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Dorsal clock neurons claw their way out to control sleep in *Drosophila*

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The drive to sleep is strongly influenced by time of day, with temporal information conveyed through the circadian clock. In pursuit of the neural mechanisms underlying this process, in this issue of *Neuron*, Sun et al. identify a novel circuit that links circadian output neurons to sleep-promoting neurons within the mushroom bodies.

Sleep comprises about one-third of our lives, and lack of it has an enormous impact on both our health and well-being. Understanding how sleep is regulated has been a major quest for neuroscientists. A long-standing view of sleep regulation describes two different processes impacting our drive to stay awake or to sleep. The sleep homeostat keeps track of how long an individual has been awake and promotes sleep in response to increasing wake time, whereas the circadian clock promotes either sleep or wakefulness at the appropriate times of day. *Drosophila melanogaster* has shown itself to be an amenable model for the study of the neural circuits that underlie sleep regulation because they have a relatively small number of neurons and a long-standing repertoire of genetics tools. In addition, accessibility to its brain connectome provides exciting perspectives in the study of sleep-regulatory circuits. The fly brain structures involved in the processes of sleep regulation mentioned previously include the central complex—mainly neurons projecting to the dorsal fan-shaped body and to ring neurons within the ellipsoid body (EB-Rs), both part of the sleep homeostat—as well as the mushroom body (MB). Within the MB, different Kenyon cells, as well as some MB output neurons (MBONs), have been shown to be either sleep promoting or sleep suppressing, with sleep-promoting Kenyon cells signaling onto cholinergic MBON and wake-promoting Kenyon cells connecting to glutamatergic MBON (Shafer and Keene, 2021).

The neuronal constituents of circadian timing have been described in great detail and comprise 75 pairs of neurons expressing circadian molecular machinery, with neurons separated into different groups depending on the neuroanatomical position and gene expression profiles. Among these groups, a thorough dissection of the physiological role was carried out for the ventral and dorsal lateral neurons (LNvs and LNds), and the dorsal-posterior neurons 1 (DN1ps) (Beckwith and Ceriani, 2015). How exactly the neurons that constitute the circadian clock provide information for promoting or inhibiting sleep at different times of the day is still an open question, both in flies and mammals. In *Drosophila*, it has been shown that LNvs, LNds, and DN1ps can modulate sleep either through the EB-Rs or through other outputs such as leucokinin-expressing and pars intercerebralis (PI) neurons (reviewed in Shafer and Keene, 2021). However, the contribution of the remaining clusters that constitute the circadian network has not been explored in depth. This is particularly the case even for the most numerous group within the circadian network, the dorsal neurons 3 (DN3s), whose role—not only in sleep regulation, but also on circadian timekeeping—has remained elusive.

In this issue of *Neuron*, Sun et al. describes a novel pathway that links DN3 neurons with the sleep-promoting MB γ lobe Kenyon cells and allows for circadian gating of sleep (Sun et al., 2022). The authors generated novel split-Gal4 drivers that allow for specific manipulation of different DN3s subtypes and also identified a subgroup of anterior-projecting DN3 neurons (apDN3s) that promote sleep in response to forced depolarization. Neuroanatomical description of the apDN3 cells suggested that their dendritic branches are adequately positioned to receive information from other circadian clusters in the posterior side of the brain, and functional experiments confirmed that apDN3s receive excitatory inputs from the DN1p cluster that have previously been shown to be involved in sleep regulation as well (Guo et al., 2018; Lamaze et al., 2018).

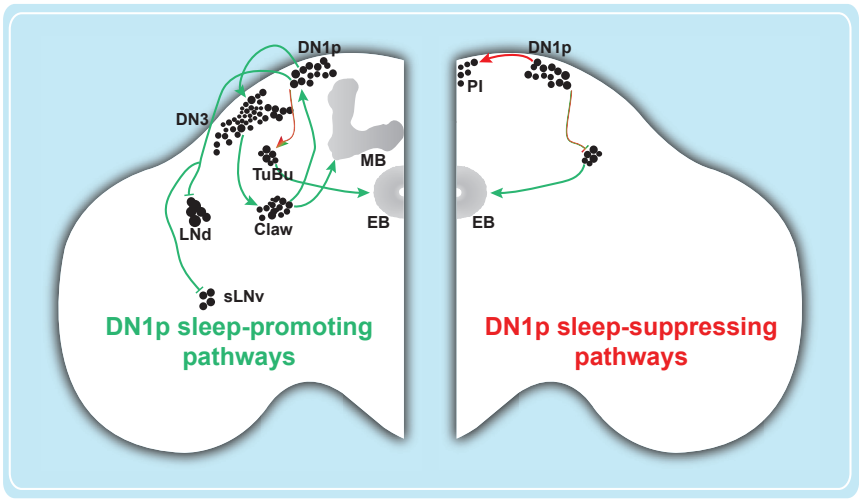

Figure 1. A DN1p sleep-regulatory hub

Sleep-promoting circuits (green lines) recruit the DN1p-apDN3-CL-MB neurons (including the recurrent excitatory loop encompassing DN1p-apDN3-CL-DN1p) as well as the inhibition of wake-promoting LNds and sLNvs. Sleep-suppressing circuits (red lines) are conveyed through connections to PI neurons. It is worth mentioning that the sleep-promoting TuBu to EB-R circuit receives information from a subset of DN1ps, although the data available describes this connection as either excitatory (Guo et al., 2018) or inhibitory (Lamaze et al., 2018), ultimately leading to opposing roles of DN1p-TuBu-EB-R circuit in terms of promoting/suppressing sleep. This ambiguity is reflected on both panels with green/red lines. It remains an open question as to whether these apparently contradicting results reflect the existence of further subdivisions within the DN1p cluster, each leading to excitatory/inhibitory responses in TuBu neurons, or to a dual (perhaps time-of-day-regulated) nature of the connection. Arrowheads indicate activating connections; blunt ends indicate inhibitory connections.

How do apDN3 cells influence sleep? By combining anterograde tracing, connectome data, and optogenetics, the authors identified a novel neuronal cluster (termed "Claw" neurons, CL) that receive excitatory glutamatergic input from the anterior projections of apDN3s and, upon activation, are able to induce sleep. Interestingly, intracellular calcium levels in claw neurons showed a time-of-day variation, with rising levels during nighttime (when flies tend to sleep more), as well as an increased number of cells with higher calcium levels during this time window. Moreover, activation of increasing proportions of claw cells showed a correlation between the number of activated CL cells and the degree of sleep induction, suggesting that the global activity of this neuronal population induces sleep in a dose-dependent manner. Although CLs do not possess a molecular clock, oscillation in their calcium levels is absent in mutants for the clock gene period. These results suggest that calcium oscillation in clock-less CL cells can be generated by timed signals propagating from upstream circadian neurons, a phenomenon previously observed in LK- and diuretic hormone 44-expressing neurons, which are important players of the circadian output mechanism (Cavey et al., 2016). Such examples of circadian neuronal activity in non-circadian neurons indicate that the spread of oscillatory neuronal physiology extends beyond the circadian network, adding a new layer of complexity to the study of *Drosophila* circuitry.

Control of sleep centers by circadian neurons has previously been shown between DN1p and ellipsoid body (EB) neurons, showing that the circadian clock can directly impact sleep-regulatory regions. To further investigate the mechanism by which the DN1p-apDN3-CL circuit induces sleep, the authors explored postsynaptic partners to CL neurons through genetic anterograde tracing. They found that neurons from the MB γ lobe act downstream of CL, and CL-triggered activation of the MB γ lobe leads to reduced locomotor activity. Through conducting epistasis experiments, the authors showed that the MB is necessary for CL-induced sleep promotion, suggesting that the DN1p-apDN3-CL circuit finally acts on MB γ lobe neurons to induce sleep.

An intriguing observation uncovered from this work is the existence of an excitatory recurrent loop within sleep-regulatory neurons. The authors found a cholinergic excitatory connection between CL and DN1p cells, forming a recurrent excitatory circuit among DN1p-apDN3-CL-DN1p neurons. When CL neurons are activated, calcium levels in DN1ps are sustainably increased, which might lead to a persistent activation of the circuit. Excitatory reciprocal connections between sleep-promoting neurons have also been described in mice (Zhang et al., 2019). Of note, the precise role of such recurrent loops in sleep-promoting circuits is still unclear, but it might represent a mechanism to stabilize and sustain the activation of these cells throughout sleep, helping to consolidate this state. Indeed, an indirect measurement of sleep depth assessed by monitoring fly locomotor behavior showed that ablation of CL neurons decreases sleep depth, although further exploration is required to clearly distinguish whether these effects are a consequence of impairing the recurrent excitatory loop itself or the connectivity between CL neurons and its downstream targets.

This work describes a novel route through which circadian DN1p neurons modulate sleep, in addition to those already reported that include sleep promotion through TuBu-EB and sleep suppression through PI, LNd, and sLNv (see references in Shafer and Keene, 2021) (Figure 1). Although originally characterized by neuroanatomical position as a single cluster, the DN1p group is composed of heterogeneous cell types (Ma et al., 2021) that target different sleep-regulatory centers to achieve a similar global sleep state (i.e., sleep induction through EB-R or MB) and even have opposing roles in terms of sleep promotion/suppression, highlighting a degree of complexity yet to be explored.

DN1ps are strategically located to integrate/receive differential modulation by environmental factors, such as temperature and light conditions, which could in turn be conveyed through different circadian dorsal neuron subcircuits to modulate sleep in specific ways. As an example, CNMamide-positive DN1ps integrate thermosensory inputs and suppress sleep through DH44-positive PI neurons in response to warm

temperatures (Jin et al., 2021). It is indeed puzzling that DN1ps impact so many different sleep-control centers, which are themselves involved in a variety of behaviors taking place across the day, such as learning and memory (in the MB), navigation and motor control (in the EB), or feeding and metabolic regulation (in the PI). Many of these behaviors are themselves able to regulate sleep by acting on these sleep-control centers, as in the case of increased sleep to facilitate memory formation, which requires MB circuits (Chouhan et al., 2020).The impact these behaviors have on sleep might be circadianly gated, for which DN1p signaling would be uniquely positioned, because they reach each of the sleep centers where such interactions would take place. The work by Sun et al. provides an exciting and detailed characterization of a novel pathway by which DN1p can function as a sleep-regulatory hub.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Value representations: Fast and slow

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Corticostriatal circuits represent value and choice during value-guided decision making. In this issue of *Neuron*, Balewski et al. (2022) show that caudate nucleus and orbitofrontal cortex use distinct value signals during choice, which are consistent with two parallel valuation mechanisms, one fast, one slow.

As you approach the traffic light, it turns yellow. Do you stop or do you keep going? What about choosing a main course from a long list of options on the menu? Do you glance at the different options and immediately know what you want, or do you carefully compare different options before settling on one? We have to make decisions like this daily. In some cases, your decision time is very limited as you have to act quickly. In other cases, you can take (almost) as much time as you want to make a decision. It has been suggested that two decision-making systems co-exist: one fast, one slow (Kahneman, 2011). The first decides with seemingly little effort, almost reflexive. A gut feeling as people often call it. The other system is more deliberative and seems to evaluate—over a certain period of time—the different options at hand and their consequences.

In looking for neural evidence to support separable decision-making systems, fast, reflexive decisions have been primarily (though not exclusively) associated with subcortical structures, more deliberative decisions with cortical structures (Wood and Bechara, 2014). Independent of which of many decision-making models one favors, the question remains how the value of different choice options is represented (and even constructed) in the brain and how these value representations enter the decision-making process and affect choice.

The orbitofrontal cortex (OFC) and the caudate nucleus (CdN) have both been linked to decision making, although the path they took toward entering decision-making models is quite different. The importance of OFC, or more generally, the frontal lobe, became clear long before modern brain-mapping techniques were developed. This insight came from