Isolated cortical computations during delta waves support memory consolidation

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Delta waves have been described as periods of generalized silence across the cortex, and their alternation with periods of endogenous activity results in the slow oscillation of slow-wave sleep. Despite evidence that delta waves are instrumental for memory consolidation, their specific role in reshaping cortical functional circuits remains puzzling. In a rat model, we found that delta waves are not periods of complete silence and that the residual activity is not mere neuronal noise. Instead, cortical cells involved in learning a spatial memory task subsequently formed cell assemblies during delta waves in response to transient reactivation of hippocampal ensembles during ripples. This process occurred selectively during endogenous or induced memory consolidation. Thus, delta waves represent isolated cortical computations tightly related to ongoing information processing underlying memory consolidation.

Most of our time spent asleep is dominated by slow oscillations (0.1 to 1 Hz), when cortical neurons synchronously alternate between a depolarized (up) state associated with high levels of endogenous activity and a hyperpolarized (down) state when neurons remain silent (1). Delta waves are large deflections of the local field potential (LFP) that correspond to the down states of the slow oscillation and are thus considered periods of generalized cortical silence. The slow oscillation plays a causal role in memory consolidation (2–5), in particular by orchestrating an information flow between the hippocampus and the neocortex (6). Indeed, delta waves tend to occur in close temporal proximity to hippocampal ripples (7), which are instrumental for memory consolidation (8, 9). Hippocampal replay of awake activity (10), biased by inputs from sensory cortices (11, 12), initiates reactivation of prefrontal cortical cell assemblies (13, 14) just before the occurrence of a delta wave (7). Cortical synaptic plasticity subsequently takes place during network reorganization early in the following up state (15, 16) and during the massive calcium entry accompanying the ensuing sleep spindle (17–19). This hippocampal-cortical dialogue (20–22) is instrumental for memory consolidation (5). However, the incursion of generalized silence (delta wave) precisely between periods of information exchange and periods of network plasticity remains puzzling.

We recorded prefrontal cortical activity in nine rats during slow-wave sleep (5). Consistent with previous reports, most delta waves were accompanied by neuronal silence. Yet, occasionally, spikes did occur during delta waves (Fig. 1A), and when considering cumulative spiking activity over all recorded delta waves, unexpected residual activity appeared at the peak of the waves (Fig. 1, B and C) (spike waveforms recorded during delta waves were not distinguishable from spike waveforms accompanying the ensuing sleep spindle (17–19), which are instrumental for memory consolidation (7)).

Fig. 1. Delta spikes. (A) Example of a nonsilent delta wave. Colored curves indicate LFPs recorded from the medial prefrontal cortex (color: recording channel). Colored vertical ticks indicate spikes emitted by simultaneously recorded prefrontal units (color: channel from which the unit was recorded). Dashed lines indicate the beginning and end of delta waves. A delta spike (black circle) occurs during the second delta wave, when the rest of the network remains silent. Black calibration bars: 0.5 s (horizontal); 1 mV (vertical). mPFC, medial prefrontal cortex. (B) Mean peri-event time histogram of the normalized firing rate of prefrontal units centered on delta waves (top curve: mean field event). The dashed white line indicates residual activity during delta waves. (C) Time distribution of the spikes emitted by each prefrontal neuron closest to each delta wave. The large peak at ~100 ms corresponds with activity in the up state. The smaller peak consists of spikes occurring during delta waves. The dashed line indicates the 15-ms upper threshold used to define delta spikes in subsequent analyses (all results were confirmed by using ±30-ms time windows).

(D) (Left) Number of units that discharged in a given proportion of delta waves (gray curve: log-normal fit with the same mean and variance as the data; error bars: 95% confidence intervals from bootstrapped data). No unit fired in 0% of the delta waves. (Right) Number of delta waves containing a given number of delta spikes. (E) No difference in average waveforms between silent (black: n = 101,161) and nonsilent (blue: n = 12,205) delta waves (Monte Carlo test, P > 0.05).

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recorded outside delta waves; fig. S1). On closer examination, neuronal activity occurred consistently in a substantial fraction of delta waves (12%), wherein one or a few neurons remained active while the rest of the population became silent (Fig. 1D). We call this unexpected persisting activity “delta spikes.”

To investigate whether delta spikes were restricted to a particular subset of neurons, we counted the number of delta waves in which each unit emitted one or more spikes. As it happened, every single recorded unit fired during delta waves, suggesting instead that persisting firing may actually constitute a widespread phenomenon (Fig. 1D and fig. S2).

We then wondered whether delta spikes tended to occur in specific delta waves with distinctive characteristics. We thus compared delta waves in which we did or did not detect cortical spikes and found no significant difference between the two groups in terms of waveform (Fig. 1E), duration, timing (fig. S3), depth (fig. S4), decreased gamma power, or coupling with hippocampal ripples and thalamocortical spindles (fig. S5). This suggests that spikes could take place during virtually all delta waves but may remain undetected given the limited number of recorded neurons relative to the entire population (fig. S6). We thus hypothesize that firing during delta waves might be an overlooked phenomenon manifested in possibly all delta waves.

These findings indicate that during any given delta wave, the cortical network becomes silent except for a small but ever-changing minority of cells. The most parsimonious explanation would be that delta spikes constitute random activity reflecting imperfect coordination in the cortical alternation between the up and down states. Yet, an alternative possibility is that this activity serves a well-defined computational function. A hallmark of cortical computation is the emergence of cell assemblies. We thus tested for the presence of recurring coactive cell ensembles, using two complementary approaches. As a first approach, we performed a standard independent component analysis (23), which identified multiple significant components that were active during delta waves (fig. S7, A and B). However, these components were likely to

Fig. 2. Hippocampal ripple activity predicts delta spikes. (A) Delta spikes and preceding hippocampal activity. Blue traces indicate LFPs from the medial prefrontal cortex. Colored ticks indicate simultaneously recorded prefrontal (blue) and hippocampal (red) spikes. Black circles indicate delta spikes emitted within ±15 ms of the delta wave peak (shaded area). In the first two delta waves, delta waves were recorded from the same unit after similar hippocampal activity patterns. HPC, hippocampus. (B) Cross-correlations (curves and shaded areas, mean ± SEM; orange, data; gray, time-shifted control) between hippocampal ripple activity (sliding window) and delta spikes (fixed, 0 s). The horizontal orange line indicates the Monte Carlo test: \( P < 0.05 \), corr., correlation. (C) Enrichment in positive correlations [comparative distribution between data and control in (B)] when hippocampal activity was correlated with subsequent prefrontal delta spikes. (D) Performance of a GLM trained to predict prefrontal activity during delta waves on the basis of preceding hippocampal ripple activity (200-ms window). Delta spikes as well as delta components were significantly predicted by multiple single-unit hippocampal activity (\( P = 0.0403 \) for delta spikes, \( P = 0.0052 \) for delta components; Wilcoxon rank sum test) but not by global hippocampal drive ignoring cell identity (summed activity, \( P = 0.2597 \) for delta spikes and \( P = 0.3258 \) for delta components; Wilcoxon rank sum test). **\( P < 0.01 \); ***\( P < 0.001 \), (Wilcoxon signed-rank tests). (E) Object responsivity index for partner (green) versus other (gray) prefrontal units (curves: cumulative distributions; inset: mean ± SEM). Only partner prefrontal units showed positive object responsivity (partner units, \( P = 0.0162 \); other units, \( P = 0.5967 \); Wilcoxon signed-rank test; partner versus other units, \( P = 0.0465 \); Wilcoxon rank sum test). *\( P < 0.05 \).
combine multiple smaller but overlapping cell ensembles, given the limited number of neurons active in any given delta wave. We thus performed a second analysis to examine cooperative activity (“peer prediction”) (24) among delta spikes, an idiosyncratic property of cell assemblies. This showed that the delta spikes of one neuron could be predicted from the delta spikes of other neurons (fig. S7C).

We then asked whether delta spikes were involved in the hippocampo-cortical dialogue underlying memory consolidation. Because delta waves typically take place precisely between hippocampal replay and cortical reorganization for memory consolidation, this hypothesis would be expected to have two implications: (i) Hippocampal activity during ripples should predict which neurons (or which assemblies) are active during the following delta wave, and (ii) this predictive bias should emerge after behavior, and predictable cortical cells should be involved in the reactivation of waking experience.

Rats were trained on a spatial memory task, and hippocampal and cortical activity was recorded during both behavior and subsequent memory consolidation during the first 2 hours.

Fig. 3. Delta waves isolate cortical computations. (A) Cross-correlations (curves and shaded areas, mean ± SEM) between hippocampal ripple activity (fixed, 0 s) and prefrontal activity (sliding window). Observed cross-correlations (orange) were significantly different from a time-shifted control (gray) for cortical activity after ripples (horizontal orange line: Monte Carlo test, P < 0.05). Delta waves (dashed line; peak occurrence rate 130 ms after ripples) tend to occur within the critical window when prefrontal activity remains correlated with the preceding ripple activity. (B) Simultaneous recording of prefrontal and hippocampal activity around a delta wave (gray-shaded rectangle). (Top) Proportion of prefrontal spikes predicted by the firing of hippocampal cells (partner spikes). (Center) Raster plot of spikes emitted by 68 simultaneously recorded prefrontal units (red ticks: partner spikes; gray ticks: other spikes). (Bottom) Simultaneously recorded LFPs in the mPFC (blue: delta wave) and hippocampus (broadband and ripple-band filtered signal; blue: ripples). During delta waves, partner spikes occurred in isolation (red ellipse). Partner spikes emitted by the same units outside delta waves (gray ellipses) formed a considerably smaller proportion of the ongoing cortical activity. Black calibration bar: 0.5 s. Filt, filtered. (C) Signal-to-noise ratio (curves and shaded areas, mean ± SEM) of partner spikes relative to other spikes around delta waves. Observed values (blue) were significantly different from a time-shifted control (gray) during delta waves (horizontal blue line: Monte Carlo test, P < 0.05).
of sleep (5). Hippocampal spiking activity during ripples was significantly correlated with cortical delta spikes that occurred immediately (50 to 200 ms) afterward (the effect was not due to data recorded from any single rat) (Fig. 2, A and B, and fig. S8). This increased correlation was due to a large proportion of positively correlated interregional pairs of neurons (Fig. 2C). Furthermore, ripple spikes were better correlated with delta spikes than with spikes occurring at similar delays during an up state (correlations were greater in the presence of a delta wave) (fig. S9). A generalized linear model (GLM) analysis showed that ensemble activity in the hippocampus

Fig. 4. Induction of memory consolidation by isolation of partner spikes. (A) (Left) Experimental protocol. Delta waves were induced by brief single-pulse electrical stimulation of deep cortical layers. Induced delta waves were triggered to isolate either partner activity (coupled stimulation; green, 130 ms after ripples) or other cortical activity (delayed stimulation; purple, 290 to 370 ms after ripples) during sleep after limited training on a spatial object-recognition task. (Right) Object discrimination index during the recall phase. Only delta waves triggered to isolate partner activity (coupled stimulation) resulted in memory consolidation and enhanced task performance. (B) Performance of a GLM trained to predict delta spikes on the basis of preceding hippocampal activity (200-ms window), measured as percent improvement relative to a shuffled control (prediction gain). Only delta waves triggered to isolate partner activity resulted in a significant prediction of delta spikes ($P = 0.0030$ for isolation of partner spikes by coupled stimulation; $P = 0.1301$ for isolation of other spikes by delayed stimulation; Wilcoxon rank sum test). **$P < 0.01$. (C) Cross-correlation (curves and shaded areas, mean ± SEM) of hippocampal activity and delta spikes (green: isolation of partner spikes by coupled stimulation; purple: isolation of other spikes by delayed stimulation; gray: time-shifted control; horizontal green line: Monte Carlo test, $P < 0.05$). (D) Enrichment in positive correlations upon isolation of partner spikes by coupled stimulation (top) but not of other spikes by delayed stimulation (bottom).
could significantly predict which cortical cells would emit delta spikes (Fig. 2D). In contrast, delta spikes could not be predicted from the combined activity of all hippocampal units that ignored cell identity (multunit activity), ruling out the possibility that delta spikes merely reflect the overall level of hippocampal excitatory drive during ripples. Finally, ripples facilitated (but did not entirely control) the formation of delta assemblies (Fig. S7).

Furthermore, the same GLM analysis applied to hippocampal and cortical ensembles showed that hippocampal activity could even predict the behavioral correlates of the prefrontal units (Fig. 2E). The delta components (Fig. 2D). We thus sought to confirm the prediction that these delta waves actually isolated partner spikes (that delta spikes did occur during induced delta waves and that they were predicted by hippocampal activity). Similar to our observations in natural sleep (above), stimulation-induced delta waves did feature spiking activity (Fig. S15), and these delta spikes were predicted by preceding hippocampal activity coinciding with the timing of ripples (Fig. 4, B to D). In contrast, slightly delaying the induction of delta waves (by ~200 ms) (Fig. 3A) to isolate nonpartner delta spikes (Fig. 4, B to D) failed to induce memory consolidation (S).

Our results challenge the generally accepted tenet that delta waves, reflecting the down states of the sleep slow oscillation, are periods of complete cortical silence (1, 6, 25), to the point that they have sometimes been defined as such (26, 27) and that occasional spikes have been routinely ignored when detected (28, 29). We focused on delta spikes and found that they are not neuronal noise due to imperfect silencing of the cortical mantle. On the contrary, they constitute a common phenomenon potentially implicating all neurons and all delta waves, and they reflect genuine processing involved in memory consolidation.

This observation also provides a mechanism for the documented but puzzling role of delta waves in memory consolidation: Synchronized silence across most of the cortex isolates the network from competing inputs while a select subpopulation of neurons maintains relevant spike patterns between episodes of hippocampo-cortical information transfer (10, 12, 14) and epochs of cortical plasticity (15, 16) and network reorganization (5, 18, 19).

Yet, in many cases, cortical activity during delta waves could not be reliably predicted from the preceding hippocampal ripple activity. Such cortical activity could instead have been related to interactions with other brain networks. This suggests that delta spikes and assemblies might constitute a general mechanism of isolated cortical computation beyond the hippocampo-cortical dialogue.

**REFERENCES AND NOTES**


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**SUPPLEMENTARY MATERIALS**

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Materials and Methods

Fig. S1 to S15

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Special moments at cortical quiet states
Delta waves are moments of widespread cortical silence that alternate with active states during slow-wave sleep. However, upon closer examination, single neuronal action potentials can be detected during delta waves. Todorova and Zugaro sought to determine whether this neuronal noise could instead be an important signal (see the Perspective by Ikegaya and Matsumoto). They found that persisting action potential firing during delta waves is an overlooked but widespread phenomenon, which could potentially involve all neurons and all delta waves. A critical role of the delta wave may be to insulate specific cortical computations taking place in response to hippocampal replay and involved in memory consolidation.

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