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Do all norepinephrine surges disrupt sleep?

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Anita Lüthi[1] ✉, Paul Franken[2], Stephany Fulda[3], Francesca Siclari[4,5,6] & Eus J. W. Van Someren[4,7,8]

ARISING FROM: C. Kjaerby et al. *Nature Neuroscience* https://doi.org/10.1038/s41593-023-01314-7 (2023). ***Excessive fragmentation of sleep by arousals negatively impacts health. In a recent study in mice, Kjaerby et al. interpret surges of norepinephrine as sleep-fragmenting arousals. As a group of researchers working on human and rodent sleep, we caution against the interpretation that all sleep-related norepinephrine surges invariably indicate arousal. More work is needed to distinguish norepinephrinergic activity characterizing sound versus fragmented sleep.***

Sleep disorders are common and pose substantial risks for physical and mental health[1,2]. Intriguingly, it is not so much the limited duration of sleep as the excessive fragmentation of sleep by arousals, occurring spontaneously or as a result of external disturbances, that seems to convey these risks. The clinical importance of fragmented sleep has motivated neuroscientific studies in mice to identify circuit mechanisms of spontaneous arousals[3–6]. Despite the progress made in this field, translating the insights obtained in mice to humans is not straightforward. For example, spontaneous arousals vary greatly in origin and in electroencephalographic (EEG) appearance in both species, and there are no established criteria for their systematic assessment in mice. We write this comment because we think that in the study by Kjaerby et al.[7] some of these issues have been overlooked, and because clarifying them will enhance the value of translational sleep research.

Monoaminergic signaling during sleep has become a topic of interest in studies of the neural basis of sleep fragmentation. With the newly available genetically encoded biosensors, monoamines can be monitored in real time in the sleeping mouse brain. Their levels vary dynamically during sleep and can at times be unexpectedly high[6,8]. The importance of these monoaminergic fluctuations in determining moments during which arousals are more likely to occur is being recognized[5,9]. A recent study published by Kjaerby et al.[7] focused on the role of norepinephrine in spontaneous arousals during sleep in mice. Through combining the latest biosensor technologies with sleep recordings, this work provides a welcome step forward in addressing the roles of monoamines for sleep. The authors confirmed norepinephrine's role in regulating the occurrence of sleep spindles[6], brief EEG rhythmic (10–15 Hz) events characteristic of non-rapid-eye-movement (NREM) sleep. They also confirmed that EEG microarousals from NREM sleep, during which the animal's muscular activity measured with electromyography (EMG) increases (referred to as $MA^{EEG\text{-}EMG}$ by Kjaerby et al.[7]), preferentially appear when the prevalence of sleep spindles is low[5].

What is problematic in our view is that the authors then go on to designate all the moments of NREM sleep during which norepinephrine levels peak and spindle density declines as microarousals, in the absence of other systematic EEG or EMG changes (referred to as MA^{NE}). In both rodents and humans, NREM sleep is a highly dynamic state. A transient decline in spectral components of global brain activity can hence naturally occur in NREM sleep[9–11] and does not per se signal arousal. From the available data (including extended data), it is not clear whether the norepinephrinergic surges called MA^{NE} microarousals by Kjaerby et al.[7] show spectral properties that are different from the spectral variations inherent to NREM sleep[5,9].

A study published before that by Kjaerby et al.[7] focused on the EEG during periods of decreasing spindle activity (which correspond to the MA^{NE}) and identified these as spectrally heterogeneous[5]. About a third of these periods contained a microarousal with EMG increases, whereas approximately 25% showed an increase of fast relative to slow EEG frequencies and an increase in heart rate, a possible microarousal in the absence of EMG increases. In the remaining approximately 45% of cases, the spectral signature was typical for NREM sleep, with an increase in delta (1–4 Hz) power and a decrease in high-frequency bands. It has

[1]Department of Fundamental Neurosciences, University of Lausanne, Lausanne, Switzerland. [2]Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland. [3]Sleep Medicine Unit, Neurocenter of Southern Switzerland, Ospedale Civico, EOC, Lugano, Switzerland. [4]The Netherlands Institute for Neuroscience, Amsterdam, The Netherlands. [5]Center for Investigation and Research on Sleep, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland. [6]The Sense Innovation and Research Center, Sion, Switzerland. [7]Department of Integrative Neurophysiology, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands. [8]Department of Psychiatry, Amsterdam Public Health Research Institute and Amsterdam Neuroscience Research Institute, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands. ✉e-mail: anita.luthi@unil.ch

also been shown that the norepinephrine-releasing neurons in the brainstem discharge action potentials during ongoing EEG slow waves (0.75–1.5 Hz)[12], which form the defining correlation of deep NREM sleep. It is unclear from the data presented by Kjaerby et al.[7] whether the norepinephrinergic MA^{NE} surges can be called microarousals based only on a concurrent decrease in spindle activity, let alone whether they can define a new type of microarousal.

Despite established guidelines in humans, arousals display marked heterogeneity in terms of EEG spectral frequency composition[13] and there is no accepted standard for scoring microarousals in rodents. In addition, mice show cortical-only microarousals that depend on cholinergic signaling[3] or local arousals in sensory cortical areas in combination with heart rate increases[5]. Therefore, experimental progress of the kind made by Kjaerby et al.[7] will be critical in future discourse on advancing rodent sleep standards such that they are of best use to understand human sleep and its disorders.

Still, we argue that a common point of departure to identify an arousal is the disappearance of physiological signatures typical for NREM sleep and their replacement by corresponding signatures of wakefulness. A complete assessment and relative weighing of EEG power in sleep- and wake-characteristic frequency bands in the brain's electrical activity is useful for this purpose, often in combination with increases in muscular or autonomic activity. The phenomenological diversity of the EEG manifestations of arousals reflects diverse underlying causes of which norepinephrinergic signaling and sleep spindle suppression is a probable but certainly not the only contributor. Therefore, we challenge the interpretation of Kjaerby et al.[7] that a reduction in sleep spindle activity alone is sufficient to define a microarousal.

In addition, the authors conclude that a decline in norepinephrine levels is required for 'sleep-dependent memory enhancement'. They use optogenetic stimulation of the norepinephrine-releasing locus coeruleus (LC) at a wake-typical frequency of 20 Hz to elevate norepinephrine levels. However, as shown by the authors, this optogenetic stimulation causes a several-fold increase in the fragmentation of NREM sleep because it induces full arousal, as made clear by the conjoint EEG and EMG activation. Optogenetically induced abnormal LC activity or fragmentation (or both) of mouse NREM sleep compromises learning[14,15]. Unless experiments are done to control for sleep fragmentation, it is unclear whether the memory deficits observed are related to the norepinephrine amplitude or result from the increased sleep fragmentation and increased time spent in wakefulness. The work illustrates the complexity of disentangling the functions of norepinephrinergic signaling from the side effects of interfering with it and presents a starting point for further research.

In summary, the findings of Kjaerby et al.[7] provide exciting insights into the norepinephrinergic modulation of sleep. However, caution is warranted when interpreting surges of norepinephrinergic activity as wake-like intrusions into sleep. This is not only a matter of definition; it strongly impacts the way results are understood. The public interpretation of these findings for instance is that norepinephrine wakes your brain more than 100 times at night and that this is perfectly normal (https://healthsciences.ku.dk/newsfaculty-news/2022/07/stress-transmitter-wakes-you-up-more-than-100-times-a-night--and-it-is-perfectly-normal/). Sleep fragmentation is deleterious for human health. Therefore, suggestions in the general media that awakenings in human sleep are 'normal' do a disservice to the efforts of health experts who work to raise awareness about the impact of fragmented sleep on health and performance.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41593-023-01313-8.

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Additional information
Correspondence and requests for materials should be addressed to Anita Lüthi.

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