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Window to the soul: Pupil dynamics unveil the consolidation of recent and remote memories

Memory enables organisms to encode, store, and retrieve information essential for interacting with and adapting to a dynamic environment. As an internal representation of the external world, memory serves as a crucial bridge between past experiences and future behaviors. However, the brain continuously forms new memories, raising the question of how new memories are integrated without disrupting previously formed ones. This challenge, termed “catastrophic forgetting” in artificial neural networks (González et al., 2020; McNaughton, 2010), arises when new information overwrites existing memories. In contrast, biological systems exhibit remarkable resilience, enabling continual learning without significant loss of prior knowledge. However, the mechanisms by which biological systems prevent catastrophic forgetting are still not fully understood. Evidence suggests that newly acquired memories are initially encoded and consolidated in the hippocampus before becoming stabilized in the cortex. A widely accepted mechanism for this process involves the repeated reactivation, or replay, of neural activity associated with prior experiences during sharp-wave ripples (SWRs), synchronous hippocampal events that primarily occur during non-rapid eye movement (NREM) sleep (Tang & Jadhav, 2022). Despite occupying only a small fraction of SWRs, memory replay includes both recent and remote experiences (Karlsson & Frank, 2009). Given NREM sleep has long been considered a homogeneous process in rodents, it has been hypothesized that the replay of new and old memories is distributed randomly throughout this sleep state (Saxena et al., 2022).

Notably, in humans, NREM sleep comprises multiple distinct stages, rather than functioning as a homogeneous state. Inspired by this phenomenon, in a recent work Chang et al. (2025) proposed an alternative solution, that there may be an undiscovered microstructure within NREM sleep, composed of discrete substates that regulate memory processing (Figure 1). Specifically, the authors proposed that recent and remote memories are reactivated within distinct substates, with certain substates facilitating the early consolidation of newly acquired information, while others prioritize the replay of remote memories. To explore this hypothesis, the authors developed an innovative experimental framework integrating electrophysiology recordings, closed-loop optogenetics, and pupillometry to precisely track brain-state dynamics in naturally sleeping mice.

Continuous recordings of pupil dynamics during NREM sleep revealed cyclic fluctuations in pupil size, characterized by rhythmic dilation and constriction with a cycle of approximately one minute. To investigate whether these oscillatory patterns were functionally linked to memory processing, the authors examined the probability of task-related memory replay during SWRs and its relationship with pupil size. The authors found a significantly negative correlation between replay probability and pupil size, suggesting that smaller pupil substates are primarily associated with the replay of recently acquired task-related experiences.

To establish a causal link between pupil size-associated sleep substates and memory replay, the authors applied a closed-loop optogenetic paradigm. This approach involved real-time detection of SWRs, which then triggered an automated feedback system to deliver light stimulation for opsin activation, enabling precise manipulation of neural activity with high temporal resolution (Fernandez-Ruiz et al., 2022). Previous studies have demonstrated that indiscriminate disruption of all SWRs during NREM sleep impairs memory consolidation and subsequent retrieval behavior (Fernández-Ruiz et al., 2019; Girardeau et al., 2009; Oliva et al., 2020). Building on this framework, the authors developed a pupil size-guided SWR closed-loop disruption system, integrating online pupil size detection with targeted SWR interference, enabling the detection and disruption of SWRs within specific sleep substates in real time.

To assess memory performance, the authors trained mice on a spatial learning task in which they were required to remember the location of a sucrose reward hidden within one of several holes on a "cheeseboard" maze. Initially, mice exhibited inefficient search patterns, but with repeated trials, they learned to navigate directly to the reward location, indicating successful memory acquisition. Following training, the mice underwent a subsequent two-hour sleep period, during which we selectively disrupted SWRs during small pupil substates while preserving SWRs during large pupil substates. To evaluate the impact of this targeted disruption on memory consolidation, the authors re-exposed mice to the same task and assessed their speed and precision in locating the original reward position. Control mice, in which SWRs were undisrupted, rapidly and accurately identified the reward location, suggesting intact memory retrieval. In contrast, mice subjected to SWRs disruption during small pupil substates exhibited profound memory deficits, failing to recall the reward location. To further test the specificity of this effect, the authors conducted a control experiment in which SWRs were selectively disrupted during large pupil substates while remaining intact in small pupil substates. These mice showed no memory impairment, suggesting that memory consolidation of recent experiences is critically dependent on SWRs occurring within the small pupil substate.

However, a fundamental question remains: do small pupil

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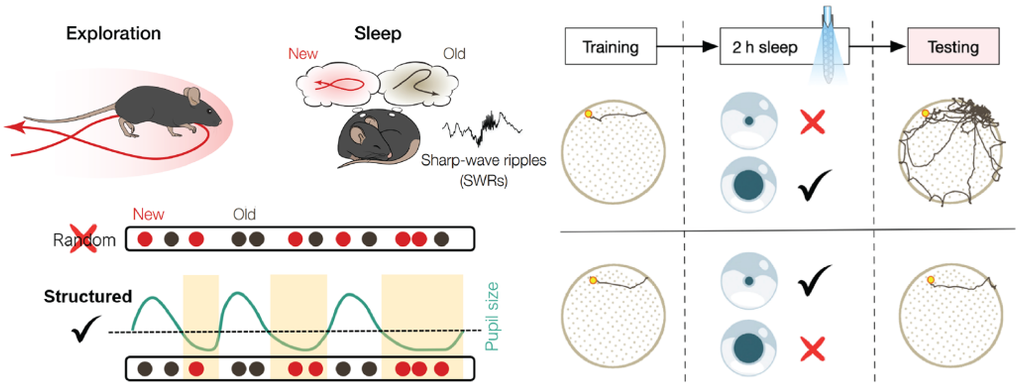

Figure 1 The pupil as a window into memory consolidation

During sleep, both newly acquired, and previously stored memories undergo reactivation. Using pupil-guided closed-loop disruption, the authors selectively disrupted memory consolidation in small pupil states but not in large pupil ones. Right: Representative mouse trajectories during small pupil substate (SWR_{pupilS}; top) and large pupil substate (SWR_{pupilL}; bottom) disruption. Yellow circles indicate reward locations in "cheeseboard" maze. Solid lines represent animal movement trajectories. Results (lower left) demonstrated that memory replay during sleep was organized into substates.

substates selectively consolidate novel memories, or do they facilitate the reactivation of both recent and remote memories? To test this, the authors modified the cheeseboard task to assess memory consolidation for both familiar and newly learned goal locations. Mice were initially trained over several days to learn one reward location (familiar goal) before being introduced to a new reward location in subsequent trials. After training, the mice underwent the same SWRs closed-loop disruption during small pupil substates, and memory recall was assessed for both the original and newly learned targets. These findings demonstrated that disruption of SWRs specifically during small pupil substates selectively impaired the consolidation of the newly acquired goal while leaving the memory of the familiar goal intact. These results suggest that small pupil substates in NREM sleep preferentially support the replay of recent memories. To further elucidate the neural mechanisms underlying the differential processing of new and old memories, we analyzed the reactivation dynamics of cell assemblies associated with each memory type during sleep. Plastic cell assemblies linked to new memories were more frequently reactivated during small pupil substates, while previously established cell assemblies related to remote memories were more often reactivated during large pupil substates. Furthermore, these functionally distinct substates were associated with different circuit dynamics: small pupil substates were characterized by stronger excitatory inputs, whereas large pupil substates were characterized by stronger local circuit inhibition (Figure 1).

In summary, this study (Chang et al., 2025) combined pupil size measurements with large-scale hippocampal neuronal recordings in mice, discovering a fundamental organizational principle of NREM sleep. Contrary to the long-standing view of NREM as a homogeneous state, these findings revealed, for the first time, the existence of a fine microstructure consisting of distinct substates that differentially support the consolidation of new and old memories. These substates regulate the selective reactivation of recent or remote memories within alternating cycles on a timescale of minutes that can be measured by pupil contraction and dilation, indicating shifts between distinct hippocampal network states. While SWRs are present throughout NREM sleep, memory replay is not uniformly distributed. Instead, reactivation of newly acquired experiences more frequently occurs during SWRs in small-pupil substates rather than in large-pupil substates. Notably, pupillometry, a widely used non-invasive technique in human cognitive research, can serve as an indicator for these NREM sleep substates. This discovery opens avenues for developing non-invasive interventions, such as targeted memory reactivation, to enhance memory performance in humans. Moreover, these insights could inform the design of biologically inspired AI algorithms, enabling continual learning while mitigating catastrophic forgetting.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

W.T. and H.C. wrote the draft manuscript. All authors revised, read, and approved the final version of the manuscript.

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