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Development: Better Sleep On It, Children

A new study has identified a neural circuit that is responsible for increasing sleep in young fruit flies. Reduced dopamine signaling to the fan-shaped body during early life promotes sleep and is critical for proper brain development.

Kazuma Murakami and Alex C. Keene

When it comes to sleep, the needs of children and adults differ dramatically. Many animals sleep more in early life and a number of factors suggest this sleep is critical for proper brain development [1,2]. In a recent study published in *Science*, Kayser *et al.* [3] examine the neural and functional basis for enhanced sleep during early life. The authors demonstrate that reduced activity in a small population of wake-promoting dopamine neurons increases sleep of young flies, and that this early life sleep enhancement is critical for proper brain development.

While significant progress has been made towards understanding how sleep is regulated, the neural basis for interactions between sleep and brain development are less well understood. Sleep affects broad aspects of physiology, immunity and behavior, and sleep loss disrupts synaptic plasticity and memory in both flies and mammals. Animals sleep more during early life when the brain is developing, suggesting that enhanced sleep in young animals may be essential for proper brain development [1,2].

In *Drosophila*, as in mammals, sleep is regulated by neural networks that include sleep- and wake-promoting neurons. Dopamine is a key modulator of arousal, and both genetic and pharmacological manipulations of dopamine function support its role as a conserved wake-promoting transmitter [4,5]. In this new study, the authors find that dopamine levels are reduced in one day old flies, raising the possibility that a reduction in dopamine signaling underlies the early life increase in sleep [3].

Dopamine is expressed in only ~200 neurons in the fly brain, and these control diverse functions including memory, sleep, and courtship [6,7]. Subsets of arousal-promoting dopamine neurons target the dorsal Fan Shaped Body (dFSB), a brain region which expresses the dDA1 dopamine receptor [8,9]. Early life sleep deprivation causes memory defects that are rescued by blocking dDA1 receptor function, suggesting that dopamine signaling is particularly important for interactions between sleep and development [10]. Kayser *et al.* find that enhancing dopamine signaling through either genetic means or activation of dopamine neurons more potently suppresses sleep in one-day-old flies than older counterparts. The authors manipulated distinct classes of dopamine neurons to localize the relevant population of neurons underlying developmentally related changes in sleep. Selective activation of the wake-promoting dopamine neurons that project onto the dorsal dFSB prevents the increased sleep observed in one day old flies, suggesting that reduced dopamine release from dFSB-innervating dopamine neurons underlies the elevated sleep observed in young flies.

A large genetic toolkit is available for analysis of neural function in *Drosophila* and three independent indicators of neural activity suggest the activity of the dFSB-innervating dopamine neurons is lower in one day old flies compared to 8–10 day old counterparts. Both Cre–luciferase, an indicator of CREB activity, and the Ca^{2+} indicator CALexA (Ca^{2+}-dependent nuclear import of LexA) revealed reduced activity in the dopamine neurons that target the dFSB. Furthermore, the authors use the DopR-Tango system to directly measure dopamine activity in postsynaptic neurons of the dFSB. DopR tango, which uses a stable fluorescent reporter as a readout for dopamine signaling, confirmed reduced dopamine signaling in the dFSB of one day old flies [3,11]. Therefore, both pre- and postsynaptic analysis of dopamine neuron function indicates that dopamine release from the wake-promoting neurons that target the dFSB is reduced in young animals.

The dFSB promotes sleep in *Drosophila* and the authors sought to manipulate neural function of this region to functionally validate its role in early life sleep enhancement [12]. Genetic activation experiments suggest that the dFSB, but not other sleep-promoting regions, are already near maximal activity levels in one day old flies, fortifying the notion that this brain region is less inhibited by dopamine neurons early in life. Indeed, detection of Ca^{2+} levels with CALexA confirmed enhanced activity in the dFSB in young flies [3]. Therefore, these findings provide physiological and behavioral evidence for a neural circuit where activity of dFSB-innervating dopamine neurons is reduced in one day old flies, enhancing activity of the dFSB and promoting sleep.

What is the function of enhanced sleep in young animals? In mammals, sleep during early life is thought to be

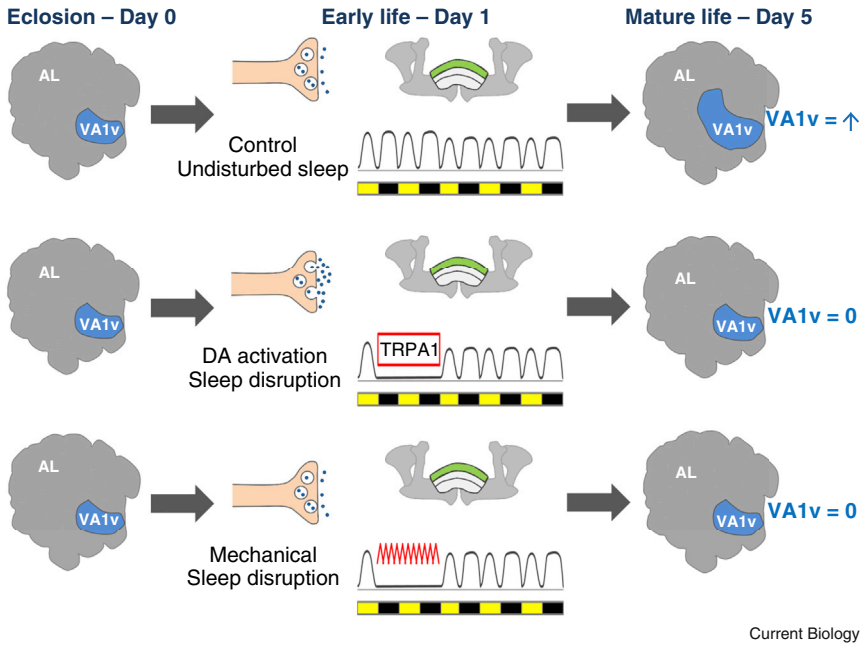

Figure 1. Schematic of dopamine-dependent modulation of VA1v development.

The VA1v (blue) glomerulus of the antennal lobe (AL) increases in size during early life. In control flies, reduced dopamine signaling to the dFSB (green) results in enhanced sleep. Sleep deprivation during post-eclosion days 1 and 2 through genetic expression of TRPA1 in dopamine neurons or mechanical disruption impairs VA1v development in mature flies. The volume of multiple antennal lobe glomeruli increases between day 0 and day 5, but changes in VA1v size are uniquely dependent on early life sleep.

important for cortical development and neuronal wiring [13,14]. Sleep regulates synapse strength and morphology, while manipulations that promote morphological plasticity enhance sleep, revealing reciprocal interactions between these processes [15,16]. Further, sleep deprivation in young animals impairs memory, revealing a critical developmental role for sleep in behavioral plasticity [10]. Kayser *et al.* find that sleep loss during early development results in reduced courtship and copulation success, indicating that early sleep is critical for development of a hard-wired behavior. The male-specific transcription factor fru^M is expressed in neurons required for male courtship behavior, including three olfactory glomeruli [17]. Interestingly, the size of the fru^M-expressing VA1v glomerulus is reduced in flies that are sleep deprived in early life. The VA1v glomerulus appears to be unique compared to other sexually dimorphic and fru^M-expressing glomeruli because its size robustly increases during early life (Figure 1) [3]. These results raise the possibility that early sleep is required for establishing the fly courtship circuitry.

Identification of the neural circuitry required for developmental changes in sleep and morphological plasticity of the brain provides a system for understanding the relationship between sleep and other biological processes. For example, the mechanism through which dopamine neurons increase activity between 1 and 5 day old flies remains to be determined. Further, the role for the VA1v glomerulus in male courtship behavior, and why this glomerulus is particularly sensitive to early life sleep deprivation are unclear. It is not known whether the reduced courtship success of flies subjected to early sleep deprivation results from changes in this sensory input pathway or other courtship circuitry within the brain.

In addition to the role of sleep during early development, it is possible that these findings provide avenues to address questions related to sleep and aging. Sleep becomes fragmented in aged flies through a mechanism that has been linked to both free radical production and insulin signaling [18,19]. Insulin signaling is a key factor regulating brain development and aging and therefore it is possible that conserved neural mechanisms underlie sleep changes in early adulthood and aged animals. Examining the functional connectivity between dopamine neurons and the dFSB in aged animals, or insulin signaling in young animals, may provide insight into age-dependent sleep fragmentation.

Taken together, this work furthers our understanding of how sleep regulates synaptic plasticity and brain development. It is possible that disruption of sleep in early life contributes to a number of neurodevelopmental disorders. For example, sleep disturbances are prevalent in children with autism, a disorder associated with altered brain development and connectivity, raising the possibility that early sleep loss contributes to the etiology of this disease [20]. The identification of dopamine as a key regulator of early life sleep opens the door to future studies examining the molecular basis for sleep-dependent regulation of brain development.

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Genomic Stability: Boosting Cohesion Corrects CIN

Chromosomal instability is a driving force for heterogeneity within tumours. A recent study shows that boosting sister chromatid cohesion corrects chromosomal instability in pRB-deficient cancer cells. This key finding provides an important lead to make tumours more susceptible to anti-cancer drugs.

Ahmed M.O. Elbatsh, René H. Medema, and Benjamin D. Rowland*

We have known for over a century that cancer cells often have chromosome numbers that deviate strongly from the healthy diploid karyotype. The German pathologist David Hansemann already noted back in 1890 that, even within a given tumour, some nuclei contain more chromatin than others [1]. We now know that this phenotype of chromosomal instability (CIN) can be found in many cancer types and is particularly abundant in solid tumours. CIN tumours are notoriously difficult to treat with anti-cancer drugs. The continuous gain and loss of chromosomes in these tumour cells is thought to be the driver for intra-tumour heterogeneity. This unstable karyotype facilitates the accelerated evolution of cancer cells such that they can easily adapt to evade the action of chemotherapeutic agents [2].

CIN is caused by errors in the segregation of chromosomes in mitosis. Chromosome segregation is controlled by an intricate cellular network, which ensures that each of the daughter cells inherits a complete copy of the genome. A key aspect of this network is sister chromatid cohesion, which is mediated by the cohesin complex. Cohesin is believed to entrap both sister chromatids of each individual chromosome inside its ring-shaped structure. Cohesin holds together the sister chromatids until the moment that all chromosomes are correctly attached to microtubules from both poles of the cell. Then the sudden destruction of cohesin allows the synchronous separation of the sister chromatids to the opposite poles. This process is tightly controlled, as premature loss of cohesion leads to segregation errors and to daughter cells with unequal karyotypes [3].

It is therefore perhaps not surprising that the cohesin complex is often found to be mutated in CIN tumours [4]. An important example is the finding that mutations in cohesin's STAG2 subunit are causative for the CIN phenotype of glioblastoma cells [5]. Cohesion is also affected by inactivation of the pRB tumour suppressor pathway. pRB inactivation leads to defects in sister chromatid cohesion and to segregation errors in mitosis, which in turn cause chromosomal instability [6,7]. Loss of pRB or its upstream regulator $p16^{ink4a}$ is a common feature of many human cancers [8]. pRB inactivation may thereby represent a common cause of CIN in a large portion of human cancers.

The CIN phenotype of tumours in essence creates two therapeutic possibilities. The first option is to augment the chromosome segregation defect such that the degree of the errors is no longer compatible with survival of the cancer cells. A potential drawback of this approach is that healthy cells will likely undergo segregation defects due to this treatment, which is dangerous by itself. A fundamentally different approach is to correct the segregation defects of CIN tumours. This treatment will not kill cancer cells directly, but it would slow down intra-tumour evolution. This could be beneficial to prevent the development of resistance to chemotherapeutic agents.

An important new study from the Dyson laboratory [9] now shows that the segregation errors caused by pRB inactivation can be corrected by boosting sister chromatid cohesion. How pRB regulates cohesion is not fully understood, but it appears to involve the Suv4-20H2 methyltransferase. pRB binds to this factor that is important for the recruitment of cohesin to heterochromatin [10,11]. Indeed, the segregation errors of pRB-deficient cells can be corrected by overexpression of Suv4-20H2. Importantly, the cohesion defect and the segregation errors can also be corrected by inactivation of cohesin's antagonist Wapl [9]. In the absence of Wapl, cohesin rings more stably associate with DNA [12]. The new paper goes on to show that