13. Thomas, S.J., Harrison, R.R., Leonardo, A., and Reynolds, M.S. (2012). A battery-free multichannel digital neural/emg telemetry system for flying insects. Biomed. Circ. Syst. IEEE Trans. *6*, 424–436.
14. Stange, G., and Howard, J. (1979). An ocellar dorsal light response in a dragonfly. J. Exp. Biol. *83*, 351–355.
15. Krapp, H.G. (2009). Ocelli. Curr. Biol. *19*, R435–R437.
16. Parsons, M.M., Krapp, H.G., and Laughlin, S.B. (2006). A motion-sensitive neurone responds to signals from the two visual systems of the blowfly, the compound eyes and ocelli. J. Exp. Biol. *209*, 4464–4474.

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Neurodegeneration: Paying It Off with Sleep

A new study in fruit flies suggests modulation of neural activity links sleep and Alzheimer's disease. Both sleep loss and amyloid beta increase neural excitability, which reinforces the accumulation of amyloid beta and shortens lifespan.

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Alzheimer's disease (AD) is a progressive, irreversible brain disorder that gradually erodes neural circuits underlying higher order cognitive functions including learning and memory. It is the most common neurodegenerative disorder of the elderly, afflicting over 35 million people worldwide [1]. Due to the absence of effective treatment options it is inevitably fatal. Thus, there is great interest in understanding the molecular and neural circuit changes that accompany AD, especially during its onset. An intriguing hypothesis [2], tested most recently by Tabuchi *et al.* in this issue of *Current Biology* [3], is that AD and poor quality sleep may be mutually enforcing, with overlap in the underlying dysfunctional mechanisms of control.

The brains of healthy, aging adults are subjected to various stressors that are thought to increase the likelihood of subsequent neural degeneration and dementia. Mounting evidence suggests a primary factor in AD pathogenesis is accumulation of amyloid beta (Aβ) protein within the brain. For example, heritable forms of AD are caused by mutations in a genetic precursor of Aβ called APP or in genes called presenilins, whose protein products process APP to Aβ. The risk of developing AD is also increased by certain alleles of the gene encoding apolipoprotein E, which may regulate clearance of Aβ [4]. Evidence suggests that an imbalance between clearance and production of Aβ results in toxic amyloid aggregates within neurons or as plaques between neurons that initially damage synapses and later cause neurodegeneration [5].

Several factors are known to modulate the toxicity of Aβ, and one of these is sleep. For example, in a mouse model of AD, knockout of the wake-promoting orexin gene reduces Aβ accumulation, an effect that is reversed by sleep deprivation [6]. In humans as well, the risk of accumulating Aβ is decreased by consolidated sleep, whereas the risk of developing certain forms of AD is enhanced by poor quality sleep. Intriguingly, insomnia is common among patients with AD, and the severity of this symptom is correlated with the degree of dementia [7]. Collectively this evidence has led to the hypothesis that AD and sleep have a bidirectional relationship that could inform understanding of both disorders and lead to improved treatment options for AD [2].

The fruit fly, *Drosophila melanogaster*, provides a powerful model system for investigating both neurodegenerative disease and sleep. Flies expressing human Aβ recapitulate several key features of AD, including Aβ accumulation, age-dependent learning impairment, and neurodegeneration [8]. *Drosophila* also show all the hallmarks of sleep in vertebrates, including elevated arousal threshold, homeostatic control, and electrophysiological distinction from wakefulness [9]. Although the mechanistic relationship between AD and sleep has eluded researchers, Tabuchi *et al.* [3] suggest that both phenomena may influence each other by altering neuronal activity (Figure 1). To examine the reciprocal relationship between AD and sleep in flies, the authors expressed different forms of Aβ throughout the nervous systems of flies. They found that Aβ reduced sleep, but only when it was expressed in pathogenic forms, especially a variant called Arctic, which encodes a membrane-tethered mutant form of human Aβ with enhanced toxicity [10].

It is common for AD patients to have reduced or disrupted sleep as well, supporting the possibility that Aβ suppresses sleep but also raising the additional possibility that poor quality sleep promotes the accumulation of Aβ. To test the latter hypothesis in flies, the authors measured Aβ levels following expression of Arctic in the mushroom bodies (MBs), a brain region required for many types of associative memory. Sleep deprivation following mechanical perturbation or thermogenetic activation of dopaminergic neurons increased Aβ levels, and sleep induction by activation of arousal-suppressing neurons decreased Aβ levels. Collectively these data suggest that waking interferes with and sleep facilitates clearance of Aβ from the brain. These experiments also support mammalian studies suggesting that sleep functions to rid the brain of metabolic wastes, including Aβ [11].

Although the cellular roles of both sleep and Aβ remain poorly understood, sleep appears to modulate synaptic strength across phyla. Studies in flies and rodents reveal the brain-wide accumulation of markers of synaptic potentiation during wakefulness which appear to dissipate during sleep [12,13]. These findings support another leading hypothesis about sleep, which is that it facilitates synaptic depression to counterbalance

net potentiation during waking and thus maintains overall homeostasis of synaptic strength across the brain [12]. If this were true then sleep's ability to suppress Aβ accumulation might be caused by a reduction in neuronal excitability, which would reduce synaptic activity. Consistent with this idea, neuronal activity positively regulates Aβ accumulation in mammals [14–17], and indeed Tabuchi *et al.* found that electrically silencing MBs with a transgenic potassium channel overcame the ability of sleep deprivation to elevate Aβ levels in the MBs. Thus, neuronal excitability acts downstream of sleep to regulate Aβ accumulation. This principle held up even when sleep was induced by activating arousal-suppressing neurons with a transgenic sodium channel: increasing excitability of these neurons overcame the ability of sleep to reduce Aβ levels in the same cells.

To determine if sleep normally regulates neuronal excitability, Tabuchi *et al.* then recorded from the large ventral lateral neurons (lLNvs) that regulate circadian behavior and sleep in flies. When synaptic transmission was blocked to isolate intrinsic excitability, the researchers found that the firing frequencies of lLNvs were elevated by sleep deprivation. Similar results were obtained in the absence of sleep deprivation when Arctic was expressed in the same neurons. Thus, both sleep deprivation and Aβ increase neuronal excitability. The authors then isolated individual currents in the same neurons at membrane potentials around threshold, where firing frequency is most likely to be affected. They found that a potassium current was reduced by sleep deprivation alone, and another potassium current was additionally reduced when sleep deprivation was combined with Arctic expression. Thus, sleep deprivation and Aβ converge mechanistically by decreasing potassium currents to increase neuronal excitability, which in turn may interfere with Aβ clearance.

Based on these findings, Tabuchi *et al.* then reasoned that, like sleep, pharmacological reduction of neuronal activity might also reduce Aβ accumulation. To test this hypothesis they fed flies the anti-convulsant levetiracetam (LEV). Strikingly, treatment with LEV reduced Aβ levels and improved longevity in Arctic transgenic flies, while having no noticeable effect on controls, suggesting that LEV protects against Aβ-induced toxicity. These results led the authors to propose that sleep loss and Aβ function in a positive feedback loop to potentiate each other's influence on hyperexcitability, which in turn interferes with Aβ clearance.

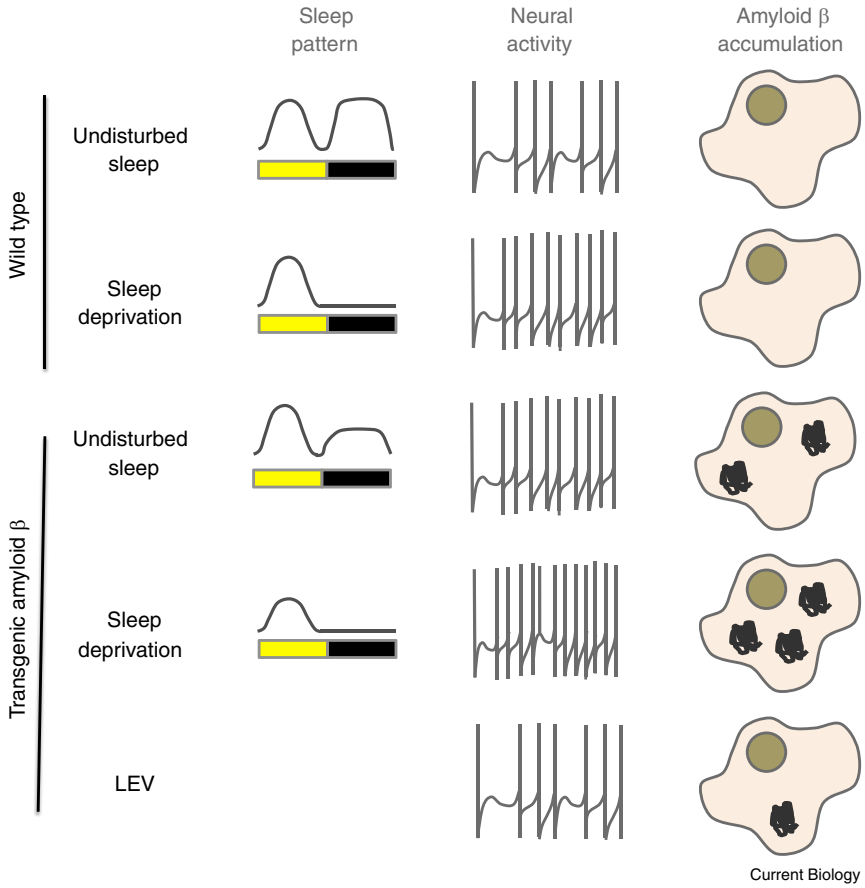

Figure 1. A model for the effects of sleep deprivation and neural activity on Aβ accumulation. Both sleep deprivation and transgenic expression of Aβ increase neural activity. Additionally, transgenic Aβ expression reduces sleep. The effects of Aβ expression on neural activity and sleep are rescued by feeding flies the anti-convulsant levetiracetam (LEV). LEV extends lifespan in Aβ-expressing flies but the effects on sleep have not been tested.

Although previous mammalian studies have correlated either Aβ-induced alterations with increased excitability or sleep changes with AD pathology, Tabuchi *et al.* provide a mechanistic link between all four phenomena. While these authors provide evidence for cell-autonomous accumulation of Aβ in neurons that is linked to sleep deprivation, other factors likely regulate Aβ buildup as well. For example, in mice, evidence suggests the sleep facilitates the removal of metabolic wastes by the brain's glymphatic system, an effect that extends to injected Aβ as well [11]. Furthermore, in mammals, Aβ plaques are found within and between neurons and therefore are unlikely to be governed exclusively by the cell-autonomous mechanisms described here. It is possible that Aβ accumulation is regulated by both cell-autonomous and systemic mechanisms, or that different regulatory mechanisms exist in flies and mammals. Addressing these questions in mammals will be important for understanding the applicability of the current findings to human disease etiology.

Another question raised by this study is whether treatment options for AD should be considered that focus on modulation of neuronal activity. Current therapies are largely palliative and attempt to boost signaling of nondegenerated synpases, whereas

many recent clinical trials for new AD medications have been focused on ways to reduce levels of toxic Aβ [1]. With respect to the latter it is notable that several Aβ-induced alterations have been recapitulated in wild-type mice following seizure induction or prevented in an AD mouse model by blocking overexcitation, thus supporting a role for neuronal hyperactivity in AD symptomology [18]. Surprisingly, among various anti-epileptics that have been tested head-to-head in a mouse model of AD, only LEV reduced hyperexcitability, remodeling of hippocampal circuits, synaptic dysfunction, and cognitive deficits [19]. It will thus be important to determine why LEV is more effective than other suppressors of neuronal activity at reducing AD symptomology and how exactly this drug mediates its effects. While attempts to slow neurodegeneration in AD patients have met little success, the finding that sleep loss and neural excitability may underlie Aβ accumulation in the fruit fly paves the way for new approaches to drug development.

References

1. Spencer, B., and Masliah, E. (2014). Immunotherapy for Alzheimer's disease: past, present and future. Front. Aging Neurosci. *6*, 114.
2. Ju, Y.E., Lucey, B.P., and Holtzman, D.M. (2014). Sleep and Alzheimer disease pathology–a bidirectional relationship. Nat. Rev. Neurol. *10*, 115–119.
3. Tabuchi, M., Lone, S.R., Liu, S., Liu, O., Zhang, J., Spira, A.P., and Wu, M.N. (2015). Sleep interacts with Abeta to modulate intrinsic neuronal excitability. Curr. Biol. *25*, 702–712.
4. Bertram, L., and Tanzi, R.E. (2008). Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses. Nat. Rev. Neurosci. *9*, 768–778.
5. Jack, C.R., Jr., and Holtzman, D.M. (2013). Biomarker modeling of Alzheimer's disease. Neuron *80*, 1347–1358.
6. Roh, J.H., Jiang, H., Finn, M.B., Stewart, F.R., Mahan, T.E., Cirrito, J.R., Heda, A., Snider, B.J., Li, M., Yanagisawa, M., *et al*. (2014). Potential role of orexin and sleep modulation in the pathogenesis of Alzheimer's disease. J. Exp. Med. *211*, 2487–2496.
7. Moran, M., Lynch, C.A., Walsh, C., Coen, R., Coakley, D., and Lawlor, B.A. (2005). Sleep disturbance in mild to moderate Alzheimer's disease. Sleep Med. *6*, 347–352.
8. Iijima, K., and Iijima-Ando, K. (2008). Drosophila models of Alzheimer's amyloidosis: the challenge of dissecting the complex mechanisms of toxicity of amyloid-beta 42. J. Alzheimers Dis. *15*, 523–540.
9. Sehgal, A., and Mignot, E. (2011). Genetics of sleep and sleep disorders. Cell *146*, 194–207.
10. Nilsberth, C., Westlind-Danielsson, A., Eckman, C.B., Condron, M.M., Axelman, K., Forsell, C., Stenh, C., Luthman, J., Teplow, D.B., Younkin, S.G., *et al*. (2001). The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. Nat. Neurosci. *4*, 887–893.
11. Xie, L., Kang, H., Xu, Q., Chen, M.J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D.J., Nicholson, C., Iliff, J.J., *et al*. (2013). Sleep drives metabolite clearance from the adult brain. Science *342*, 373–377.
12. Tononi, G., and Cirelli, C. (2014). Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. Neuron *81*, 12–34.
13. Yang, G., Lai, C.S., Cichon, J., Ma, L., Li, W., and Gan, W.B. (2014). Sleep promotes branch-specific formation of dendritic spines after learning. Science *344*, 1173–1178.
14. Kamenetz, F., Tomita, T., Hsieh, H., Seabrook, G., Borchelt, D., Iwatsubo, T., Sisodia, S., and Malinow, R. (2003). APP processing and synaptic function. Neuron *37*, 925–937.
15. Cirrito, J.R., Kang, J.E., Lee, J., Stewart, F.R., Verges, D.K., Silverio, L.M., Bu, G., Mennerick, S., and Holtzman, D.M. (2008). Endocytosis is required for synaptic activity-dependent release of amyloid-beta in vivo. Neuron *58*, 42–51.
16. Cirrito, J.R., Yamada, K.A., Finn, M.B., Sloviter, R.S., Bales, K.R., May, P.C., Schoepp, D.D., Paul, S.M., Mennerick, S., and Holtzman, D.M. (2005). Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. Neuron *48*, 913–922.
17. Bero, A.W., Yan, P., Roh, J.H., Cirrito, J.R., Stewart, F.R., Raichle, M.E., Lee, J.M., and Holtzman, D.M. (2011). Neuronal activity regulates the regional vulnerability to amyloid-beta deposition. Nat. Neurosci. *14*, 750–756.
18. Palop, J.J., Chin, J., Roberson, E.D., Wang, J., Thwin, M.T., Bien-Ly, N., Yoo, J., Ho, K.O., Yu, G.Q., Kreitzer, A., *et al*. (2007). Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron *55*, 697–711.
19. Sanchez, P.E., Zhu, L., Verret, L., Vossel, K.A., Orr, A.G., Cirrito, J.R., Devidze, N., Ho, K., Yu, G.Q., Palop, J.J., *et al*. (2012). Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. Proc. Natl. Acad. Sci. USA *109*, E2895–E2903.

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Animal Cognition: Bumble Bees Suffer 'False Memories'

The existence of 'false memories', where individuals remember events that they have never actually experienced, is well established in humans. Now a new study reports that insects similarly form illusory memories through merging of memory traces.

Judith Reinhard

Memories define us as individuals. We accumulate them throughout our lives, lay some of them down as long-term memories that last a life-time, while other memories are short-lived and only last for minutes or days. Although we tend to believe that our memories are precise recollections of past events, memories are a fickle thing. No two people have the exact same memories even if they experience the same events, which is why eye witness records are notoriously unreliable. Memories may change over time as the brain recalls and re-consolidates them, resulting in various types of 'false memories', where individuals remember events or objects that they have never actually been exposed to or merge different memory traces into an illusory memory [1–3]. For example, when presented with a list of words all pertaining to fruit, people would remember the word 'apple' even if it was not on the list. While the existence of such memory errors is well established in humans [2,3], it has never before been shown in animals, although many animal species from insects to vertebrates are known for their sophisticated learning and memory abilities. Now, a new study by Kate Hunt and Lars Chittka [4], published in this issue of *Current Biology*, has revealed that an insect with a small brain, the bumble bee, also suffers false memories. The study suggests that memory traces for different visual stimuli such as a colour and a black-and-white pattern are merged