Sleep: Astrocytes Take their Toll on Tired Flies

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Calcium signalling in astrocytes modulates sleep, yet how astrocytes communicate with neural circuits that control sleep is unclear. A new study now uncovers a calcium-dependent relay between astrocytes and neurons that promotes sleep homeostasis in fruit flies.

Sleep is a near-universal behaviour in metazoan genera as diverse as *Hydra* and *Homo* [1, 2]. From clubbers to child carers of our own species, sleepless nights produce an all-too-familiar feeling: a deep urge to nod off during the following day. This homeostatic response is a fundamental feature of sleep, yet the mechanisms that enable it remain largely mysterious.

Much work in both vertebrate and invertebrate models has focused on identifying neural circuits that control the multifaceted aspects of sleep, including sleep homeostasis [3, 4]. However, neurons are not the only inhabitant of the brain. On the contrary, in mammals, glia (encompassing astrocytes, oligodendrocytes, and microglial cells) are roughly as prevalent as neurons. The most common glial cell-type are astrocytes. The elongate processes of individual astrocytes are interconnected via gap junctions, allowing astrocytes to form a syncytial network throughout the brain. Their processes also envelop synapses, placing astrocytes in a prime position to sense the activity of their neuronal neighbours and induce corresponding changes in synaptic transmission and plasticity. Conversely, synaptic transmission itself can increase intracellular calcium within nearby astrocyte processes [5], illustrating that neurons and astrocytes communicate with each other in a bi-directional manner.

Two recent studies have shown that this communication plays an important role in sleep. Examining astrocytes in regions of the mouse cortex, the authors found that the pattern of calcium fluctuations in astrocytic processes varied across sleep and wakefulness [6, 7], and that suppressing calcium release from intracellular stores of astrocytes disrupted homeostatic responses to sleep deprivation [7]. These works suggest that astrocytes are capable of sensing sleep need and transmitting this information to sleep-regulatory neurons in a calcium-dependent manner. Yet the mechanistic basis of such neuro-glial communication is unclear. Using a series of state-of-the-art approaches, Blum *et al*., now tackle this issue in the fruit fly, *Drosophila* [8].

Blum *et al*., first examined whether calcium levels in *Drosophila* astrocytes vary with sleep need. Using fluorescent calcium sensors, they found that prolonged wakefulness or mechanically induced sleep deprivation elevated intracellular calcium and increased the frequency of calcium spikes in astrocytes. Of note, increases in intracellular calcium following naturally occurring wakefulness were mainly observed in astrocytic processes, consistent with the above studies in the mouse cortex [6, 7].

What channels gate these increases in intracellular calcium? By reducing the expression of a range of calcium channels in astrocytes using RNA interference, Blum *et al*., identified an important role for Ca-α1D, an L-type voltage-gated calcium channel, and further showed that Ca-α1D knockdown in astrocytes suppressed homeostatic increases in sleep following sleep

deprivation. These results suggest that, similarly to their mammalian counterparts [7], calcium levels in *Drosophila* astrocytes encode sleep need. Consistent with this hypothesis, elevating intracellular calcium in astrocytes via a heat-activated TrpA1 cation channel induced a sleep state that persisted far beyond the period of TrpA1 activation.

Taking advantage of the utility of *Drosophila* to perform unbiased genetic screens, Blum *et al*., next identified tyramine receptor II (TyrRII), a G-protein coupled receptor activated by biogenic amines, as an important protein acting downstream of Ca-α1D. TyrRII expression in astrocytes is increased following sleep deprivation in a Ca-α1D-dependent manner, and this upregulation is required to elevate astrocytic calcium and drive homeostatic increases in sleep following sleep deprivation.

Does this astrocytic pathway influence the excitability of neural circuits known to regulate sleep? Indeed it does. The authors previously identified a population of neurons in the fly brain called ellipsoid body R5-neurons, whose excitability scales with sleep need and which promote sleep following sleep deprivation [9]. Blum *et al*., found that increasing astrocytic calcium enhanced the excitability of R5-neurons, and that silencing these neurons partially suppressed the sleep-promoting effect of elevating astrocytic calcium, placing these two cell-types in a common circuit. Excitingly, they also found that Spätzle (Spz), a functional analog of the mammalian cytokine Interleukin-1 (IL-1), is transcriptionally upregulated in response to elevated astrocytic calcium levels, and acts as a secreted ligand to facilitate communication between astrocytes and R5-neurons.

Drosophila homozygous for *spz* mutations exhibit defects in dorso-ventral patterning during development, resulting in embryos morphologically reminiscent of the German egg noodles after which Spz is named [10]. Spz also plays an important role in the innate immune response, and functions in both pathways by acting as a ligand for a receptor called Toll [11, 12]. Consistent with this, knockdown of Toll in R5-neurons suppressed increases in their excitability, as well as homeostatic increases in sleep, following sleep deprivation.

Collectively, these results suggest a mechanism (Figure 1) in which Ca-α1D and TyrRII initially act in a positive feedback loop to elevate astrocytic calcium levels and enhance the capability of astrocytes to sense extracellular monoamines that accumulate during wakefulness. The resulting secretion of Spz then increases the excitability of R5-neurons via Toll, thus promoting sleep following sleep deprivation.

Blum *et al*.'s work is exciting not only due to their identification of this hitherto undescribed means of neuro-glial communication, but because their results yield a series of fascinating questions that promise to drive new avenues of investigation. For example, how are astrocytic Ca-α1D channels initially activated during wakefulness to set this signalling cascade in motion? Elevated extracellular K^+ or glutamate activate L-type calcium channels in mammalian astrocytes via membrane depolarisation and astrocytic glutamate receptors respectively [13]. Long periods of neuronal firing and synaptic release during waking periods may thus eventually provide sufficient spill-over of K^+/glutamate to kick-start the Ca-α1D-TyrRII positive feedback loop, driving sustained elevations in astrocytic calcium levels. This paradigm argues against the notion that a single key sleep-promoting substance (or somnogen) induces sleep homeostasis. Instead, it suggests that numerous indicators of neuronal activity, including K^+, glutamate, and monoamines such as dopamine, serotonin, and octopamine (the *Drosophila* equivalent of noradrenaline), may be synergistically sensed by astrocytes that, in turn, modulate the excitability of sleep-promoting neurons.

Secondly, how does signalling downstream of Toll alter the excitability of R5-neurons? Canonical Toll signalling results in the activation of two transcription factors, Dif and Dorsal

[14]. Do these transcription factors influence the activity of sleep-regulatory neurons, and if so, what transcriptional programs do they induce to do so?

One surprising finding by Blum *et al*. is that increasing astrocytic calcium also paradoxically *reduces* the excitability of a population of wake-promoting cells called large LN_v (l-LN_v) neurons [15]. Can Spz secreted from astrocytes induce opposing alterations in the excitability of different neurons? An array of Toll receptor homologues that may bind Spz are expressed in the *Drosophila* nervous system, each of which exhibits a unique expression pattern [16]. Cell-specific complements of distinct Toll receptors may thus allow astrocytes to differentially impact the excitability of wake- versus sleep-promoting circuits. Alternatively, specificity may be encoded at the level of astrocytes themselves. Recent studies have revealed a surprising degree of molecular and functional diversity between hippocampal and striatal astrocytes in the mouse brain [17]. Since it is currently unclear whether the inhibition l-LN_v neurons by astrocytes is Spz-dependent, it remains possible that functionally distinct populations of astrocytes may modulate R5- and l-LN_v neurons by secreting different sleep-modulatory signalling molecules.

Finally, comparing Blum *et al*.'s results to prior work in mice suggests that vertebrate and invertebrate astrocytes influence sleep through both analogous and distinct pathways. Akin to the sleep-promoting role of Spz, IL-1 secretion from mammalian astrocytes also enhances slow wave sleep [18], showing that immuno-modulatory ligands influence sleep in both species. Conversely, murine and *Drosophila* astrocytes appear to utilise different mechanisms to increase intracellular calcium during wakefulness: IP_3 receptor- and STIM1-dependent Ca^{2+}-release from the endoplasmic reticulum in mice [6, 7], and influx of extracellular Ca^{2+} through L-type calcium channels in *Drosophila* [8]. It is noteworthy that mammalian L-type calcium channels have been linked to IL-1 secretion in the context of astrocyte activation during inflammatory responses to mechanical nerve injury, rather than in relation to sleep [19]. Since the most commonly used protocol for sleep depriving flies involves intermittent mechanical shaking throughout the night, it will be interesting to test whether the pathway identified by Blum *et al*., also gates homeostatic changes in sleep induced by more subtle methods of sleep deprivation [20].

Regardless of these questions, Blum *et al*.'s study elegantly points us towards a more integrated view of how diverse cell-types in the nervous system coordinate to induce the restorative power of a good nap. Something I suspect many readers could do with!

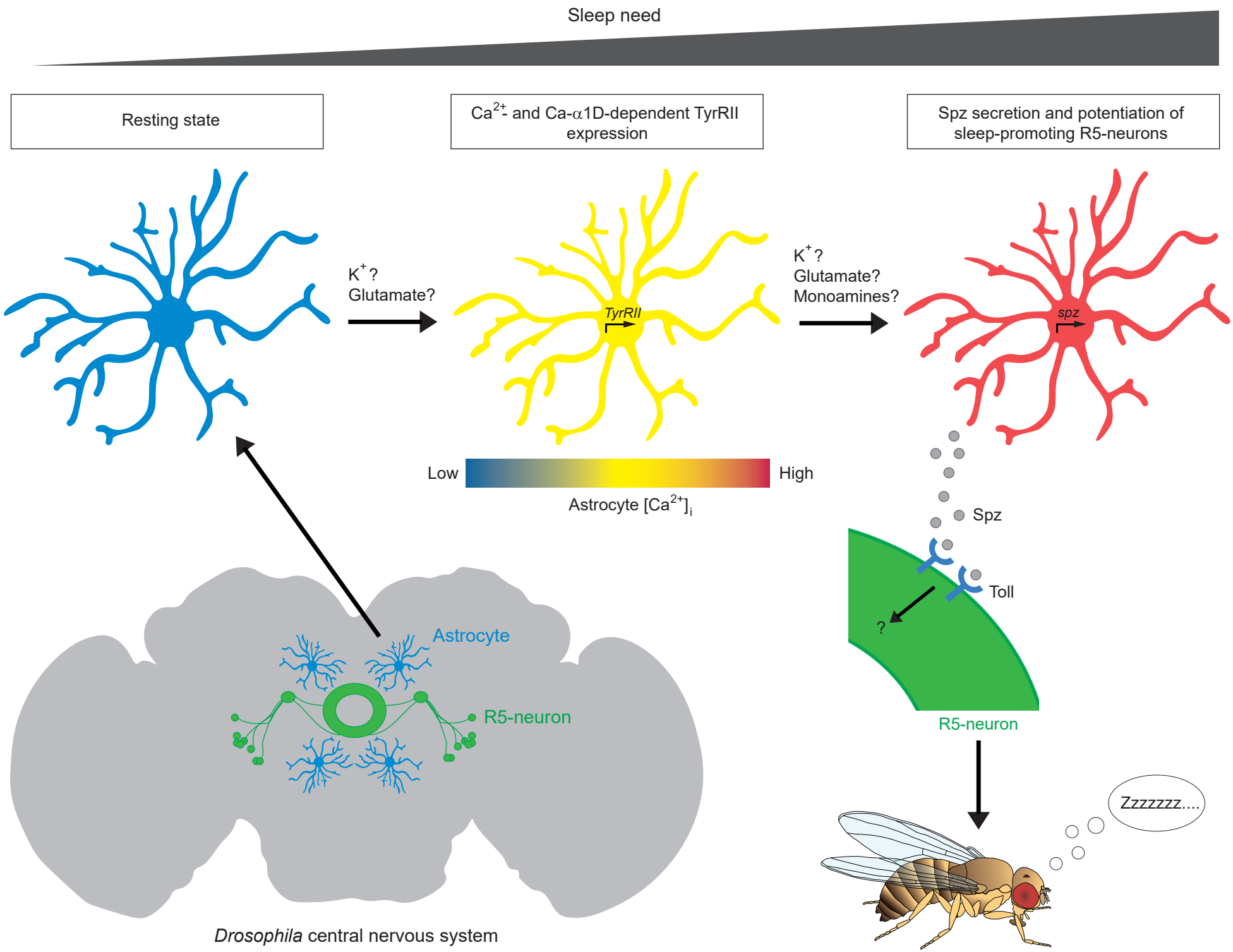
Figure 1: Mechanistic model linking wake-induced increases in astrocytic Ca^{2+} levels to the activity of sleep-promoting neurons.

A schematic of the *Drosophila* brain shows the approximate location and morphology of sleep-promoting R5-neurons, alongside neighboring astrocytes. Prolonged wakefulness activates astrocytic Ca-α1D channels, kick-starting a positive feedback loop that sequentially elevates astrocytic Ca^{2+} levels and leads to secretion of Spz. This ligand then acts via Toll receptors to enhance the excitability of R5-neurons, sending flies to sleep. Intriguing questions arising from Blum et al.'s study are highlighted (?). Astrocyte and *Drosophila* images are modified from Scidraw.io (doi.org/10.5281/zenodo.3926048 and doi.org/10.5281/zenodo.392591).

References

1. Kanaya, H.J., Park, S., Kim, J.H., Kusumi, J., Krenenou, S., Sawatari, E., Sato, A., Lee, J., Bang, H., Kobayakawa, Y., et al. (2020). A sleep-like state in Hydra unravels conserved sleep mechanisms during the evolutionary development of the central nervous system. Sci Adv *6*.
2. Sehgal, A., and Mignot, E. (2011). Genetics of sleep and sleep disorders. Cell *146*, 194-207.
3. Tomita, J., Ban, G., and Kume, K. (2017). Genes and neural circuits for sleep of the fruit fly. Neurosci Res *118*, 82-91.
4. Weber, F., and Dan, Y. (2016). Circuit-based interrogation of sleep control. Nature *538*, 51-59.
5. Di Castro, M.A., Chuquet, J., Liaudet, N., Bhaukaurally, K., Santello, M., Bouvier, D., Tiret, P., and Volterra, A. (2011). Local Ca2+ detection and modulation of synaptic release by astrocytes. Nat Neurosci *14*, 1276-1284.
6. Bojarskaite, L., Bjornstad, D.M., Pettersen, K.H., Cunen, C., Hermansen, G.H., Abjorsbraten, K.S., Chambers, A.R., Sprengel, R., Vervaeke, K., Tang, W., et al. (2020). Astrocytic Ca(2+) signaling is reduced during sleep and is involved in the regulation of slow wave sleep. Nat Commun *11*, 3240.
7. Ingiosi, A.M., Hayworth, C.R., Harvey, D.O., Singletary, K.G., Rempe, M.J., Wisor, J.P., and Frank, M.G. (2020). A Role for Astroglial Calcium in Mammalian Sleep and Sleep Regulation. Curr Biol.
8. Blum, I., Keles, M., Baz, E.-S., Han, E., Park, K., Luu, S., Issa, H., Brown, M., Ho, M., Tabuchi, M., et al. (2020). Astroglial Calcium Signaling Encodes Sleep Need in Drosophila. Curr Biol *This issue*.
9. Liu, S., Liu, Q., Tabuchi, M., and Wu, M.N. (2016). Sleep Drive Is Encoded by Neural Plastic Changes in a Dedicated Circuit. Cell *165*, 1347-1360.
10. Morisato, D., and Anderson, K.V. (1994). The spatzle gene encodes a component of the extracellular signaling pathway establishing the dorsal-ventral pattern of the Drosophila embryo. Cell *76*, 677-688.
11. Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J.M., and Hoffmann, J.A. (1996). The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell *86*, 973-983.
12. Hu, X., Yagi, Y., Tanji, T., Zhou, S., and Ip, Y.T. (2004). Multimerization and interaction of Toll and Spatzle in Drosophila. Proc Natl Acad Sci U S A *101*, 9369-9374.
13. Letellier, M., Park, Y.K., Chater, T.E., Chipman, P.H., Gautam, S.G., Oshima-Takago, T., and Goda, Y. (2016). Astrocytes regulate heterogeneity of presynaptic strengths in hippocampal networks. Proc Natl Acad Sci U S A *113*, E2685-2694.
14. Valanne, S., Wang, J.H., and Ramet, M. (2011). The Drosophila Toll signaling pathway. J Immunol *186*, 649-656.
15. Sheeba, V., Fogle, K.J., Kaneko, M., Rashid, S., Chou, Y.T., Sharma, V.K., and Holmes, T.C. (2008). Large ventral lateral neurons modulate arousal and sleep in Drosophila. Curr Biol *18*, 1537-1545.
16. Li, G., Forero, M.G., Wentzell, J.S., Durmus, I., Wolf, R., Anthoney, N.C., Parker, M., Jiang, R., Hasenauer, J., Strausfeld, N.J., et al. (2020). A Toll-receptor map underlies structural brain plasticity. Elife *9*.
17. Chai, H., Diaz-Castro, B., Shigetomi, E., Monte, E., Octeau, J.C., Yu, X., Cohn, W., Rajendran, P.S., Vondriska, T.M., Whitelegge, J.P., et al. (2017). Neural Circuit-Specialized Astrocytes: Transcriptomic, Proteomic, Morphological, and Functional Evidence. Neuron *95*, 531-549 e539.
18. Krueger, J.M., Walter, J., Dinarello, C.A., Wolff, S.M., and Chedid, L. (1984). Sleep-promoting effects of endogenous pyrogen (interleukin-1). Am J Physiol *246*, R994-999.

19. Cheli, V.T., Santiago Gonzalez, D.A., Smith, J., Spreuer, V., Murphy, G.G., and Paez, P.M. (2016). L-type voltage-operated calcium channels contribute to astrocyte activation In vitro. Glia *64*, 1396-1415.
20. Geissmann, Q., Garcia Rodriguez, L., Beckwith, E.J., French, A.S., Jamasb, A.R., and Gilestro, G.F. (2017). Ethoscopes: An open platform for high-throughput ethomics. PLoS Biol *15*, e2003026.


Sleep need
Resting state
Ca2+- and Ca-α1D-dependent TyrRII expression
Spz secretion and potentiation of sleep-promoting R5-neurons
K+?
Glutamate?
TyrRII
K+?
Glutamate?
Monoamines?
spz
Low
High
Astrocyte [Ca2+]i
Spz
Toll
?
R5-neuron
Astrocyte
R5-neuron
Zzzzzzz....
Drosophila central nervous system