also accompanied by increased extracellular levels of neuromodulators, such as dopamine (Creese, 1983), which are implicated in the facilitation of memory consolidation in neocortex (Schicknick et al., 2012). Amphetamine also reduces extracellular gamma-aminobutyric acid (GABA) concentrations (Bourdelais and Kalivas, 1990) and stimulates glutamate release (Karler et al., 1994; Kelley and Throne, 1992). These mechanisms are believed to be responsible for enhanced cortical plasticity after amphetamine injection (Boroojerdi et al., 2001; Tegenthoff et al., 2004). Amphetamine can also improve performance in tasks requiring attention (Grilly et al., 1989), and attention is associated with enhanced desynchronization and enhanced representation of salient stimuli (Harris and Thiele, 2011; Marguet and Harris, 2011). Similarly, desynchronization induced by tail pinch and carbachol infusion into the posterior hypothalamus involves activation of the cholinergic system (Boucetta and Jones, 2009; Duque et al., 2000; Manns et al., 2000; Marguet and Harris, 2011), which is known to modulate diverse plastic processes in the hippocampus and neocortex (for review, see Picciotto et al., 2012). Multiple studies also show that acetylcholine enhances plasticity during presentation of specific sensory stimuli, allowing those specific sensory stimuli to evoke stronger or more prominent neuronal response (Dykes, 1997; McLin et al., 2002; Metherate and Weinberger, 1990). Thus, we suggest that the brain is more plastic in the desynchronized (attentive-like) state, which may result in better "encoding" of tactile stimuli that, in turn, results in stronger reverberation during subsequent spontaneous activity. It remains to be determined if increased attention in the awake state could have an analogous enhancement of stimulus-evoked neural reorganization.

We also investigated what plasticity mechanisms may be involved in replay activity, and we found that it was suppressed by application of an NMDA receptor antagonist. Those results are in line with studies showing that the consolidation of recent information into long-lasting memories appears to depend on NMDA function both during and shortly after an experience (Wang et al., 2006). For instance, localized interference of NMDA receptor function after an experience impairs recall tested many hours or days later, as has been shown in a number of brain structures including hippocampus (Shimizu et al., 2000), auditory cortex (Schicknick and Tischmeyer, 2006), and prefrontal cortex (Tronel and Sara, 2003). NMDA receptor antagonism also blocks experience-dependent expansion of hippocampal "place fields" (Ekstrom et al., 2001). Further, NMDA receptors play a crucial role in the modification of neural connectivity during or following experiences. NMDA antagonists attenuate experience-driven reorganization of the body map in S1 of awake animals (Jablonska et al., 1999) and retard value-related changes of neural firing in orbitofrontal cortex of behaving animals (van Wingerden et al., 2012). These data suggest that neural reactivation causes formation of long-term memories via NMDA-dependent changes in synaptic strength. The pattern reactivation phenomena we describe here is also dependent on NMDA receptors and is therefore consistent with the mechanisms of memory consolidation in the awake state.

Previous studies have suggested that "reverberating" patterns are similar to spontaneous patterns that precede specific sensory experience. This phenomenon is termed "preplay" and was elegantly shown in hippocampal cortex by Dragoi and Tonegawa (2011). Similarly, in Euston et al. (2007) in Figure 1, the pretask spiking patterns in medial prefrontal cortex have obvious similarity to patterns during the task and patterns replayed after the task. The data presented here are consistent with these results and suggest that repeated stimulation induces only gradual changes to existing spiking patterns (note that, in Figures 2D and 6A–6E, similarity of evoked patterns to preceding spontaneous activity is consistently above 0). For that, the relationship between stimulus-evoked (or reverberating) sequences

to prior patterns occurring spontaneously is a very important question. We have previously shown that stereotypical patterns of population activity are associated mostly with the beginning of UP states (Luczak and Barthó, 2012; Luczak et al., 2007) and that stimulus-evoked patterns have strikingly similar temporal structure to such spontaneous patterns (Luczak et al., 2009). Furthermore, even in desynchronized brain states, population activity is composed of bursts of population activity with similar temporal structure to patterns during UP states in synchronized states (Luczak et al., 2013). Similar sequential patterns with stereotyped spatiotemporal dynamics have been also observed in vitro (Mao et al., 2001; Cossart et al., 2003; Ikegaya et al., 2004; MacLean et al., 2005), suggesting that network UP states could be circuit attractors. Together, these in vitro and in vivo studies suggest that connectivity patterns at the local level impose significant constraints on activity propagation (Luczak and Maclean, 2012), thus leading to formation of similar sequential population patterns both spontaneously and during stimulation (although different stimuli produce slightly different variations of that sequential pattern; Luczak et al., 2013). The results presented here are consistent with these ideas, and we suggest that repeated stimulation may induce stimulus-specific changes in the underlying neuronal connectivity, especially when stimuli are presented in desynchronized brain states. We speculate that these neuroanatomical changes could be the reason why spontaneous activity, which propagates through the same cortical circuits as evoked activity, becomes more similar to previously presented evoked patterns.

We also speculate that the reverbatory activity described here may relate to memory formation in behaving animals. Although the mechanisms underlying memory formation processes are still not well understood, there is a body of theoretical work going back to Hebb (1949) and Marr (1971) that predicts reverberation (Hebb) and/or reactivation (Marr) as fundamental components of memory consolidation. Such phenomena have since been observed in the hippocampus and cortex of behaving animals (Euston et al., 2007; Wilson and McNaughton, 1994). These observations, like ours, are consistent with the theory but do not demonstrate that memory depends on this replay. However, more recent evidence suggests a direct link between replay and memory. In hippocampus, the reverberation (reactivation) is associated with SPWR events, and studies have now shown that memory is impaired when SPWRs are disrupted immediately following training (Girardeau et al., 2009; Ego-Stengel and Wilson, 2010). Furthermore, there are individual differences in reactivation and memory performance, and these are correlated (Gerrard et al., 2008). These data suggest that the replay of task-related activity is involved in memory processes. Note also that our experiments follow the same general design as "classic" reactivation experiments (Wilson and McNaughton, 1994). We have a control period before an experience, a repetitive experience, followed by a test period. We show that the activity in the test period resembles the activity in the repetitive experience after controlling for any pre-existing similarity. The only difference is that the animal is not actually behaving but rather under anesthesia. By the fundamental definition of memory as a recapitulation of neural activity evoked by an experience, this is memory. Thus, we suggest that replay of stimulus-evoked patterns observed in desynchronized brain states in urethane-anesthetized rats could be a useful model for studying mechanisms of memory.

EXPERIMENTAL PROCEDURES

Surgery and Recording

We used surgery and recording procedures that have been previously described in detail (Luczak et al., 2007; Schjetnan and Luczak, 2011). Briefly, for somatosensory experiments, 11 Long Evans rats (400–900 g) were anesthetized with urethane (1.5g/kg intraperitoneally [i.p.]). Rats were then placed in a stereotaxic frame, and a window in the skull was prepared over primary somatosensory cortex (S1) hindlimb area (anteroposterior 1 mm; mediolateral 2 mm; dorsoventral 1.5 mm). For auditory experiments, eight Long Evans rats (250–350 g) were anesthetized with urethane (1.5g/kg i.p.) and placed in a nasal restraint that left the ears free. A window in the skull (2 × 3 mm) was prepared over the primary auditory cortex (Luczak et al., 2007; Marguet and Harris, 2011). For all recordings, we used silicon probes consisting of eight shanks (200 μm shank separation): each shank had four recording sites in a tetrode configuration (20 μm separation between sites; 160 μm^2 site area; 1–3 MOhm impedance; NeuroNexus Technologies; see Supplemental Experimental Procedures for recording details). The locations of the recording sites were determined to be layer five in S1 and in A1 based on histological reconstruction of the electrode tracks (Figure S1), electrode depth, and firing patterns. Desynchronization of brain state in the urethane auditory experiments was induced by applying (1) 30 s to 1 min of pressure to the base of the tail (tail pinch; n = 2), repeated 5–10 times in a 40 min period (Marguet and Harris, 2011) or (2) by the application of 2 μl of carbachol (10 μg/μl; n = 6) at a rate of 0.5 μl/min infused through a guide cannula (30G) implanted into the right posterior hypothalamic nucleus (Figure S1A; Bland et al., 1994). Every 5–10 min over 40 min of that experimental condition, an additional 1 μl of carbachol was infused to prevent reoccurrence of synchronized brain state. After tail pinch or carbachol activation, animals were injected with amphetamine (1 mg/kg d-methamphetamine HCl [Sigma] dissolved in the sterile saline at a concentration of 10 mg/3 ml i.p.), and after waiting 20 min for the effect of amphetamine to stabilize, we recorded 40 min of neuronal activity. Then, rats were injected with an NMDA antagonist (MK801; 0.1 mg/kg i.p.), and after waiting 20–30 min for drug effects to stabilize, we again recorded for 40 min. During each experimental condition, we recorded 10 min of spontaneous activity, followed by 20 min of stimulation, followed by 10 min of spontaneous activity (see details in sections below and in Figures 1 and 5).

The experimental procedures for the awake, head-fixed experiment have been previously described (Luczak et al., 2009). Briefly, a headpost was implanted on the skull of the animal under ketamine-xylazine anesthesia, and a crainiotomy was performed above the auditory cortex and covered with wax and dental acrylic. After recovery, the animal was trained for 6–8 days to remain motionless in the restraining apparatus. On the day of the surgery, the animal was briefly anesthetized with isoflurane, the dura was resected, and, after recovery period, recording began. Only experiments where the animal stayed motionless for at least 1 hr, indicated by stable, clusterable units, were included in this study (three/seven rats). All experiments were carried out in accordance with protocols approved by the University of Lethbridge Animal Welfare Committee and the Rutgers University Animal Care and Use Committee and conformed to NIH Guidelines on the Care and Use of Laboratory Animals.

Tactile Stimulation

The time course of the experimental protocol is illustrated in Figures 1A and 1B. It consisted of two 40 min periods: in the first period, the rat was only under urethane anesthesia, and in the second period, the animal was additionally injected with amphetamine (1 mg/kg). Each recording period consisted of 10 min of spontaneous activity, followed by 20 min of tactile stimulation, and then another 10 min of spontaneous activity. The tactile stimulation consisted of 600 repetitions of 1 s stimulation at 20 Hz followed by 1 s without stimulation. The tactile stimulator consisted of a plastic rod attached at one end to a membrane of a speaker controlled by a computer. The other end of the rod was placed in contact with left hind limb.

Auditory Stimulation

For auditory stimulation in anesthetized animals, the time course of experimental protocol was similar to that for tactile experiments in S1, and it is illustrated in Figures 5A–5D. After 10 min of recording spontaneous activity, tones were presented for 0.5 s interspersed with 1 s of silence. This timing allowed for more off-to-on transitions of tones, which evoked the greatest response than would be possible with the same period using tones of 1 s duration. Thus, 800 repetitions of tone stimuli were presented in the 20 min stimulation period. For each experimental condition, we used a different tone frequency during stimulation (urethane only: 1 kHz; tail pinch or carbachol: 1.5 kHz; amphetamine: 2.2 kHz; MK801: 3.2 kHz). For experiments with awake, head-restrained rats, auditory stimulation was presented for over 40 min in each animal. The pattern of stimulation consisted of repetitions of tones for 1 s followed by 1 s of silence. Activity occurring 200 ms after stimulus offset and before the next stimulus onset was regarded as spontaneous. Stimuli consisted of pure tones tapered at the beginning and the end with a 5 ms cosine window. In data sets from awake animals, we did not have extended spontaneous periods preceding or following stimulation period. Experiments took place in a single-walled sound isolation chamber (IAC) with tones presented free-field (RP2/ES1, Tucker-Davis).

Latency

In order to quantify temporal relations among neurons, we calculated the mean spike latency as described previously (Luczak et al., 2009). Briefly, for each neuron, latency is defined as the center of mass of a cross-correlogram of that neuron with the summed activity of all other simultaneously recorded cells (multiunit activity [MUA]) within a time window of 100 ms (Figure 2A). Before calculating the center of mass, cross-correlograms were smoothed with a Gaussian kernel with SD = 5 ms and normalized between zero and one to discard effects of baseline activity. Thus, this measure estimates the time when the corresponding neuron is most likely to fire with respect to the population activity. In addition to analysis of cross-correlograms between single neurons and multiunit activity as described above, we also calculated latency from pair-wise cross-correlograms to look at temporal relations between neurons in more details (Figures 2E, white bars, and 6F–6O). To validate performance of latency measure on noisy data, we conducted analyses on simulated data (Figure S2). We also confirmed latency measure stability over time (Figures S3A–S3F).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and six figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2013.06.013.

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