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## **Sleep: Switching off the off-switch to wake up**

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### **40-word summary:**

What are the synaptic drives controlling the sleep-wake circuitry in the mammalian brain? Venner *et al.* found that GABAergic cells in posterior lateral hypothalamus inhibit sleep-promoting anterior hypothalamic cells to cause waking, whereas their inhibition augments sleep.

**Main Text:**

The movie “Awakenings” (1990), starring Robert de Niro as a 40-year old *encephalitis lethargica* victim waking up after three decades of somnolence, is a captivating documentary of a life without control over sleep and wakefulness. This movie, adapted from the award-winning book of the late Oliver Sacks (1933-2015), undoubtedly selects a historically unique and dramatic case of human debilitation. However, remind yourself of the last day after an all-nighter to get a flavor of how life would be if sleep took over uncontrollably. Indeed, repeated intrusion of sleep into waking, as well as the converse, disruption of sleep by awakenings, often indicate a need for neurological consultation. These symptoms also bring to light an obvious, yet neurobiologically non-trivial, capability of our brain: it keeps sleep and wake strictly segregated and ensures that transitions occur rapidly, similar to on-off switches of the lights in our houses at day or night onset. A study in this issue of *Current Biology*, authored by Venner *et al.* [1], presents a novel synaptic pathway through which this vital switch is made possible.

Neuroanatomic and lesioning studies for most of the past century have uncovered groups of neurons, located mainly in the brainstem and hypothalamus, that are implicated in vigilance state control [2]. Focusing here on waking and non-REM sleep, these fall broadly into wake- and non-REM sleep-active neuronal subgroups. Each increases action potential discharge during its preferred vigilance state and each receives dense inhibitory input from its counterpoised partner. Such a circuitry ensures rapid and complete state transitions: overcoming inhibition on one side leads to more inhibition on the antagonist side, reinforcing the winner’s activity. A current influential working model of the sleep-wake switch is analogous to a “flip-flop” electronic circuit. This is a bistable device that will remain in one state until a trigger causes it to switch to the other state ([3], Figure 1A). But which are the triggers of the transition? Multiple regulatory inputs arising from circadian, homeostatic and allostatic systems [3, 4] need to be coordinated to decide between sleep and wakefulness.

Furthermore, limbic and cognitive variables modulate sleep-wake states. Little is known about the wiring of all these synaptic inputs into the sleep-wake switch. Do they conform to the “flip-flop” model proposing that sleep-wake transitions need synaptic excitation of wake-promoting groups, whereas sleep-promoting groups are excited for the reverse transition?

Optogenetic and chemogenetic techniques equip neurons with optic or ligand actuators such that light pulses or designer drugs control their electrical activity. These techniques are ideal to probe the sleep-wake control elements directly and to set their activity in causal relation with the vigilance state. Currently available studies have indeed successfully woken up sleeping mice, for example through illuminating hypothalamic orexinergic neurons [5] or brainstem noradrenergic neurons within the locus coeruleus (LC) [6], corroborating the power of these wake-promoting cell types as part of the “flip-flop” model.

The optogenetic dissection of the sleep-promoting circuit, in contrast, is just about to start. A small cluster of neurons within the preoptic hypothalamus facing the bottom surface of the brain, the ventrolateral preoptic (VLPO) area, shows a strong increase in firing during sleep and densely innervates many wake-promoting brain areas [7]. The VLPO area has been a prominent element in the “flip-flop” model, essentially accounting for the multiple and diversified inhibition of wake-promoting groups during sleep [3]. However, the VLPO area does not contain a neurochemically and functionally homogeneous cell population [8, 9], its lesion dramatically, but not fully reduces sleep time [10], and it is also involved in thermoregulation [11] and, possibly, parental behaviors [12]. More recent studies have also shown that the VLPO area is not the only brain region to contain sleep-active or, indeed, sleep-promoting activity [13, 14]. It is nevertheless an overdue task to dissect apart the neural identities of the sleep-promoting cell groups within the VLPO area, to identify their synaptic inputs, and to evaluate the role of the VLPO area in the “flip-flop” switch.

The recent paper by Venner *et al.* takes a first step towards this question. The authors focus on the posterior lateral hypothalamus (LH), known to contain wake-promoting orexinergic neurons and REM sleep-promoting neurons expressing melanin-concentrating hormone [7]. Both these neuropeptidergic projections are widely regarded as hierarchically superior regulatory nodes for their respective vigilance state. Not only do these excite the neural networks underlying the polysomnographic features of wake and REM sleep, but they are also involved in the corresponding nutritional and emotional attributes. Within this same region of the hypothalamus, Venner *et al.* manipulated intermingled GABAergic neurons, some of which were previously reported to be sleep-active [15, 16]. Anterograde tracing revealed dense projections to the VLPO area, with additional projections found in brainstem areas related to sleep-wake control (see also [17]). As would be expected based on their GABAergic nature, chemogenetic activation of these neurons potently promoted waking, while inhibition slightly enhanced non-REM sleep without modifying REM sleep. Changes in VLPO *c-fos*-staining, a marker gene reflecting neural activity, paralleled the bidirectional manipulation of the LH GABAergic neurons. The authors document the chemogenetically induced vigilance state through showing that EEG spectral changes, as well as behavioral correlates, corresponded to those of natural vigilance states. They also find that wake promotion caused a post-waking increase in sleep depth, similar to that found after a period of sleep deprivation. This latter observation is consequential because it shows that their manipulation set in motion natural homeostatic sleep regulation.

A technical aspect of this paper that deserves mention is the skillful use of viral injections for transfection of the LH GABAergic neurons. Typically, viral injections are done via bulk application of liquid droplets containing virus particles, typically hundreds of nanoliters. The precision of the pipette targeting and droplet size determine the infected brain area. Therefore, in brain regions with closely apposed and heterogeneous cell groups, which is

common in hypothalamus, large injection sites risk going beyond the cells or zone of interest. Here, the authors used amongst the tiniest viral injections described so far (7-66 nl) and correlated the anatomy and change in the response variable (i.e., induced waking duration) for each individual mouse. The “hot spot” of effective LH-VLPO projections was ~150  $\mu\text{m}$  in diameter and separate from GABAergic neurons of the dorsally located *zona incerta* or the anterior basal forebrain, which are also involved in thalamocortical state changes [2, 16]. This approach hence sets a standard for future viral injection strategies for small brain regions with cellular heterogeneity. Differences in the exact viral targeting strategies could contribute, for example, to the reported discrepancies to another recent work stating that lateral hypothalamic GABAergic projections can promote arousal through projections to the thalamic reticular nucleus [18].

Venner *et al.* [1] have undoubtedly identified a powerful and clearly delineated inhibitory input to the VLPO area, which is consistent with previous cellular recordings [15, 16] and this will require an elaboration of the “flip-flop” model. Sleep-wake transitions could be schematized as a balance, wherein waking would be promoted once the weight of wake-promoting overcomes that of sleep-promoting areas. In addition, according to Venner *et al.* [1], decreasing the weight of sleep-promoting areas via external synaptic inhibition also enables wakefulness (Figure 1B). Such external control of the sleep-wake balance might impart greater flexibility in the integration of the multiple physiological variables in the sleep-wake control. For example, why does a sleeping hungry mammal wake up more easily than a well-fed mouse? Such conditions could involve hypothalamic control of the VLPO area. Thus, this paper is the first to demonstrate that a synaptic inhibition of the VLPO area can efficiently turn-off the off-switch to promote arousal.

Many new stimulating questions come up by the work of Venner *et al.* [1]. First, which are the physiological conditions that drive the LH-VLPO projection? Where in the hierarchy

of sleep-wake control lies this projection, in particular also with respect to other non-REM sleep promoting areas [13, 14]? What about REM sleep control [19]? What are the neuronal and molecular identities of the VLPO neurons that are targeted by LH-GABAergic neurons? The power of chemogenetic tools for these question becomes very clear in this landmark study. Trans-synaptic tracing, molecular profiling combined with sleep manipulation will help for further advances in the genetic and circuit dissection of the VLPO. These results will provide clues to more specific pharmacological and behavioral sleep therapies.

1. Venner, A., Anaclet, C., Broadhurst, R.Y., Saper, C.B., and Fuller, P.M. (2016). A novel population of wake-promoting GABAergic neurons in the ventral lateral hypothalamus. *Curr. Biol. in press*.
2. Brown, R.E., Basheer, R., McKenna J.T., Strecker, R.E., and McCarley, R.W. (2012). Control of sleep and wakefulness. *Physiol. Rev.* 92, 1087-1187.
3. Saper, C.B., Fuller, P.M., Pedersen, N.P., Lu, J., and Scammell, T.E. (2010). Sleep state switching. *Neuron* 68, 1023-1042.
4. Luppi, P.H., Peyron, C., and Fort, P. (2016). Not a single but multiple populations of GABAergic neurons control sleep. *Sleep Med. Rev. in press*.
5. Adamantidis, A.R., Zhang, F., Aravanis, A.M., Deisseroth, K., and de Lecea, L. (2007). Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* 450, 420-424.
6. Carter, M.E., Yizhar, O., Chikahisa, S., Nguyen, H., Adamantidis, A., Nishino, S., Deisseroth, K., and de Lecea, L. (2010). Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nat. Neurosci.* 13, 1526-1533.
7. Szymusiak, R., and McGinty, D. (2008). Hypothalamic regulation of sleep and arousal. *Ann N.Y. Acad. Sci.* 1129, 275-286.

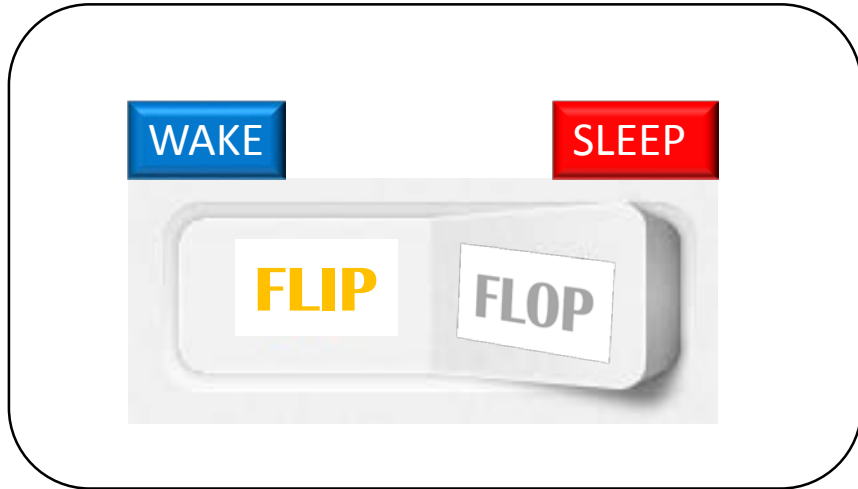
8. Szymusiak, R., Alam, N., Steininger, T.L., and McGinty, D. (1998). Sleep-waking discharge patterns of ventrolateral preoptic/anterior hypothalamic neurons in rats. *Brain Res.* *803*, 178-188.
9. Modirrousta, M., Mainville, L., and Jones, B.E. (2004). Gabaergic neurons with  $\alpha$ 2-adrenergic receptors in basal forebrain and preoptic area express c-Fos during sleep. *Neuroscience* *129*, 803-810.
10. Lu, J., Greco, M.A., Shiromani, P., and Saper, C.B. (2000). Effect of lesions of the ventrolateral preoptic nucleus on NREM and REM sleep. *J. Neurosci.* *20*, 3830-3842.
11. Boulant, J.A. (2000). Role of the Preoptic-Anterior Hypothalamus in Thermoregulation and Fever. *Clin. Inf. Dis.* *31 Suppl 5*, S157-S161.
12. Dulac, C., O'Connell, L.A., and Wu, Z. (2014). Neural control of maternal and paternal behaviors. *Science* *345*, 765-770.
13. Zhang, Z., Ferretti, V., Guntan, I., Moro, A., Steinberg, E.A., Ye, Z., Zecharia, A.Y., Yu, X., Vyssotski, A.L., Brickley, S.G., *et al.* (2015). Neuronal ensembles sufficient for recovery sleep and the sedative actions of  $\alpha_2$  adrenergic agonists. *Nat. Neurosci.* *18*, 553-561.
14. Anaclet, C., Ferrari, L., Arrigoni, E., Bass, C.E., Saper, C.B., Lu, J., and Fuller, P.M. (2014). The GABAergic parafacial zone is a medullary slow wave sleep-promoting center. *Nat. Neurosci.* *17*, 1217-1224.
15. Hassani, O.K., Henny, P., Lee, M.G., and Jones, B.E. (2010). GABAergic neurons intermingled with orexin and MCH neurons in the lateral hypothalamus discharge maximally during sleep. *Eur. J. Neurosci.* *32*, 448-457.
16. Koyama, Y., Takahashi, K., Kodama, T., and Kayama, Y. (2003). State-dependent activity of neurons in the perifornical hypothalamic area during sleep and waking. *Neuroscience* *119*, 1209-1219.



17. Gervasoni, D., Peyron, C., Rampon, C., Barbagli, B., Chouvet, G., Urbain, N., Fort, P., and Luppi, P.H. (2000). Role and origin of the GABAergic innervation of dorsal raphe serotonergic neurons. *J. Neurosci.* *20*, 4217-4225.
18. Herrera, C.G., Cadavieco, M.C., Jego, S., Ponomarenko, A., Korotkova, T., and Adamantidis, A. (2016). Hypothalamic feedforward inhibition of thalamocortical network controls arousal and consciousness. *Nat. Neurosci.* *19*, 290-298.
19. Sapin, E., Bérrod, A., Léger, L., Herman, P.A., Luppi, P.H., and Peyron, C. (2010). A very large number of GABAergic neurons are activated in the tuberal hypothalamus during paradoxical (REM) sleep hypersomnia. *PLoS One* *5*, e11766.

**Figure 1. Schematic representations of models for the transitions between sleep and wakefulness.** (A) A “flip-flop” switch analogous to the equally named electronic circuit has been shown to account for the rapid and complete transitions between sleep and wakefulness (wake) [3]. The schematic illustrates this through a single switch with a “flip” and a “flop” side that represent wakefulness and sleep, respectively. The neural circuits behind this switch are wired such that activating one side automatically deactivates the other side through mutually inhibitory connections. (B) A model using a balance, in which wake- and sleep-promoting cell groups act as the weight to tilt the balance towards one or the other side. The green tower symbolizes the hypothalamic GABAergic neurons identified by Venner *et al.* [1] that antagonize or increase the weight of the sleep-promoting VLPO area to cause wakefulness or sleep, respectively.

A



B

