Neural substrates of wakefulness are probed with classic and emerging technologies

By Viviana Gradinaru

The need for sleep is recognized across the animal kingdom: Short-term sleep deprivation affects proper functioning, and extended sleep deprivation is fatal. However, animals can override the physiological drive to sleep when necessary or advantageous. Some can choose to sleep for shorter periods to avoid predators. Others, like the great frigatebird, can skip sleep altogether for weeks to maximize mating.

In humans, the expression “to sleep with one eye open” denotes the ability to retain residual consciousness of the outside world during the behavioral quiescence of sleep. Salient or alerting cues can rouse a slumbering person to a fully awake and vigilant state. This capability holds obvious evolutionary benefits because it allows us to avoid danger or attend to the needs of our offspring.

Within the past several years, our group at the California Institute of Technology has sought to explore the mammalian neural circuits underlying the ability to override sleep, which were, until recently, a neurobiological puzzle.

DOPAMINE IN SLEEP AND AROUSAL

In flies, dopamine (DA) cells were recently identified as the locus of behavioral arousal and sleep-wake switching (1). In mammals, too, basic and clinical observations were converging upon DA as a crucial modulator of wakefulness, with DA reuptake inhibitors (e.g., amphetamines and modafinil) decreasing sleep and sleep disturbances emerging as an early symptom of neurodegenerative diseases characterized by DA cell loss, such as Parkinson’s disease. But characterizing the specific DA neurons responsible among the heterogeneous mammalian DA groups has been challenging. With little evidence from single-unit recordings that the well-studied DA neurons of the midbrain ventral tegmental area (VTA) and substantia nigra compacta (SNC) nuclei might alter their activity across sleep-wake cycles, graduate student Ryan Cho and postdoctoral fellow Jennifer Treweek instead chose to examine the DA neurons of the dorsal raphe nucleus (DRN) (DRN\textsuperscript{DA}), a small and comparatively understudied population of DA neurons (see the first image) (2).

A decade-old study (3) hinted at the role of DRN\textsuperscript{DA} in promoting wakefulness. Research conducted by Lu et al. in 2006 showed that chemically lesioning these cells in rats caused a 20% increase in sleep and that these cells express cFos after wakefulness, indicating that they are wake-active. DRN\textsuperscript{DA} neurons are also known to degenerate in patients with multiple systems atrophy and Lewy body dementia, disorders commonly associated with excessive daytime sleepiness (4).

DIGGING INTO THE DRN

With all this in mind, we set out to explore DRN\textsuperscript{DA} function in mice, with a particular interest in determining how these neurons might signal and/or determine wakefulness in a variety of scenarios. Using fiber photometry to measure cell activity through calcium imaging, we observed that DRN\textsuperscript{DA} cells were activated by arousal-evoking salient cues, irrespective of their hedonic value (2). By combining fiber photometry with polysomnography (electroencephalography/electromyography to determine sleep states), we found that DRN\textsuperscript{DA} bulk activity fluctuated across sleep-wake cycles and was highest during wakefulness. Interestingly, the duration of the waking episodes correlated with changes in DRN\textsuperscript{DA} activity.

Both endogenous and optogenetically driven DRN\textsuperscript{DA} firing were associated with arousal from sleep. Indeed, 1 hour of DRN\textsuperscript{DA} stimulation during a period of high sleep pressure resulted in a threefold increase in the time spent awake, and DRN\textsuperscript{DA} activation promoted immediate arousal, even when animals were sleep deprived. DRN\textsuperscript{DA} inhibition through chemogenetics, on the other hand, opposed wakefulness, even in the presence of salient stimuli (e.g., mate or predator). Last, optogenetic DRN\textsuperscript{DA} inhibition time-locked to a tone reduced the probability that the sound would immediately wake the animal from sleep.

Our results are evidence that DRN\textsuperscript{DA} neurons in the dorsal raphe nucleus control arousal. To achieve optogenetic and chemogenetic activity modulation of dopamine neurons in the dorsal raphe nucleus (DRN), we stereotaxically delivered adeno-associated viruses (AAVs) encoding E1a-DIO-Chr2-eYFP (green) and hSyn-DIO-hM4Di-mCherry (red) into the DRN of a tyrosine hydroxylase (TH):IRESCre mouse. TH staining (blue) indicates dopamine neurons. (Related to (2)).

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neurons and the input-output circuits in which they play a part comprise a system in the mammalian brain capable of modulating sleep-wake states with an awareness of the outside environment.

Given their biological importance to survival and fitness, the neural substrates for overriding the physiological sleep drive likely involve multiple distributed circuits. Our work on DRN DA cells—as well as recent work on DA cells in the VTA (5)—is just beginning to reveal these circuits.

TOWARD NEW THERAPIES FOR DISTURBED SLEEP
The ability to rouse from sleep in response to alerting stimuli is an evolutionarily conserved survival strategy from the primitive jellyfish Cassiopea (6) to humans. However, in modern human populations, malfunctioning arousal-promoting circuits may trigger negative sequelae such as insomnia or hypersomnia. For example, in those suffering from depression, excessive sleepiness and a lack of motivation to wake up despite normal circadian rhythms and the absence of a homeostatic sleep deficit can occur.

To effectively address such sleep disturbance and other related pathologies, one may need to manipulate cells simultaneously across distributed networks. Strategies that specifically target DA activity jointly across the DRN, VTA, and other potential targets may prove to be optimal.

TECHNIQUES FOR STUDYING SLEEP AND AROUSAL
We have developed viral vectors that can cross the blood-brain barrier, enabling noninvasive brainwide transduction of specific cell types and regions in mice (see the second image) (7, 8). Combined with chemogenetics and other methods under development, this will allow us to modulate DA activity across multiple deep-brain structures in a minimally invasive manner. We also aim to bring new technologies to bear to extend our knowledge of DRN DA circuitry. New techniques for sparse cell labeling and morphological reconstruction (8) will help us elucidate downstream DRN DA projections. Single-cell recording at depth by two-photon endoscopy should enable us to determine whether the DRN DA population is uniformly excited by salience (positive and negative) or whether individual cells intermingled within the DRN are oppositely tuned to stimuli with positive and negative valences.

Our long-term goal is to use our tissue clearing (9) and viral vector tools (7, 8) to delineate arousal-promoting circuitry. We believe that this will not only answer important questions about brain architecture and function but also aid in the identification of targets to improve sleep and alertness.

REFERENCES

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