

mechanisms for O=O bond formation. In 2016, a study reported a room-temperature structure of PSII following two light flashes, hence enriched in the S_3 state of the OEC (11). It reported no new S_3 -state electron density around Mn1, as one would expect for a newly bound water. This result directly conflicts with the proposed Siegbahn model and EPR studies. Shortly following this study, another group reported an S_3 structure with new electron density near Mn1 and O5 that was assigned to a newly bound oxygen (O6) (12). This was considered support for the S_3 -insertion substrate water model. However, the study showed close proximity (0.15 nm) between O5 and O6, consistent with O-O bond formation producing a peroxide at the S_3 state, which would in turn be associated with Mn reduction in the S_2 -to- S_3 transition. This is in conflict with EPR and x-ray spectroscopic data (3, 9, 14, 15). A more recent study reports a new oxygen “Ox” bound both to Mn1 and the Ca ion of the OEC (13), and with a 0.21 nm distance to O5, much longer than the reported 0.15 nm (12) and too long to represent the O-O bond of a peroxide.

Suga *et al.* have now cryotrapped serially flashed microcrystals of PSII with the goal of achieving more accurate interatomic distances, especially between O5 and O6. In the S_2 state, they observed an open cubane structure with a five-coordinate Mn1. At the S_3 state, a flip in the side chain of the monodentate carboxylate ligand Glu¹⁸⁹ (12) provides room for O6 to insert and bind to Mn1 and Ca. To address the crucial O5-O6 distance, Suga *et al.* calculated difference maps

as a function of modeled O5-O6 distance and found the smallest residual density at a distance of about 0.19 nm. Their full residual density analysis suggests an oxyl/oxo pairing for O5 and O6, which they consider evidence for an oxyl/oxo coupling mechanism in the O=O bond formation at the final S_4 state. Glu¹⁸⁹ appears to gate water insertion into the OEC from the “O1” water channel of the PSII reaction center.

Although there are still open questions as to the detailed assignments of substrate waters and the exact modes of water oxidation and O=O bond formation, the results from different studies are starting to converge, and the structural results appear to be closely in line with mechanistic proposals supported by computational chemistry and spectroscopy. The field anxiously awaits possible time-resolved structures detailing the O=O bond formation through the S_4 -to- S_0 transition. ■

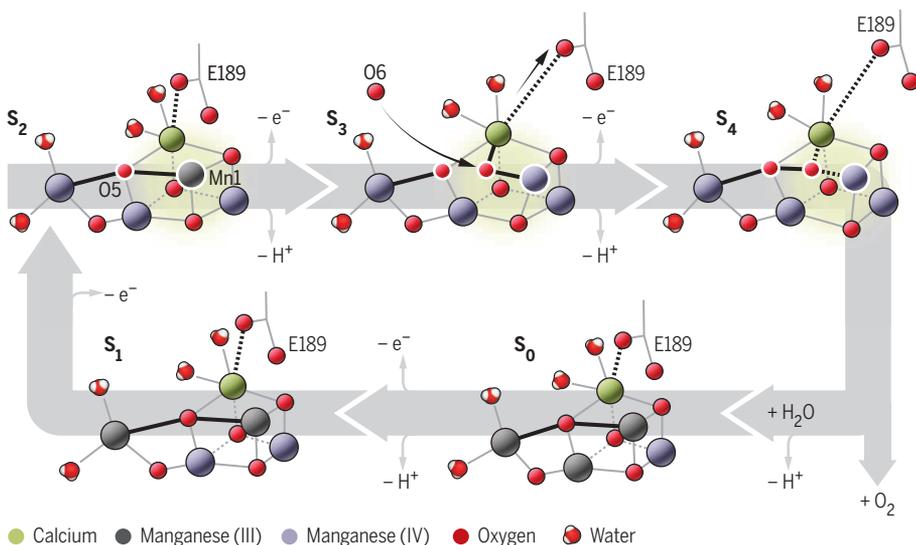
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The S states in the oxygen-evolution reaction

The oxygen-evolving complex is photo-oxidized through a series of S states to produce molecular oxygen from water. In the final steps before O=O bond formation, a new oxygen, O6, binds to the vacant site at Mn1. After a final photo-oxidation event, O5 and O6 appear poised to form an O=O bond, releasing molecular oxygen, reducing the cluster, and beginning the catalytic cycle anew. Glutamic acid at position 189 is noted as E189.



NEUROSCIENCE

Spikes in the sleeping brain

Memory is replayed and consolidated under low background noise during deep sleep

By Yuji Ikegaya^{1,2} and Nobuyoshi Matsumoto¹

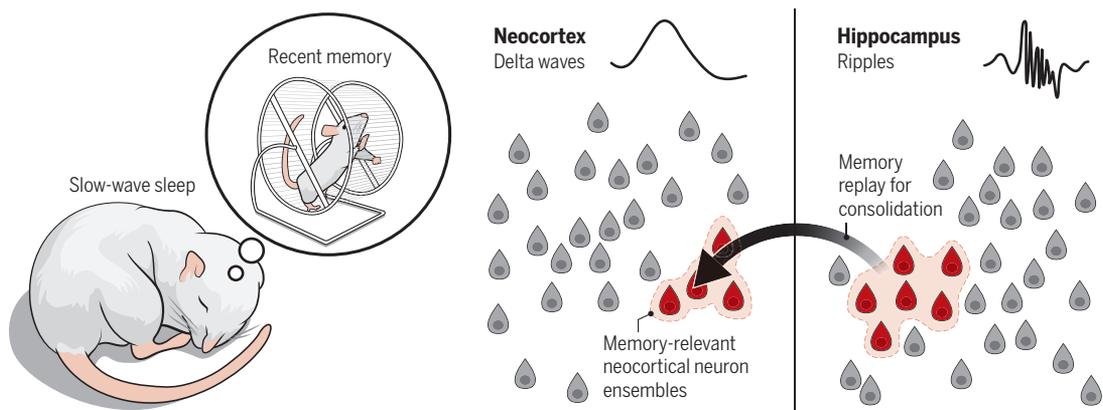
Memories of recent experiences are transferred and fixed in long-term storage in the neocortex during sleep. A key aspect of this process is the occurrence of ripples, which are high-frequency oscillations in neuronal activity in the hippocampus (1, 2). Ensembles of hippocampal neurons that have been activated during an experience emit synchronized neuronal activity (spikes) during ripples in subsequent sleep (3). These “replayed” memories propagate to the neocortex during slow-wave states (4), which are characterized by alternating “bright” states with active neurons and “dark” states, called delta waves, without active neurons (5). Researchers reasoned that hippocampal-cortical dialogue occurs during the bright states and that delta waves represent intermittent neocortical “sleep” to recover from synaptic fatigue (6) or increased potassium conductance (7) and have no active function. On page 377 of this issue, Todorova and Zugaro (8) challenge this view, demonstrating that a small number of neurons are reactivated during delta waves.

Todorova and Zugaro recorded activity, both spikes from multiple neurons and local field potentials, simultaneously in the rat dorsal hippocampus and the medial prefrontal cortex, which is believed to store long-term memories. They reconfirmed that the prefrontal cortex becomes inactive during each delta-wave epoch but also discovered that a few neurons remain active and occasionally emit synchronous spikes during delta waves (which they called “delta spikes”) (see the figure). The neurons recorded in this study constitute only a small portion of the total cells in the prefrontal cortex. Thus, it is important to ask how frequently delta spikes occur. The local field

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Hippocampal-cortical dialogues during sleep

The hippocampus communicates with the neocortex and activates experience-associated neocortical neuron ensembles during delta waves, low-cerebral activity states that emerge intermittently during slow-wave sleep. This selective activity focuses memory consolidation.



potential waveforms, which reflect the synchronous activity of a group of neurons, do not differ depending on whether delta waves are accompanied by delta spikes in the recorded neurons. Moreover, the probability of detecting delta spikes is proportional to the number of simultaneously recorded neurons. Extrapolating these observations suggests that delta spikes accompany virtually all delta waves.

Delta spikes are not shots in the dark or remnants of ripples that accidentally fail to disappear during delta waves. Rather, delta spikes occur in spatiotemporally designed, specifically recursive configurations and seem to be actively generated in the brain. Todorova and Zugaro annotated delta spikes as behavioral correlates. As rats were trained in a spatial memory task, behaviorally relevant neurons became preferentially activated in delta waves during the following sleep period. The neuronal replay in the prefrontal cortex appears to be instructed by hippocampal ripples, because the set of prefrontal neurons that emit a delta spike in a given delta wave can be statistically predicted from the set of hippocampal neurons activated in the ripple immediately preceding the delta wave. The spike correlations of cell pairs between the hippocampus and the medial prefrontal cortex remain high for hundreds of milliseconds, indicating that hippocampal-cortical dialogue occurs within a short time frame, during which the neocortex serves as a temporary reservoir of hippocampal information. As a result, memory-relevant delta spikes are fired mainly in the early phase of each delta-wave event.

Delta spikes consist mainly of behaviorally relevant neurons, whereas behaviorally irrelevant neurons are more likely silenced during delta waves. Reducing irrelevant

information improves the signal-to-noise ratio of neuronal information and thereby helps isolate cortical computations to avoid deleterious cross-talk. That is, the neocortex quiets down during delta waves and focuses attention on the content to be memorized, consistent with the authors' previous prediction that delta waves "isolate target synapses from competing inputs, allowing selective reorganization of the network" (9). This leads to the possibility that facilitating delta spikes in ripple-associated neurons could enhance memory consolidation. Consistent with this idea, task performance in mice is enhanced when artificial delta waves containing ripple-coupled delta spikes are repeatedly induced using brief single-pulse electrical stimulation of deep cortical layers during sleep after an object-discrimination task. This suggests that delta spikes mediate memory consolidation.

"...task performance in mice is enhanced when artificial delta waves...are repeatedly induced..."

How do delta spikes contribute to memory consolidation? It is unknown what delta waves give rise to in prefrontal neuronal circuits. Delta spikes are transient and thus are unlikely to work as persistent memories by themselves. So, what form of long-lasting plasticity is induced by delta spikes? Moreover, activity patterns in individual task-associated neocortical neurons change over a period of days after learning (10). Do delta spikes also have an instructive role in the dynamic reorganization of neuronal identities? Another line of evidence suggests that delta waves are involved in forgetting rather than memory consolidation (11). Thus, drifting delta spikes could contribute to deactivation of memory traces.

Another question is how the dorsal hippocampus and the medial prefrontal cortex communicate during slow-wave sleep. Direct synaptic connections are unlikely

to exist between these brain regions. On the basis of anatomical evidence of axonal projections, candidates for the relay station include the ventral hippocampus (12), a neocortical area via the entorhinal cortex (13), and the retrosplenial cortex via the subiculum (14). Recording or ablation of neuronal activity in these brain regions is necessary to answer this question.

Delta waves are a hallmark of the entire neocortex during sleep, and delta spikes may occur in cortical regions other than the medial prefrontal cortex. Do delta spikes have a role in processes other than memory consolidation? Todorova and Zugaro have successfully linked their unexpected discovery to the current knowledge of memory and sleep, but future investigations are still required to uncover the precise mechanisms and functions of delta spikes. ■

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