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Journal Club

"A broken heart" becomes sleepless, literally

Sun-Kyung Lee, Seung Hyun Kim, and Joohong Ahnn

Department of Life Sciences, Research Institute for Natural Sciences, Research Institute for Convergence of Basic Science, College of Natural Sciences, Hanyang University, Seoul 04763, Republic of Korea

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Humans spend one-third of their lives sleeping. Attaining sufficient sleep at the right times is essential for survival and proper maintenance of healthy bodies and minds. The sleep-wake cycle is tightly controlled by the hormone melatonin, which is produced and secreted by the pineal gland in the brain in response to darkness and is tightly associated with the expression of circadian clock genes (Huh, 2022; Lynch et al., 1975). Therefore, its level is high at night but low during the day. Diurnal synthesis and secretion of melatonin by the pineal gland are in sync with the Earth's day and night cycle (Cajochen et al., 2003; Kim et al., 2023). The pea-sized pineal gland receives input from neurons in the superior cervical ganglia (SCG) located in the neck, which also contain neurons innervating the heart (Bowers et al., 1984; Végh et al., 2016; Ziegler et al., 2018).

Many patients with heart disease have been widely reported to complain of sleep disturbance, and their melatonin levels tend to be low (Thosar et al., 2018). These unwanted disruptions greatly burden the patients. Ziegler et al. (2023) reported that these patients have profoundly lost the SCG neurons that innervate the pineal gland, which are neighboring other SCG neurons innervating the heart, due to an inflammatory reaction caused by the accumulation of inflammatory macrophages in the SCG (Covassin and Somers, 2023).

First, they collected postmortem pineal glands from patients with heart disease and healthy controls and stained them for the sympathetic marker enzyme tyrosine hydroxylase. They found that the axonal density was significantly reduced and reasoned that loss of innervation to the pineal gland may be responsible for the low level of melatonin, which is often observed in patients with heart disease. They established mouse models of cardiac hypertrophy and failure with transverse aortic constriction (TAC), which induces left ventricular pressure overload (Rockman et al., 1991). The concentration of melatonin in the blood of the mice subjected to TAC was lower than that of the controls, and as a result, their sleep-wake cycle was disrupted, including respirometry to survey metabolic rates, nutrient uptake, body mass, and activity. However, the loss of diurnal rhythmicity is not associated with changes in total energy expenditure and activity, which suggests that the pathological disruption was specific and not due to the general sickness of the mice.

Subsequently, they generated mice in which sympathetic neurons were genetically labeled by cross-breeding dopamine beta-hydroxylase-Cre mice with tdTomatoflox mice and applied TAC to these mice (Pirzgalska et al., 2017). The significantly reduced sympathetic axonal density of the pineal gland was prominent. Furthermore, the SCG in mice subjected to TAC was larger than in controls and showed extensive fibrotic scars, which suggests pathological hypertrophy and likely irreversible damage (Lim et al., 2023). These pathological changes were also observed in human SCG samples. In addition, ultrasound images indicate that SCG sizes in patients with hearts are significantly increased compared to healthy controls.

To dissect and quantitatively assess the cellular basis for the observed changes in SCG, single-cell and single-nuclei RNA sequencing of mouse ganglia was performed (Hong et al., 2023; Park and Jung, 2022). Five of the main cell types were sympathetic neurons, Schwann cells, fibroblasts, endothelial cells, and immune cells. The snRNA sequence identified 2 distinct cell clusters among sympathetic neurons, the smaller of which selectively expressed melatonin receptor 1A (Mtnr1a). Mtnr1a^{+} cell clusters were assigned as bona fide pineal gland-innervating neurons because the target organs of sympathetic innervation typically secrete specific guidance cues that facilitate selective axon growth during embryonic development, and specific innervation (Cho et al., 1996; Scott-Solomon et al., 2021). Most of the detected immune cells were macrophages. Spatial sequencing of mouse SCG cryosections using 140 RNA probes allowed for transcriptome mapping with single-cell resolution. Automated machine learning-based cell segmentation combined with uniform manifold approximation and projection clustering yielded 6 distinct cell clusters, including 2 major subclusters of sympathetic neurons, 1 of which expressed a set of marker genes highly similar to the bona fide pineal gland-innervating neurons. They stained all sympathetic neurons in mice, in which membrane cholesterol was extracted to facilitate deep penetration of antibodies to immunolabel markers, then generated a 3-dimensional dataset of sympathetic innervation and traced the sympathetic innervation originating from the SCG, through the upper neck, cranial cavity, and pineal gland (Hongcheng et al., 2023). Subsequently, they determined a set of marker genes for the major cell populations in the SCG, which enabled the genetic deconvolution of deep RNA-seq data of mouse and human ganglia (Newman et al., 2015). They found that mice subjected to TAC had a marked increase in macrophage cells and a significant reduction in neurons innervating the sympathetic pineal gland well before the

A damaged heart induces sleep disturbance by denervating the pineal gland, thereby lowering melatonin levels.

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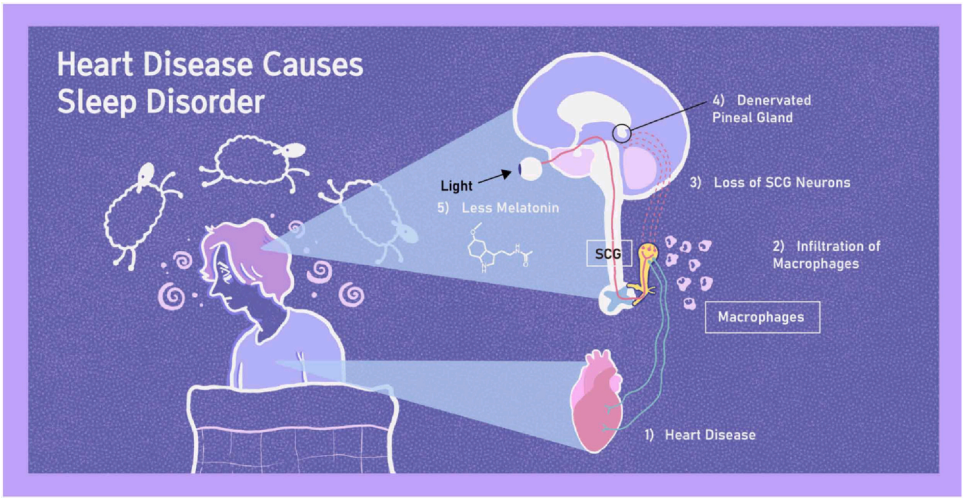

Fig. 1. Heart disease disturbs sound sleep at night due to reduced melatonin production linked to a decrease in the number of axons that sprout from the superior cervical ganglia (SCG) in the neck and lead to the pineal gland in the brain, which results from a response mediated by macrophages accumulated in the SCG.

mice developed decompensated heart failure. These changes were highly specific because they did not observe macrophage accumulation in noncardiac innervating ganglia or elevated levels of general inflammation markers.

The loss of neurons innervating sympathetic pineal glands rapidly progressed toward an almost complete loss of Mtnr1a^{+} neurons and was possibly affected, by electron-dense alterations in the axon initiation segment (Luebke and Wright, 1992). Transcriptome analysis of human SCG autopsy samples from patients with heart disease revealed that in patients with heart disease significant macrophage infiltration of neurons induces the macrophages and loss of the sympathetic pineal gland. In addition, immunodetection in these samples confirmed the disease-associated accumulation of macrophages.

To assess the effects of denervation of the pineal gland, the SCG was surgically removed. The mice without SCG showed a disrupted sleep-wake cycle. However, the normal cycle was completely restored by melatonin supplementation. Transcriptome profiling revealed that the communication network between macrophages and neurons innervating the sympathetic pineal gland is most prominent at the early stages of cardiac disease, 5 days after TAC (Efremova et al., 2020). They injected the macrophage inhibitor clodronate into the SCG of mice treated with TAC to deplete macrophages and interfere with the presumed harmful macrophage-neuron interaction. The local injection of clodronate into the SCG resulted in increased sympathetic axonal density within the pineal gland and melatonin levels, thus preventing denervation and dysfunction.

Do macrophages and neurons negatively interact during cardiac disease? To answer this question, sympathetic neurons and macrophages were cocultured in an ex vivo system. Sympathetic neurons cultured with proinflammatory, so-called M1-like macrophages inhibited neurite outgrowth and eventually died after nicotine stimulation nicotine stimulated. In this setting, when macrophage activation was blocked by cobra venom factor, a broad-spectrum complement inhibitor, neurons grew neurites and survived. Thus, local macrophage inhibition may be therapeutically effective in heart disease (Haihua et al., 2018).

Damaged heart muscle weakens the pineal gland in the brain, to which it appears to have no direct link. This study links sleep-wake disorder commonly observed in heart disease with loss of melatonin secretion under the control of SCG, in which inflammatory macrophages infiltrate and inhibit neuronal innervation onto the pineal gland. Neuron-associated macrophages play a critical role in human physiology (Lee and Kim, 2023). The SCG in a heart disease patient is like “an electrical switch box,” in which one bad wire sparks a fire spreading to another wire (https://dzhk.de/en/news/latest-news/article/cause-of-sleep-disturbance-in-cardiac-disease-identified/). Therefore, this study may provide insights into the pathology of other diseases in organs linked through the ganglia that act as switch boxes and help in the search for new drugs. Enlarged ganglia may be a diagnostic indicator of heart failure and can be easily checked by a standard ultrasound of the neck. “A broken heart” essentially becomes sleepless leading to a swollen neck (Fig. 1). It makes sense, does not it?

AUTHOR CONTRIBUTIONS

S.K.L. and J.A. conceived commentary concepts, wrote the manuscript, and secured funding. S.H.K. provided expertise and feedback.

DECLARATION OF COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ORCID

Sun-Kyung Lee: https://orcid.org/0000-0001-5368-0722
Seung Hyun Kim: https://orcid.org/0000-0003-1140-410X
Joohong Ahnn: https://orcid.org/0000-0003-2229-3580

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