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Diving Deep for Sleep: How pH and Blood Volume in the Lateral Hypothalamus Impact REM

Properties of REM Sleep Alterations With Epilepsy

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It is usually assumed that individuals rest during sleep. However, coordinated neural activity that presumably requires high energy consumption is increased during REM sleep. Here, using freely moving male transgenic mice, the local brain environment and astrocyte activity during REM sleep were examined using the fibre photometry method with an optical fibre inserted deep into the lateral hypothalamus, a region that is linked with controlling sleep and metabolic state of the entire brain. Optical fluctuations of endogenous autofluorescence of the brain parenchyma or fluorescence of sensors for Ca2+ or pH expressed in astrocytes were examined. Using a newly devised method for analysis, changes in cytosolic Ca2+ and pH in astrocytes and changes in the local brain blood volume (BBV) were extracted. On REM sleep, astrocytic Ca2+ decreases, pH decreases (acidification) and BBV increases. Acidification was unexpected, as an increase in BBV would result in efficient carbon dioxide and/or lactate removal, which leads to alkalinization of the local brain environment. Acidification could be a result of increased glutamate transporter activity due to enhanced neuronal activity and/or aerobic metabolism in astrocytes. Notably, optical signal changes preceded the onset of the electrophysiological property signature of REM sleep by ~20-30 s. This suggests that changes in the local brain environment have strong control over the state of neuronal cell activity. With repeated stimulation of the hippocampus, seizure response gradually develops through kindling. After a fully kindled state was obtained with multiple days of stimuli, the optical properties of REM sleep at the lateral hypothalamus were examined again. Although a negative deflection of the detected optical signal was observed during REM sleep after kindling, the estimated component changed. The decrease in Ca^{2+} and increase in BBV were minimal, and a large decrease in pH (acidification) emerged. This acidic shift may trigger an additional gliotransmitter release from astrocytes, which could lead to a state of hyperexcitable brain. As the properties of REM sleep change with the development of epilepsy, REM sleep analysis may serve as a biomarker of epileptogenesis severity. REM sleep analysis may also predict whether a specific REM sleep episode triggers post-sleep seizures.

Commentary

The reliable cyclicity of non-rapid eye movement (NREM) sleep, REM sleep, and wake is vital for optimal brain function and overall health. The neurobiological processes that regulate this cyclicity are not completely understood nor how these processes are impacted by neurological disorders such as epilepsy. The study by Ikoma and colleagues dives deep into the brain, into the lateral hypothalamus, and provides insight into how epileptogenesis changes this local environment and how these changes may impact REM sleep.[1]

The lateral hypothalamus is a key regulatory region that houses neurons expressing important peptides including orexin. Orexin neurons integrate signals of physiology, behavioral state, metabolism, and environment, then exert regulatory adaptations to maintain homeostasis or establish a new homeostasis. The process of information integration and output is very dynamic and constant. By projecting to many regions including the cortex, thalamus, other hypothalamic regions, midbrain and brainstem, orexin influences many functions including sleep-wake transitions and cardiorespiration. Importantly, the function of orexin neurons depends on astrocytes.[2-4]

In the study by Ikoma and colleagues, changes in the pH of astrocytes and the brain blood volume were examined in the lateral hypothalamus during REM sleep. Using an established preclinical model of epileptogenesis mice were rapidly kindled via a series of electrical stimulations. Sequential stimulations over multiple days cause a reduction in seizure threshold and ultimately induce a severe seizure. Before and after kindling, changes in pH and blood volume were assessed via fiber photometry in transgenic mice that expressed optical sensor proteins in astrocytes. Fiber photometry allows for relatively stress-free continuous recording in deep brain structures.

A significant finding of the study was that a drop in astrocytic pH in the lateral hypothalamus preceded REM sleep.

The acidification of the astrocytes occurred seconds prior to the onset of REM sleep (which was determined by changes in cortical electroencephalography (EEG) and electromyography (EMG)). In addition, experimentally reducing the pH of astrocytes in the lateral hypothalamus for more than 30 seconds induced behavior (freezing) and frequency band changes reminiscent of REM sleep. In humans, REM sleep is known to be associated with hypoventilation-induced mild hypercapnia (low pH) and a subsequent increase in cerebral blood flow.[5] This study found that the astrocytic acidification during REM sleep was associated with increased blood volume deep in the lateral hypothalamus.

An intriguing finding was that this relationship between pH and blood volume during REM sleep was dysregulated in animals that were kindled. In kindled animals, the drop in astrocytic pH was more pronounced, cortical REM sleep durations were shortened, and there was minimal increase in brain blood volume.

An important technical finding of the study was that in addition to local autofluorescence changes during REM sleep, they identified other confounding variables that caused changes in fluorescence, factors for which previous studies have not accounted. Through a series of careful, systematic experiments and calculations, the authors described a novel analytical method to be able to confidently attribute a change in fluorescence to one variable. This degree of insightful analysis is critical in preclinical studies and will have a significant impact on future fluorescent *in vivo* studies.

While this study correctly identified rapid kindling as a model of epileptogenesis because it induces neuroplastic changes that lower seizure threshold, it does not model epilepsy because mice do not have spontaneous recurring seizures, and is thus a limitation. A second limitation is that because it is unclear how much time elapsed between the kindling-induced seizure and the observed effects, it is difficult to distinguish if the effects were a result of the epileptogenic process or the seizure. Finally, 3 of the conclusions should be regarded as speculative and were not directly tested. First, while acidification of astrocytes in the hypothalamus may promote hyperexcitability, whether it contributes to seizures will require more research. Second, while the analysis of sleep architecture is important in epilepsy, use of REM sleep analysis as a biomarker of epileptogenesis may be premature because many things can cause shorten REM duration, and it is not common for patients to be assessed during the epileptogenic period. Third, whether REM sleep analysis can be used to predict post-sleep seizures is an intriguing idea, but will require further research.

This study expands our understanding of REM sleep neurobiology and brain vascular physiology by proposing a cyclic axis, interlacing astrocytic pH in the lateral hypothalamus—REM sleep onset—hypoventilation—hypercapnia (increased CO_2)—increased local brain blood volume. This study also furthers our understanding of how kindling may induce neuroplastic pathology in the lateral hypothalamus, a region that contributes to establishing and resetting homeostatic thresholds.[6-11] With chemosensing orexin neurons and their prolific projections, including to key cardiorespiratory centers, the consequences of this neuroplasticity may be widespread. The idea that there may be a disconnect in the lateral hypothalamic astrocytic pH—REM sleep duration—ventilation—blood gases—local brain blood volume axis—is intriguing for a couple of reasons. First, the ratio of blood flow to metabolism influences central chemoreceptor activity,[5] thus a disconnect may impair central chemoresponses. In addition, shortened REM duration may mechanistically contribute to REM deficiency associated with specific epilepsy syndromes.

The study by Ikoma and colleagues advances our appreciation of local hypothalamic influence on EEG activity and blood volume. The description of the processes involved in shortening REM duration in a well-used model of epileptogenesis provides a platform from which future studies can dive into the mechanistic end. In addition, future studies can determine for how long after a seizure these effects persist. Further, whether changes in astrocytic pH alter the function of lateral hypothalamic chemosensitive orexin neurons would be interesting, as these neurons project to respiratory centers and influence inspiration, expiration, and breathing frequency. Whether a dysfunction in this axis impacts responses to hypercapnia-hypoxia challenges, such as those associated with seizures and apnea, would be relevant not only to epilepsy but also to sudden unexpected death in epilepsy.

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Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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