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Neuroethology: Regulation of pre-sleep behaviors

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Animals exhibit species-specific behaviors before transitioning from wake to sleep. A new study characterizes pre-sleep behaviors in mice and shows that these behaviors are regulated, at least in part, by neurons in the lateral hypothalamus.

Before going to bed, most people prepare for sleep by engaging in a series of specific pre-sleep behaviors: relocating to a dedicated room, changing clothes, shutting off lights, laying under blankets, and so on. These behaviors are thought to help us transition from wakefulness to sleep by de-arousing the brain, limiting exposure to environmental stimuli, and facilitating the physiological transition from activity to rest[1,2]. It turns out that humans are not unique in these pre-sleep rituals — many animal species, from invertebrates to primates, prepare for sleep by relocating to a dedicated space, assuming a stereotypical posture appropriate for sleep, and engaging in other species-specific behaviors[3,4]. Although a tremendous amount of research has focused on the neuronal regulation of sleep and wakefulness[5–7], not much is known about the role and central regulation of pre-sleep behaviors. A new study reported in this issue of *Current Biology* by Sotelo *et al.*[8] has directly investigated pre-sleep behaviors in mice by characterizing behaviors that occur immediately prior to sleep onset, demonstrating that these behaviors are important for sleep quantity and quality, and showing that these behaviors are regulated, at least in part, by a discrete population of neurons in the lateral hypothalamus. Taken together, this study establishes a neuroethologically relevant mouse model of pre-sleep behavior.

To characterize pre-sleep behaviors in mice, Sotelo *et al.*[8] defined and documented several distinct behaviors throughout periods of sustained wakefulness and measured how these behaviors changed in the 20 minutes prior to sleep onset. While many dynamic behaviors, including walking, digging, and eating/drinking, decreased prior to sleep, grooming and nest-building behaviors increased significantly. Nest building is particularly ethologically relevant because nests allow for warmth and safety during periods of inactivity. Mice naturally build nests in the wild[9], and laboratory mice are commonly provided with nesting material in the form of cotton-fiber 'nestlets' or crinkled paper strands (as used in this study, see Figure 1A)[10]. Just before sleep, mice increased the time spent sorting, fluffing, and fraying their nesting material. Gradually, they remained motionless in their nests and transitioned to sleep.

Is this nest-building important for sleep itself? To determine the impact of nest building on sleep quantity and quality, Sotelo *et al.*[8] removed nesting material from mouse cages and measured sleep parameters in mice using electroencephalography (EEG), a method

of measuring brain activity to characterize sleep/wake states. Removing nesting material from mouse cages increased the time spent awake and decreased the time spent in the two canonical states of sleep, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. In addition to reducing sleep quantity, the absence of nesting material also affected sleep quality by fragmenting the natural duration and cycles of NREM and REM sleep. Furthermore, mice without nesting material exhibited a decrease in the EEG delta power, a hallmark of cortical activity during NREM sleep, suggesting lower intensity ('lighter') states of sleep. Importantly, these sleep deficits were observed even if animals were provided with warmth or solid protective shelters, suggesting that it is the activity of nest-building itself that promotes sleep as opposed to the secondary benefits of having a nest.

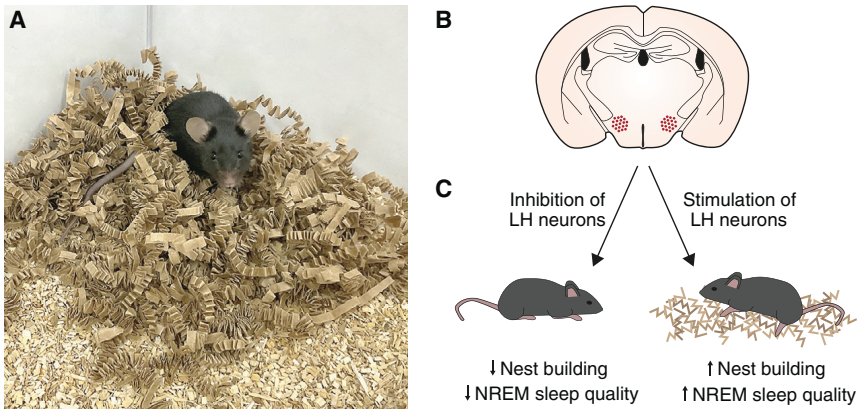

Figure 1. Manipulation of TRAPed LH neurons affects nest building behavior and NREM sleep quality.
(A) A C57BL/6J strain mouse engaging with crinkled paper nesting materials, as used in Sotelo *et al.*[8]. (B) Coronal mouse brain diagram depicting the location of TRAPed pre-sleep-activity neurons (red) in the lateral hypothalamus. (C) Summary of LH manipulation experiments performed in Sotelo *et al.*[8]. Chemogenetic inhibition of LH neurons decreased pre-sleep nest building and decreased NREM sleep quality. Stimulation of LH neurons increased pre-sleep nest building and increased NREM sleep quality.

A major advance in the work of Sotelo *et al.*[8] was the identification of candidate neuronal populations that regulate nest-building behavior. To identify relevant neuronal populations, the authors utilized the Targeted Recombination in Active Populations (TRAP) technique[11], a neuroscience method that fluorescently labels neurons active within an experimentally defined time window — in this case, during the periods when mice exhibited pre-sleep nest-building behavior. Although multiple populations of neurons throughout the brain were fluorescently labelled, only neurons in the lateral hypothalamus (LH) exhibited a significantly higher number of labelled neurons during nest building compared to during sleep or sleep deprivation, indicating that these neurons were specifically active during pre-sleep behaviors (Figure 1B). That these neurons are located in the LH is compelling, as the LH is well known to integrate interoceptive and environmental cues to regulate sleep/wake behaviors[12–15]. For example, the LH contains a population of neurons that produce hypocretin (Hcrt) neuropeptides that integrate metabolic information and regulate wakefulness and arousal[16]. The LH also contains a distinct population of neurons that produce melanin-concentrating hormone (MCH) neuropeptides that promote sleep[17]. Surprisingly, the TRAPed neurons were nearly completely distinct from Hcrt and MCH neurons, indicating that these neurons constitute a separate and novel population.

If the TRAPed LH neurons play an important role in regulating pre-sleep behaviors, one would hypothesize that inhibiting their activity would decrease nest building and subsequently disrupt sleep quality (Figure 1C). Indeed, artificially inhibiting the targeted LH neurons using chemogenetic methods caused a reduction in the duration of nest building, while not affecting other behaviors such as eating, drinking, or grooming. This inhibition also caused a decrease in the delta power of NREM sleep and a fragmentation of NREM sleep that resulted in a greater number of NREM sleep episodes and an increase in the total duration of NREM sleep. A valid interpretation of these results is that inhibiting TRAPed LH neurons ultimately causes shallower, lower quality NREM sleep episodes for which the brain tries to compensate by increasing the total duration of NREM sleep.

In contrast with these inhibition experiments, chemogenetically stimulating the targeted LH neurons caused an increase in the duration of nest-building behavior without significantly affecting other behaviors (Figure 1C). Delta power increased during NREM sleep, indicating a deeper, sustained NREM sleep state. However, paradoxically, the stimulated mice exhibited an increased duration of wakefulness and decreased duration of NREM sleep. This result may be a consequence of the temporal resolution of chemogenetic methods that continuously stimulated the targeted LH neurons for hours — presumably these neurons are only active during pre-sleep behaviors, but stimulation lasted during sleep itself. Future studies could stimulate these LH neurons only during pre-sleep behaviors and cease stimulation at sleep onset.

Taken together, these seminal findings by Sotelo *et al.*[8] establish nest building in mice as a model of pre-sleep behavior and serve as compelling evidence that LH neurons play an important role in regulating this behavior. Several interesting questions remain. First and foremost, what is the identity of the TRAPed LH neurons? The authors showed that these LH neurons do not express Hcrt or MCH — perhaps genetic sequencing methods could be used to identify and target these neurons for future studies. What are the real-time activity patterns of these neurons? The authors used histological techniques to identify pre-sleep-active LH neurons, yet correlating their activity patterns across behavioral states using millisecond-scale techniques such as electrophysiology or calcium imaging will be necessary to firmly establish these neurons as regulating pre-sleep states. What are the functionally relevant circuits that regulate pre-sleep behaviors? The authors mapped

widespread downstream axonal projections from TRAPed LH neurons to other neuronal populations throughout the brain, many of which showed an increase in activity (as measured using the TRAP technique) during nest building. Future studies could test the necessity and sufficiency of these circuits to identify their specific roles in nest building and sleep regulation.

A final broad, speculative question is whether the role of the LH in regulating pre-sleep behaviors is generalizable not only across different strains of mice, but even across different species. The LH is conserved across many vertebrate organisms, with homologous populations of neurons (such as Hcrt and MCH neurons) identified in primates, rodents, birds, and fish[18,19]. It would be fascinating if the LH regulates pre-sleep behaviors in a variety of organisms that each exhibit different pre-sleep rituals. Do we have homologous LH neurons that are active as we switch off the lights and curl up under the covers? The formative findings of Sotelo *et al.*[8] lay the groundwork for comparative ethological studies of pre-sleep behaviors in other model organisms and provide behavioral scientists with many interesting future directions to sleep on.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Tissue architecture: Two kinesins collaborate in building basement membrane

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Basement membranes are essential for tissue architecture and development. A new study reveals that two microtubule motors, kinesin-3 and kinesin-1, work collaboratively to direct basement membrane protein secretion in the *Drosophila* follicular epithelium for correct tissue movement.

The basement membrane is a self-organized extracellular sheet composed of extracellular matrix proteins that surrounds animal tissues and organs. Basement membranes mediate attachment of cells to maintain/change tissue shape, serve as barriers between tissues, and function as signaling platforms that are essential for tissue