SLEEP

Cortical norepinephrine GRABs a seat at the sleep table

A new study shows that infra-slow cortical norepinephrine oscillations shape the micro-structure of sleep and transitions to micro-arousals, wakefulness or rapid eye movement (REM) sleep. Prolonged descending phases of these oscillations promote the occurrence of spindle-rich intermediate sleep, which is involved in memory consolidation.

Juan Facundo Morici and Gabrielle Girardeau

Sleep is a temporary and reversible loss of consciousness and is present throughout the animal kingdom. A common assumption is that for such a vulnerable state to have been preserved throughout evolution, it must sustain functions that are absolutely crucial for the survival of the individual. Indeed, sleep has been shown to be involved in the health and maintenance of most bodily and cognitive functions, including memory. This role of sleep is likely to have co-evolved with safeguards that allow quick arousal in case of danger. Wakefulness, REM and non-REM (NREM) sleep, and the transitions between these brain states, are under the tight control of a complex network of structures and neuromodulatory systems, including noradrenergic neuromodulation controlled by the locus coeruleus (LC)[1]. The norepinephrine (NE) neurotransmission system is crucial for the control of arousal and is the main target of many wake- and sleep-promoting medications[2]. In addition to REM and NREM states, sleep has a fine micro-architecture that includes very brief awakenings interspersed within NREM sleep, called micro-arousals. Excessive micro-arousals that lead to pathological fragmentation of sleep impair memory performance[3], but micro-arousals have also been shown to contribute to the homeostatic maintenance of cortical firing rates[4]. How the micro-architecture of sleep and the fluctuations in arousal levels affect sleep-dependent cognitive processes is still under investigation. In this issue of *Nature Neuroscience*, Kjaerby et al[5]. report correlational and causal evidence that, in mice, LC-mediated NE neurotransmission in the medial prefrontal cortex (mPFC) is involved in shaping sleep micro-architecture and thereby influences memory consolidation.

Historically, LC activity and the resulting NE levels were thought to be high during wakefulness, intermediate or low during NREM sleep and absent during REM sleep[6]. However, as the booming development of increasingly specific genetically encoded fluorescent sensors for neuromodulators[7] has allowed neuromodulatory systems to be dissected with unprecedented precision, a more recent body of work, including the paper by Kjaerby et al[5], has revealed a more complex picture. The LC is indeed completely silent during REM sleep but it shows irregular, burst-like activity during NREM sleep[8], and the overall level of this activity is higher than during quiet wakefulness. But how does the LC activity sustain the NE levels that govern

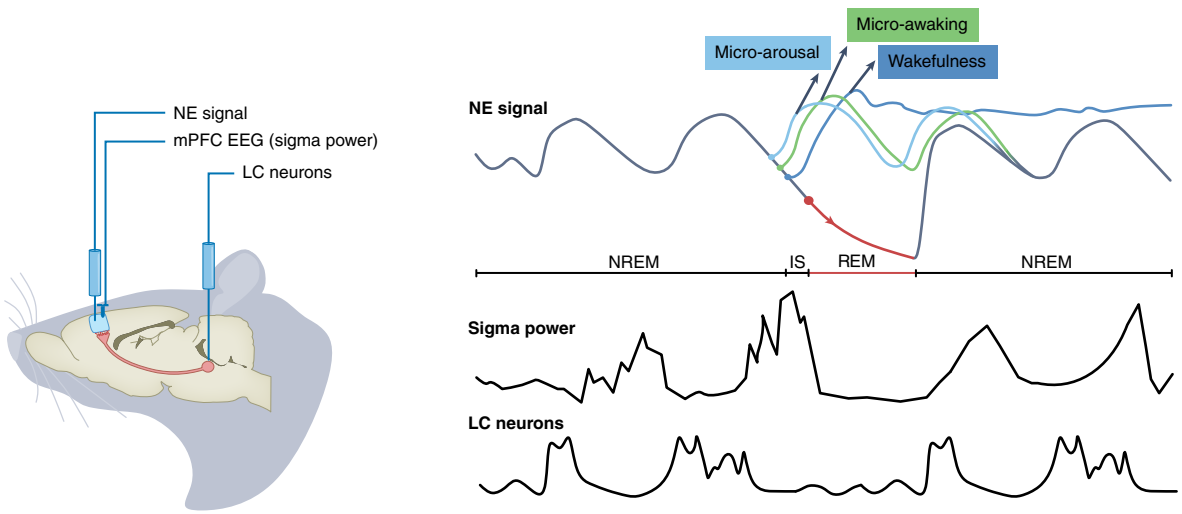

Fig. 1 | Schematic representation of sleep architecture orchestration by NE-mediated mPFC activity. Left, experimental setup combining fiber photometry in the mPFC and LC with EEG recordings to correlate activity of LC noradrenergic neurons with NE level and sigma power in the mPFC during sleep. Right, schematic traces of NE signal in the mPFC, sigma power, and calcium signal in LC neurons. Along the infra-slow NE oscillation, the longer the descent, the higher the arousal in the next state (micro-arousal, micro-awakening or full awakening). Below a certain NE level, NE levels keep decreasing while the brain enters spindle-rich intermediate sleep (IS) with enhanced sigma power and then transitions to REM sleep.

arousal state and sleep micro-structure? Kjaerby et al[5]. performed challenging dual-fiber photometry recordings of both LC neuronal activity and NE levels in the prefrontal cortex, using the NE-specific sensor $GRAB_{NE2m}$. They established that the irregular activity of the LC during NREM sleep underlies infra-slow oscillations of NE levels in the prefrontal cortex, similar to those observed in the thalamus[9].

Importantly, the authors found that these slow (30–50 s) oscillations shape the microarchitecture of sleep. Specifically, the length of the descending phase of the NE oscillation in the mPFC influenced which of four brain states was most likely to follow: a micro-arousal characterized by a drop in sigma power; a micro-arousal characterized by both a drop in sigma power and muscle activity (which could be considered as a micro-awakening); full awakening; or REM sleep (Fig. 1). The longer the descent, the higher the chance of awakening at the beginning of the next oscillatory cycle, when the LC resumes its activity; however, a sustained descent of NE levels past the micro-arousal and awakening 'checkpoints' preceded a transition to REM sleep (Fig. 1). The authors confirmed this observation by applying tones at random times during the mice's sleep in order to trigger NE release: tones applied when NE levels were low were more consistently followed by awakenings, and the subsequent NE rise in the mPFC was larger. This finding is surprising and somewhat at odds with previous reports that external inputs at higher NE levels facilitate awakening[10].

Spindles are waxing and waning 10–15 Hz oscillatory patterns that emerge from local networks within the thalamus and propagate to the neocortex. The density of spindles can be approximated by the overall power in the sigma band calculated from the cortical EEG. Spindles contribute to plasticity and memory consolidation by synchronizing the activity of neurons within the cortex and with other areas such as the hippocampus[11,12]. Kjaerby et al[5]. provide evidence that the oscillations in the levels of NE in the mPFC during NREM sleep are negatively correlated with oscillatory changes in sigma power[13], as previously established for thalamic NE levels[9]. The authors also showed that the prolonged descent in NE levels that leads to REM sleep was associated with a specific increase in sigma power that corresponds to the spindle-rich intermediate sleep that precedes REM episodes[14]. Together, these findings suggest that inputs from the LC to the mPFC orchestrate the transitions from NREM sleep to REM sleep or microarousals and the related variations in sigma power.

If spindles, microarousals and NE levels are all important players in the sleep orchestra, how are they linked to the mnemonic function of sleep? In other words, what are the consequences for memory of disrupting this sequentially organized micro-architecture? To try and answer these questions, Kjaerby et al[5]. used bidirectional optogenetic modulation of the LC using the inhibitory and excitatory opsins Arch and ChR2, respectively. First, they inhibited LC neurons in sleeping mice for 2 min periods. This effectively prolonged the NE descent and increased the chances that the mice would enter a spindle-rich intermediate sleep episode followed by REM sleep. The wake-up rate was also increased upon termination of the stimulation. Because optogenetic inhibition with Arch is known to induce a rebound of activity after termination of the light stimulation, it is unfortunately impossible to determine whether these awakenings are a physiological rebound of the LC activity arising after the brain detects lower NE levels or a consequence of the release of the optogenetic inhibition.

The authors then inhibited the LC during sleep after the mice had been trained on the classical object-recognition learning paradigm. In this task, the mice are exposed to two objects in a familiar environment and, after a delay, they are returned to the same environment in which one object has been replaced with a different one. Mice that remember the previous objects will preferentially explore the new object. Mice that received LC inhibition during sleep expressed an increased preference for the novel object, indicating that LC inhibition effectively enhanced the consolidation of the memory for the objects. Individual performances correlated with the increase in sigma power, but not with any of the REM sleep parameters, confirming the role of cortical spindles in memory consolidation.

To firmly establish a causal relationship between NE dynamics in the mPFC and memory consolidation, the authors performed the opposite manipulation. This time, they set up a closed-loop system to automatically stimulate LC neurons when NE levels in the mPFC were crossing gradually decreasing thresholds; the goal here was to decrease the amplitude of the oscillation. Although it is notoriously difficult to selectively manipulate specific parameters of a brain oscillatory pattern without abolishing the oscillation completely, the optical activation of LC neurons strongly altered the infra-slow oscillation and kept the NE levels within a certain range, leading to a global decrease in sigma power, an increase in microarousals resulting in fragmented NREM sleep, and a decrease in REM sleep duration. The authors showed that optogenetic stimulation during sleep after training on the object recognition task significantly decreased performance in the recall test the next day. Although REM sleep is not expected to be involved in the consolidation of this task, it is unknown whether the impairment in performance is due to a decrease in the sigma power alone or because the general architecture of sleep, including REM sleep, was modified by the manipulation. Nevertheless, the result was corroborated in an experiment in which extracellular NE levels were elevated through administration of the NE reuptake inhibitor desipramine. Spindles are believed to be important not only for episodic memory, but also for procedural memory[15], and it would be informative to investigate whether this and other types of memory are affected by these manipulations.

The study by Kjaerby et al[5]. definitely establishes the LC-driven infra-slow NE oscillation as an important mechanism for shaping sleep micro-structure, the related variations in cortical spindles, and their influence on memory consolidation. It also raises further questions as to the generation of this oscillation: is there a pacemaker or an internal clock that controls LC activity? Is there a feedback mechanism that senses cortical NE levels and other parameters, like the homeostatic pressure for REM and NREM sleep, and switches the LC on when NE levels are low? Identifying the parameters that determine, along the descending phase of the oscillation, whether the system will move to a microarousal, awakening, or keep descending into spindle-rich intermediate sleep and subsequent REM sleep might be the next challenging and exciting step in the quest to better understand sleep. ❐

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References

1. Poe, G. R. et al. *Nat. Rev. Neurosci.* **21**, 644–659 (2020).
2. Mitchell, H. A. & Weinshenker, D. *Biochem. Pharmacol.* **79**, 801–809 (2010).
3. Bonnet, M. H. & Arand, D. L. *Sleep. Med. Rev.* **7**, 297–310 (2003).
4. Watson, B. O., Levenstein, D., Greene, J. P., Gelinas, J. N. & Buzsáki, G. *Neuron* **90**, 839–852 (2016).
5. Kjaerby, C. et al. *Nat. Neurosci.* https://doi.org/10.1038/s41593-022-01102-9 (2022).
6. Osorio-Forero, A., Cherrad, N., Banterle, L., Fernandez, L. M. J. & Lüthi, A. *Int. J. Mol. Sci.* **23**, 5028 (2022).
7. Wu, Z., Lin, D. & Li, Y. *Nat. Rev. Neurosci.* **23**, 257–274 (2022).
8. Eschenko, O., Magri, C., Panzeri, S. & Sara, S. J. *Cereb. Cortex* **22**, 426–435 (2012).

9. Osorio-Forero, A. et al. *Curr. Biol.* **31**, 5009–5023.e7 (2021).
10. Hayat, H. et al. *Sci. Adv.* **6**, eaaz4232 (2020).
11. Peyrache, A. & Seibt, J. *Philos. Trans. R. Soc. Lond. B* **375**, 20190230 (2020).
12. Dickey, C. W. et al. *Nat. Commun.* **12**, 1027 (2021).
13. Lecci, S. et al. *Sci. Adv.* **3**, e1602026 (2017).
14. Glin, L. et al. *Physiol. Behav.* **50**, 951–953 (1991).
15. Lustenberger, C. et al. *Curr. Biol.* **26**, 2127–2136 (2016).

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Competing interests
The authors declare no competing interests.

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FUNCTIONAL CONNECTIVITY

Traveling and standing waves in the brain

Studying the natural wanderings of the living brain is extremely challenging. Bolt et al. describe a new framework for considering the brain's intrinsic activity based on the geophysical concepts of standing and traveling waves.

Javier Gonzalez-Castillo

Economists, politicians, CEOs, scientists: they all strive to understand complex dynamical systems so that they can predict — and hopefully improve — their future outcomes. They collect relevant data and morph it into actionable information using meticulously selected analytical tools. Yet, over time, conflicting views will probably emerge from the same data, owing to either unaccounted-for noise or methodological discrepancies. When this happens, it is key to promptly identify and address these issues. This is what Bolt et al.[1] set out to do in this issue of *Nature Neuroscience* for the field of resting-state functional MRI (fMRI), and more generally, for our understanding of intrinsic brain dynamics.

Scientists often study the living brain by asking people to lie still inside an MRI scanner for a few minutes while functional (for example, blood-oxygen level-dependent (BOLD)[2] or vascular space occupancy (VASO)[3]) data are being acquired. This experimental practice, commonly known as resting-state fMRI, has become ubiquitous in cognitive neuroscience owing to its simple setup and low demands for participants. By the end of a single resting-state scan, researchers will have a set of time-series recorded from thousands of small locations across the brain (voxels). Different physiological (for example, neuronal activity, cardiac, respiration) and non-physiological (for example, head motion, hardware instabilities) sources contribute variance to these recordings; fMRI practitioners first try to isolate neuronally induced fluctuations from all others[4]. Next, they face the challenge of summarizing and interpreting such vast amounts of data. Some proceed by exploring average levels of inter-regional synchronicity using methods of varying complexity, ranging from pair-wise Pearson's correlation[5] to nonlinear dimensionality reduction[6]. Bolt et al. refer to those approaches as 'zero-lag' analyses, as they are not able to account for inter-regional delays. By contrast, recent complementary modeling work that considers variable delays in inter-regional synchronicity (termed 'time-lag' analyses) have provided additional exciting insights[7]. Yet, as Bolt et al. succinctly state in their abstract's opening sentence, this methodological explosion has translated into "seemingly disparate insights into [the] large-scale organization of the human brain." Importantly, Bolt et al. propose a solution: constructing a unifying view of methods and observations by turning our attention to the geophysical concepts of standing and traveling waves.

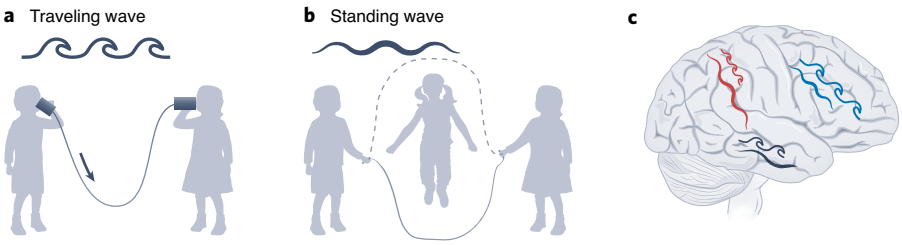

Fig. 1 | Wave types in the real world and the brain. a,**b**, Examples of traveling waves (**a**) and standing waves (**b**) in the real world. **c**, Schematic of the proposed framework to understand resting-state intrinsic fluctuations as a combination of three spatiotemporal patterns with varying levels of standing and traveling behaviors.

What are standing and traveling waves? Traveling waves are like talking to someone using two cans connected by a string (Fig. 1a). By talking into one can, you generate a sound wave that travels through the string and reaches your interlocutor. This is possible because the string provides a means for the sound wave's energy to be transferred across the distance from point A (your mouth) to point B (their ear). By contrast, standing waves are like jumping rope (Fig. 1b). As you get ready to jump, your friends start to move the rope up and down at a constant rate. This motion initially generates traveling waves between both; yet, because the ends of the rope are anchored, the rope almost instantly engages on a regular up-and-down movement with the maximum deflection always occurring at the rope's mid-point. That 'in-place' pattern is a standing wave. According to Bolt et al., intrinsic brain activity can be better conceptualized by relying on methods that accommodate a mixture of traveling and standing waves, instead of using zero-lag and time-lag analyses, each of which accounts for only one these two interlinked components.

This newly proposed framework turns out to be quite powerful. It allows Bolt and