RESEARCH HIGHLIGHT

Treating insomnia with 40 Hz light flicker

P. Lorenzo Bozzelli[1,2] and Li-Huei Tsai[1,2,3]✉

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A new study published in *Cell Research* reports that 40 Hz light flicker induces a somnogenic effect mediated by adenosine-ENT2 signaling. Remarkably, 40 Hz light flicker was shown to improve the sleep quality of children with insomnia, offering a potential non-pharmacological therapeutic approach for the treatment of sleep disorders.

Sleep disruptions are prevalent in various brain disorders, yet the field's limited understanding of the underlying mechanisms has hindered the advancement of effective therapies.[1] Furthermore, emerging evidence has implicated sleep disturbances as potential drivers of disease, with sleep alterations often preceding disease onset by several years,[1] highlighting the importance for developing new effective sleep therapies.

The exploration of 40 Hz sensory stimulation has garnered great interest as a non-invasive therapeutic in the treatment of various neurodegenerative diseases.[2] In a new study from Zhou et al.,[3] the authors sought to identify the underlying mechanisms through which non-invasive sensory stimulation exerts a protective effect. The authors focused on adenosine signaling given that gamma oscillatory activity in the brain is an energetically demanding process requiring ATP metabolism that results in increased extracellular adenosine. Adenosine is known to reduce neuronal activity, and accumulation of adenosine increases sleep pressure.[4] As such, it is the blockade of adenosine receptors that underlies the wakefulness-promoting effects of caffeine.[4]

To examine adenosine levels in response to various light flicker frequencies, the authors used the G protein-coupled receptor (GPCR) activation-based adenosine sensor ($GRAB_{Ado}$) in various brain regions. When compared to the other frequencies that were tested in the primary visual cortex (V1), 40 Hz light flicker resulted in maximal extracellular adenosine levels, which were sustained for several hours after the cessation of the light stimulation. Additional experiments identified the cellular source of adenosine to be both excitatory and inhibitory neurons, but not astrocytes, suggesting that both neuronal subtypes interact to generate adenosine, similar to excitatory and inhibitory neuronal interactions giving rise to gamma oscillations.[5]

In response to neuronal activity, microglia have recently been shown to tightly regulate adenosine signaling, providing inhibitory feedback to neurons through CD73-dependent conversion of AMP to adenosine.[6] Zhou et al. found that extracellular adenosine levels were unaltered after 40 Hz light flicker in the *CD73*-knockout (KO) mouse, suggesting that an alternate pathway is involved in the 40 Hz light flicker-induced increase in extracellular adenosine.[2]

Given that intracellular adenosine can also be directly effluxed from neurons via ENT1/2 transmembrane efflux transporters, the authors examined ENT1/2 as a possible alternate pathway responsible for the 40 Hz light flicker-induced increase in extracellular adenosine. In comparing extracellular adenosine after 40 Hz light flicker between wild-type (WT) mice and either *ENT1*-KO or *ENT2*-KO mice, the effect was abolished only in the *ENT2*-KO mice, with a slight reduction observed in the *ENT1*-KO mice. Importantly, intracellular levels of adenosine were increased in all the mouse groups, demonstrating that it is primarily ENT2 that is responsible for adenosine efflux following flicker light stimulation.

Given the somnogenic effects of accumulating adenosine, the authors then examined the effects of 40 Hz light flicker on sleep induction in mice at different periods of their light cycle. Exposure to 40 Hz light flicker, but not the other tested frequencies, toward the end of the light cycle (sleep period of mice) resulted in increased sleep durations over the next 3 h of the dark phase, and both increased slow-wave sleep (SWS) and rapid-eye-movement sleep (REMS) when compared to mice who received normal light. Importantly, during the dark cycle (awake period in mice) there were no differences in SWS delta power density nor changes in theta power density during REMS, suggesting that 40 Hz light flicker can promote sleep without negative side effects such as fatigue during awake states. Furthermore, the authors found that the sleep-inducing effects required ENT2, as the WT and *ENT1*-KO mice, but not the *ENT2*-KO mice, displayed an increased total amount of sleep, and an increase in SWS during the first 2 h after the cessation of the 40 Hz light flicker.

To determine the brain regions mediating the somnogenic effect of 40 Hz light flicker, the authors conducted neuronal ablation studies. The somnogenic effect was lost upon ablation of V1 neurons, while the effect was mostly spared upon neuronal ablation within the superior colliculus (another region that had also demonstrated an increase in extracellular adenosine following 40 Hz light flicker). In contrast to neuronal ablation, additional experiments confirmed that inhibition of V1 neurons using DREADDs (designer receptors exclusively activated by designer drugs) was sufficient in promoting somnogenic effects. Furthermore, direct infusion of adenosine into V1, thereby increasing extracellular adenosine levels to inhibit neuronal activity, was also sufficient in promoting SWS. These experiments using chemogenetics and direct infusion of adenosine highlight the remarkable findings of 40 Hz light flicker as a non-invasive/non-pharmacological alternative able to increase extracellular levels of adenosine in the brain.

Finally, the authors performed sleep polysomnography in children with insomnia following 40 Hz light flicker. Thirty minutes

[1]Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, USA. [2]Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA. [3]Broad Institute of MIT and Harvard, Cambridge, MA, USA. ✉email: lhtsai@mit.edu

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of 40 Hz light flicker administered at nighttime reduced sleep onset latency, promoted sleep efficiency, and reduced wake time after sleep onset in subjects when compared to their previous baseline one night prior. Importantly, 40 Hz light flicker did not affect the number of times the subject woke during the night, suggesting that the exposure to 40 Hz flickering light promoted sleep quality. Also, 40 Hz light flicker did not alter the ratio of deep to light sleep in the subjects, suggesting that there were no sleep rebound effects.

This work provides exciting evidence for additional benefits of 40 Hz light flicker as a non-pharmacological therapeutic for the treatment of sleep disorders. Future studies examining other neuromodulators and neuropeptides that regulate sleep will also be of interest in this context, including noradrenaline, dopamine, acetylcholine, and orexin.[7] For instance, it would be illuminating to identify the 40 Hz light flicker's effect on noradrenaline and associated brain regions that are critical in modulating sleep–wake transitions, such as the locus coeruleus (LC). LC activity is markedly reduced during sleep states which corresponds to reduced levels of noradrenaline.[8] Interestingly, the decrease in noradrenergic tone during sleep regulates the waste-clearing glymphatic system, such that cerebrospinal fluid flux is the highest during sleep states.[9] These new findings on the somnogenic effects of 40 Hz light flicker converge with recent findings that 40 Hz sensory stimulation promotes glymphatic clearance of amyloid β,[10] linking glymphatic function and sleep homeostasis in response to 40 Hz sensory stimulation. These findings are important in furthering our understanding of the underlying mechanisms involved in 40 Hz sensory stimulation and in identifying future potential therapeutics to offer relief to individuals affected by sleep disorders.

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COMPETING INTERESTS

L.-H.T. is a scientific co-founder and scientific advisory board member of Cognito Therapeutics, Inc.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Li-Huei Tsai.