News Release

Title: Neural networks drive circadian cAMP rhythms

Key Points

- New optical imaging probe to visualize intracellular cAMP and extracellular peptide secretion
- The cAMP rhythms in the suprachiasmatic nucleus, the central circadian clock, are produced by a neural network
- Network-driven cAMP rhythms regulate the cellular molecular clock and behavioral rhythms

Summary

The research team led by Dr. Daisuke Ono of Graduate School of Medicine, Nagoya University, collaborating with Yulong Li of Peking University and Takashi Sugiyama of Evident Corporation found that circadian rhythms of intracellular cAMP in the SCN are regulated by neuropeptide-related neural networks. These achievements were published online Science Advances on January 4, 2023.

We show sleep and wakefulness approximately every 24 hours, even in the absence of light and time information. This is due to the existence of an internal circadian clock in our bodies. The circadian clock is present in all cells composed of our body, but its center is located in the suprachiasmatic nucleus (SCN) in the brain. It has been suggested that circadian rhythms are regulated by the transcription and translation feedback loop involving clock genes and their protein products. However, it remains unclear how the circadian rhythms of intracellular cAMP in the SCN are regulated. In this study, we developed a new probe to visualize intracellular cAMP and extracellular peptides and succeeded in cAMP and peptide secretory rhythm imaging of the SCN for the first time in the world. Furthermore, it was clarified that the circadian rhythm of cAMP is generated by a neural network mediated by neuropeptides, and that cAMP generated by this network regulates the intracellular molecular clock.

Although circadian rhythms have been thought to be regulated by mechanisms involving transcription and translation via clock genes, this study revealed that intercellular networks also play an important role in the formation of circadian rhythms.

Research Background

Life on earth is a 24-hour cycle, with various cellular functions fluctuating every 24 hours in response to the environment, which repeats itself every 24 hours. This approximately 24-hour rhythm is called the circadian rhythm and is regulated by the circadian clock. It is thought that individual cell in our body is equipped with a circadian clock and that the

circadian clock is maintained through a feedback loop involving transcription and translation of a group of genes called clock genes. In mammals, the central circadian clock is located in the SCN in the hypothalamus of the brain. The electrical legion of the SCN results in the loss of various physiological functions, indicating that the SCN is important for circadian rhythms.

Second messengers are known as systems that transmit signals from outside the cell to the cell. Among them, cAMP is known as an important molecule related to various cellular functions. In the SCN, it has been suggested that a circadian rhythm exists in cAMP, which functions as a second messenger, while a transcription-translation rhythm exists in the clock genes. However, it remains unclear how this rhythm is generated and how it relates to the transcriptional and translational rhythms of clock genes. In this study, we have developed a new imaging tool to visualize intracellular cAMP and combined it with optical manipulation of cAMP to elucidate the function of cAMP in the central circadian clock.

## Research Results

To visualize cAMP in the SCN, we developed a novel bioluminescent cAMP probe using firefly luciferase protein from Okinawa (Okiluc-aCT). Next, brain slices of the SCN were prepared, and Okiluc-aCT and GCaMP6s, a fluorescent calcium ion ($Ca^{2+}$) probe, were expressed using adeno-associated virus and time-lapse imaging was performed. We observed a distinct circadian rhythm in these two second messengers, but the spatiotemporal patterns were different. This suggests that cAMP and $Ca^{2+}$ have different functions in the SCN. Therefore, we administered tetrodotoxin, an inhibitor of membrane potential-dependent sodium channels, to inhibit neural network function by blocking neuronal firing, and measured the two rhythms. The results showed that the $Ca^{2+}$ rhythm was still present after tetrodotoxin administration, but the cAMP rhythm was abolished. This result suggests that the circadian rhythm of $Ca^{2+}$ is regulated by an intracellular mechanism, while that of cAMP is controlled by the neural network.

Next, we focused on extracellular signaling molecules that regulate the cAMP rhythm. The SCN contains several types of cells that express different transmitters. Among them, we focused on the vasoactive intestinal peptide (VIP). The VIP receptor is known to modulate cAMP. We found that pharmacological inhibition of VIP signaling abolished the cAMP rhythm, indicating that the intracellular cAMP rhythm is regulated by extracellular VIP. If this is correct, then there should be a circadian rhythm in VIP release as well. To verify this, we introduced a G-protein-coupled receptor-activation-based (GRAB) VIP sensor using green fluorescent protein. Time-lapse imaging of the GRAB sensor expressed in the SCN revealed a clear circadian rhythm. Furthermore, this VIP release rhythm was abolished by the tetrodotoxin administration. These results indicate that VIP is rhythmically released depending on neuronal activity and that the VIP release rhythm regulates the intracellular cAMP rhythm.

Finally, to determine how this cAMP affects the rhythm of transcription and translation feedback loop in a cell, we introduced light-inducible adenylate cyclase (bPAC). We

expressed bPAC in the SCN slice, measured the protein level of the clock gene Per2 by luminescence imaging, and then irradiated the cells with blue light to verify the effect of cAMP on the circadian rhythm. The results showed that manipulation of intracellular cAMP by blue light shifted the transcription-translation rhythm. Furthermore, when bPAC was expressed in the SCN of freely moving mice and the rhythm of cAMP was manipulated via optical fiber, the behavioral rhythm was also shifted. These results indicate that intracellular cAMP has a time-dependent input into the transcription and translation feedback loop.

**Research Summary and Future Perspective**

Many studies have shown the importance of transcription-translation mechanisms of clock genes for cellular functions. In this study, our group has shown that neural networks regulate the intracellular cAMP rhythm and that this rhythm influences the transcription-translation feedback loop. It has been reported that intracellular second messenger systems such as cAMP are important for various cellular functions. In the future, we would like to elucidate the ancestral circadian clock, which is independent of clock genes and exists universally in life.

**Publication**

Network-driven intracellular cAMP coordinates circadian rhythm in the suprachiasmatic nucleus
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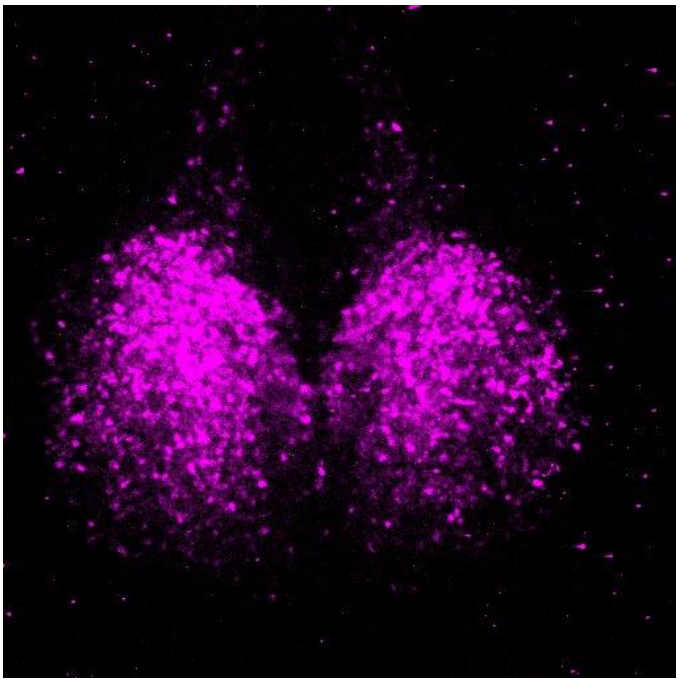

Bioluminescence imaging of the SCN using newly developed cAMP probe, Okiluc-aCT

Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Sci_230105.pdf