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Learning and Memory: Do Bees Dream?

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In mammals, evidence for memory reactivation during sleep highlighted the important role that sleep plays in memory consolidation. A new study reports that memory reactivation is evolutionarily conserved and can also be found in the honeybee.

In the early days of sleep research, many believed that sleep was a state of relative brain inactivity that occurred passively upon the removal of sensory input [1]. While sufficient evidence had accumulated by the early 1970s to demonstrate that sleep was actively regulated [1,2], questions remained about the extent to which passive or active neuronal processes predominated during sleep. These questions were largely laid to rest following the discovery by Wilson and colleagues that the sequence of hippocampal place cell activity that had been observed during prior waking was replayed during sleep [3]. In many ways, the data showing memory reactivation during sleep was revolutionary. Not only did this seminal study identify an important neuronal process that occurred during sleep, it led to a resurgence in the efforts to better understand the relationship between sleep and memory in general [4]. The possibility that reactivation of waking experience during sleep might improve memories led to a series of elegant human studies in which subjects were presented cues (e.g., odors or sounds) during learning and then again during subsequent sleep to improve memory [5,6]. A new study by Zwaka *et al.* [7] reported in this issue of *Current Biology* has the potential to be equally ground breaking. In their current study, the authors report that memory reactivation during sleep is evolutionarily conserved in the honeybee. These exciting findings have the potential to be as influential as the original report of reactivation in rodents [3] since it suggests that replay/reactivation is conserved throughout the animal kingdom and can be evaluated in organisms that are genetically tractable.

There are a shocking number of similarities between invertebrate and human sleep [8,9]. Surprisingly, while the relationship between sleep and memory has been clearly demonstrated in the fly [10–13], it has remained unclear whether invertebrates are also able to reactivate memories during sleep like humans and rodents.

In the current study, Zwaka *et al.* demonstrate that reactivation of memory during sleep can also exist in the honeybee. The choice to evaluate replay/reactivation during sleep in the bee is very creative. First the bee is known to be quite clever and as a consequence has been a favorite model for memory research for several decades. Moreover, the bee was one of the first invertebrates shown to have a sleep-like state [14]. Most importantly, the bee expresses three different sleep stages that can be identified by behavioral characteristics which can be monitored in real-time. The deepest stage of sleep is associated with complete immobility of the antennae. This is important because, in contrast to other invertebrate models, sleep depth can be identified without having to disturb the animal [15]. Thus, one can monitor sleep depth in real-time and present stimuli during different stages of sleep to test the hypothesis that sleep plays a role in reactivation of experiences acquired during prior waking.

The authors chose to evaluate the behavior of bees during the classical conditioning of the proboscis extension response (PER). In this learning paradigm, an individual honeybee is presented with a sugar reward (unconditioned stimulus, US) that induces PER. They are also presented with a thermal stimulus as a conditioned stimulus (CS). Typically, memory is assessed the following day by presenting the CS to the bees in the absence of the US. This training protocol results in memory (i.e., extension of the proboscis when only the thermal stimulus (CS) is provided). In order to investigate replay/reactivation in honeybees, the authors provided a context odor constantly during training when the bees were awake. The authors then monitored subsequent sleep in individual bees and provided the same context odor to each individual bee when they were in the deepest stage of sleep. Bees that were exposed to the context odor during sleep showed enhanced memory retention the day after training; bees in which the context odor was not provided during deep sleep did not display as robust a memory. To rule out confounding factors, the authors conducted an elegant series of experiments to determine whether the improved memory was context-odor specific or an artifact of odor presentation. They report that presenting bees with an incongruent odor during sleep did not improve memory. Thus, memory consolidation can be improved by presenting the context odor during sleep. These data suggest that the odor reactivates the learned information. Similar results have been identified in humans, in which the stimulus must be presented during the deepest stages of sleep [5].

Together these data show that memory in honeybees can be improved by the presentation of contextual odor cues during sleep. These data are remarkably similar to the data showing hippocampal replay-like processes that have been found in rodents and suggest that they likely operate in bees as well. The reactivation of memory in bees reveals a striking and surprising degree of conservation in the mechanisms of sleep-dependent memory-processing between humans and insects.

Do bees dream? The data suggest that the existence of replay in deep-sleep indicates that bees might have dream-like experiences analogous to non-REM dreams in humans. If true, this would provide novel insights into the evolution of cognition. However, the relationship between cued or spontaneous replay events and dreaming remains nebulous. For example, cue-induced memory reactivations during sleep did not appear to be incorporated into dreams [5,6]. Indeed, a recent review suggests that dreams, particularly in REM sleep, might serve as a 'preplay' of possible future outcomes, rather than a replay of specific past experiences [16]. Regardless, if bees do dream it would certainly provide novel insights into the evolution of dreams.

Will these replay and reactivation phenomena extend beyond honeybees? Elegant genetic studies in *Drosophila* have described the circuitry supporting memory acquisition in exquisite detail [17], fly sleep was demonstrated to cycle through dynamic sleep stages [15], and the proboscis extension reflex is widely utilized including in memory studies [18]. Thus, fly neurobiology appears to be excellently positioned to immediately build on this exciting breakthrough by permitting the direct observation of memory replay [19].

Looking to the future, sleep was recently characterized in *Aplysia* [20], which has long been used as a model for memory research. It would thus certainly be of interest to determine if replay/reactivation processes exist in *Aplysia* as well.

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