Research Highlight #2006

Dr. Ashley Ingiosi
The Ohio State University

Astrocytes play a starring role in sleep regulation

Dr. Ingiosi's research focuses on the role of astrocytes, a type of glial cell in the brain, in sleep and sleep homeostasis. Sleep plays a critical role in overall mammalian health, although many aspects of sleep regulation are not yet understood. When Dr. Ingiosi was choosing her graduate project at the University of Michigan, she wanted to contribute novel advancements to her field. She noticed that most of what was known about sleep was based on the study of neurons, however, there was another brain cell type—glia—that was potentially important to sleep and there were not many in vivo studies looking at these non-neuronal cell types in the brain.

> "Astrocytes were traditionally viewed as support cells for the neurons that play a passive role in the brain because they're not electrically excitable. We usually measure neuronal activity via changes in electrical signals, but astrocytes don't have that same sort of electrical behavior, so we haven't really had the tools to study them for a long time, which is probably one reason why we hadn't really studied them. Also because they were given a passive, supportive role, people didn't think that they were as important."

When Dr. Ingiosi started her postdoc work using calcium imaging on astrocytes to study sleep, she did not know if there were other cell types besides neurons in the brain whose activity was changing with sleep states or sleep need. Through her research, Dr. Ingiosi discovered that astrocyte activity indeed does change dynamically with sleep state. In her 2020 paper published in the journal, *Current Biology*, she provided some of the first in vivo evidence of this, showing astroglial calcium signals change dynamically with sleep, wake, and sleep loss. Indeed, astroglial calcium encodes changes in sleep need, and synchrony of astroglial calcium signals changes with vigilance state and sleep need.

> "This is the first evidence we have in vivo that there are other types of cells whose activity changes with different sleep/wake states and our study is the only one that has looked at astroglial calcium with respect to sleep need, along with a similar study in flies. The really cool thing is that our study in mice and the study in flies had the same outcome, which means that this is probably an important mechanism of sleep regulation because it's been conserved from flies to mice."

This exciting finding led Dr. Ingiosi to continue focusing on astrocytes during her postdoc work. Now as she moves into her new position as Assistant Professor at The Ohio State University, she will start investigating how astrocytes interact with neurons to influence the sleep/wake behavior and which signaling pathways in the astrocytes are important.

Using multiphoton microscopy and miniscopes

To effectively study the role of astrocytes in sleep and sleep regulation, Dr. Ingiosi uses a couple of different imaging tools. Electroencephalography (EEG) is a useful for monitoring brain waves and changes across sleep states. However, because astrocytes are not electrically excitable like neurons, EEG cannot provide a direct measure of astroglial activity. Since astrocytes use calcium to mediate many

ABOUT THE RESEARCHER

Ashley Ingiosi, Ph.D., is a BRAIN Initiative K99/R00 investigator and Ruth L. Kirschstein postdoctoral fellow in the laboratory of Marcos Frank, Ph.D., at Washington State University in Spokane. She earned a Ph.D. in Neuroscience and a B.S. Biopsychology & Cognitive Science and General Biology from the University of Michigan. In Fall 2022, Dr. Ingiosi will begin as an Assistant Professor in the Department of Neuroscience at The Ohio State University.

Website: Visit Dr. Ingiosi's website

Recent Publications

Ingiosi, A. M., Hayworth, C. R., Harvey, D. O., Singletary, K. G., Rempe, M. J., Wisor, J. P., & Frank, M. G. (2020). A role for astroglial calcium in mammalian sleep and sleep regulation. *Current Biology*, 30(22). https://doi.org/10.1016/j.cub.2020.08.052

functions, genetically coded calcium indicators can be used to look into how astrocyte activity changes across states and with sleep need. To investigate the calcium indicators, fluorescence microscopy is necessary. In the work of Ingiosi et al., 2020, she used Bruker's Ultima Investigator multiphoton microscopy for this investigation.

> "The big advantage of the two-photon microscope is that we get really good spatial resolution where we can see the fine processes of an astrocyte. An astrocyte itself isn't big, and they don't really send off long projections like neurons do, they've got a dense network of processes that surround the cell body. These processes interact with other neurons or with the vasculature. With two-photon we can see what's happening in different parts of the cell, which is interesting because the calcium activity in an astrocyte isn't homogeneous. The cell body primarily uses different sources of calcium than the processes use. Then we're able to take that calcium activity and align it with our EEG activity so we can know exactly what's happening in the astrocytes with their calcium activity, and how that aligns with how we define sleep/wake behavior."

The two-photon microscope has the advantage of the highest resolution to see fine structures and how activity can be compartmentalized within a single cell, but this technique requires a head-fixed mouse. Dr. Ingiosi paired her experiments with the two-photon microscope with a miniscope, which is a small, lightweight microscope that is mounted on the head of the animal so it can run around and engage in natural sleep behaviors. While it is advantageous to image in a freely behaving mouse, the miniscope lacks high resolution imaging capabilities beyond cellular resolution. Therefore, pairing two-photon microscopy with a miniscope is beneficial for investigating calcium signaling in astrocytes in mice.

Studying the role of astrocytes in sleep regulation has important implications for building an understanding of sleep disorders. Sleep is regulated through a two-process model, which includes Process C and Process S. Process C is the circadian process that regulates arousal throughout the day, therefore dictating when we sleep. Scientists have a good understanding of how this is regulated due to the discovery of core clock genes, and it was discovered that astrocytes have a role in this process. On the other hand, Process S determines the amount and intensity of sleep need. Process S describes the "sleep debt" that is accrued while we are awake and paid off when sleep occurs. Unlike Process C, Process S is more elusive.

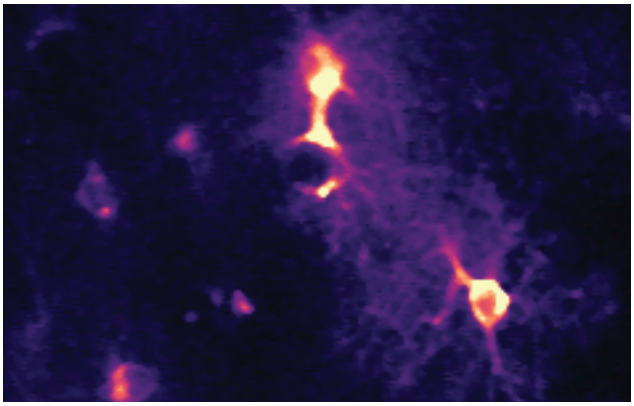

FIGURE 2

Two-photon image of astrocytes in the frontal cortex expressing the genetically encoded calcium indicator GCaMP6f.

> "We still don't have like a good handle on Process S. We don't really have "Process S genes" like we have Process C genes. This is where digging into other types of cell types, genes, and pathways, can get us closer to figuring out what might be the underlying processes for sleep need. Because sleep need can be disrupted with sleep disorders, trying to figure out I how that process is regulated under normal conditions can give us clues as to what's changing when there are problems."

Sleep is essential for mammalian health, therefore studying the processes and discovering novel mechanisms in sleep regulation is critical research. By combining imaging techniques, including multiphoton microscopy and miniscopes, to investigate understudied astrocyte processes Dr. Ingiosi treads new frontiers in research on sleep regulation in mammals.

Learn more

To discover more about multiphoton microscopy and Bruker's Ultima multiphoton microscopes, visit: https://www.bruker.com/Ultima

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