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RESEARCH HIGHLIGHT

A Longer Siesta? DN1s in Control!

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Sleep is an essential behavior that is conserved in most animal species. Two molecular mechanisms regulate sleep: circadian clocks for the timing of sleep, and a homeostatic process for the amount of sleep [1]. The fruit fly, *Drosophila melanogaster,* exhibits robust sleep behavior and serves as an excellent model in which to study the neuronal and molecular mechanisms of sleep.

The *Drosophila* brain is comprised of ~150 clock neurons, which can be divided into different groups based on their localization and function [2]. Four small ventrolateral neurons (sLNvs) expressing the neuropeptide pigment dispersing factor (PDF) are responsible for the morning activity around dawn, and thus are called morning neurons. Evening activity is modulated by the 5th sLNv (PDF-negative) and dorsal lateral neurons (LNds) [3]. There are also three groups of dorsal neurons (DN1s, DN2s, and DN3s) and one group of lateral posterior neurons (LPNs). DN1s can modulate fly activity in response to different environmental conditions, such as light and temperature [4]. Although it has been demonstrated that circadian neurons play important roles in promoting arousal [5–7], little is known about the promotion of sleep through circadian neurons.

The recent work of Guo *et al.* [8] clarifies this uncertainty by elucidating the function of DN1 neurons, which promote midday siesta and night sleep. Using optogenetics to manipulate neuronal activity of a subgroup of DN1s, the researchers observed an increase in midday siesta sleep with DN1 stimulation and a decrease in midday siesta sleep with DN1 inhibition. They also found a decrease in both siesta and night sleep when blocking DN1 neurotransmitter release. The authors demonstrated that DN1s are responsible for midday siesta sleep and night sleep. Guo *et al.* further established the presence of synaptic connections between LNds (E cells) and sLNvs (M cells) with DN1s. The authors aimed to identify the functionality of these sLNv-DN1 and LNd-DN1 synapses through Ca^{2+}-sensitive activity fluorescence. Intriguingly, they found that when DN1 neurons were activated, the firing of both LNds and sLNvs was reduced. These results explain the dialogue at the synapse and led the researchers to ask: what mediates this inhibitory response and the observed day sleep phenotype? To answer this, they first checked for the presence of glutamate in DN1 neurons. They co-stained DN1 neurons with the vesicular glutamate transporter (VGLUT) and showed a significant overlap. Interestingly, they found that the metabotropic glutamate receptor mGluRA undergoes robust circadian oscillations in the E cells, peaking at midday. Furthermore, Guo *et al.* discovered that the role of DN1s in the promotion of midday siesta is through the inhibition of E cells *via* glutamate release. They first demonstrated a glutamate-sensitive response in E cells by both reducing E cell activity in the presence of glutamate and by removing glutamate-induced inhibition with the co-application of glutamate and a mGluRA antagonist to E cells. They next reduced the amount of postsynaptic E cell mGluRAs and observed a removal of day sleep promotion under DN1 stimulation. These findings show that DN1 activation promotes the inhibition of LNds and sLNvs through glutamate to increase daytime siesta.

Furthermore, the researchers took advantage of a recently developed CaLexA-luciferase system in which neuronal activity is reported by bioluminescent signals in freely-moving flies [9]. It is known that there are remarkable differences of sleep pattern between male and female flies:

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males have a more pronounced siesta than females. Interestingly, the authors noted a significant daytime increase of DN1 neuronal activity in males compared to females, which correlates well with the siesta difference. Compellingly, this sexual dimorphism in the *Drosophila* circadian network is the first to be identified. The authors further characterized the sensitivity of DN1 neurons and sleep by assaying DN1 activity in response to environmental input. Previously, other researchers have reported increased day sleep with warmer temperatures [10, 11]. When Guo *et al.* tested the thermosensitivity of DN1s and daytime sleep, they found a marked increase in DN1 activity and daytime siesta sleep at higher temperatures. In addition, when they removed DN1 output by expression of the neurotransmitter blocker TNT, they found a significant withdrawal of temperature-promoted daytime siesta sleep. Their results demonstrate both that DN1 activity is increased at higher temperatures and that DN1 activity is required for temperature-induced siesta sleep. The authors' observations illustrate the power and throughput potential of the CaLexA-Luc assay for future research on neuronal activity.

Guo *et al.*'s findings of the role of DN1s in promoting midday siesta and night sleep, as well as regulating the sexual dimorphism and thermosensitivity of fly sleep, and CaLexA-Luciferase assays, open an abundance of avenues for further research. It would be interesting to test if neuron-specific temperature-sensitive *period* splicing occurs in DN1 neurons and is responsible for the siesta changes at high temperatures [10]. In addition, researchers may further characterize why the sexual dimorphism in sleep exists. Are there gender-specific neuronal networks that further modulate the effects of DN1s? All of these questions and many more can be explored with the framework that Guo *et al.* have established.

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