News & views

Neuroscience

How an anxious heart talks to the brain

Yoni Couderc & Anna Beyeler

During periods of anxiety, the brain affects the heart, but does a racing heart also talk to the brain to cause anxiety-related behaviour? Use of a light-stimulated pacemaker in mice shows that it does, and pinpoints a brain region involved. **See p.292**

Have you ever been so anxious that you could feel your heart racing in your chest? This tachycardia is one of the main symptoms of anxiety[1], and can be so intense that the person experiencing it sometimes mistakes it for a heart attack. Experimental research has revealed numerous pathways that convey signals from the brain to the heart. But in both clinical psychiatry and fundamental neuroscience, the reverse – the effect of heart rate on emotions – has remained a debated question for almost a century[2]. Hsueh *et al.*[3] address this issue on page 292 , identifying a mechanism by which the brain detects heart rate, and showing how this, in turn, controls emotional behaviour.

Interoception is the continuous perception by the brain of internal signals in the body, including those from the respiratory, gastrointestinal and cardiac systems[4]. In people who have anxiety disorders, sensitivity to these internal signals – especially heart rate – is altered[1]. Studies in animals have highlighted the link between cardiac changes and emotional states[5,6], but whether an increased heart rate contributes directly to anxiety had remained unclear.

So far, only nonspecific electric shocks, vagal-nerve stimulation and pharmacological approaches – all of which involve major side effects – have been used to increase or decrease heart rate and to evaluate its effect on emotions. Researchers have lacked tools with the necessary temporal and spatial resolution to adequately investigate the effects of heart rate on anxiety.

The first of Hsueh and colleagues' breakthroughs was the development of such a tool: a non-invasive optical pacemaker. It was based on the systemic delivery into mice of a viral vector carrying a gene that encodes a light-sensitive protein, the opsin ChRmine. When illuminated with red light, positively charged ions flow through this protein, which depolarizes cells that express it. In the authors' experiments, the cells targeted were heart muscle cells, the depolarization of which triggers muscle contraction.

By mounting a red micro-light-emitting diode (micro-LED), blinking at a defined frequency, to a vest worn by mice, the authors could apply their 'optogenetic' strategy to control heart rate (Fig. 1). ChRmine has been used previously to precisely control specific neural circuits in deep regions of the brain without the need for intracranial surgery[7]. Hsueh and colleagues have extended the applications of this molecular tool by using it to control the activity of (to pace) an entire organ and to determine any heart-to-brain influences on anxiety.

The authors applied their approach to test whether an increase in heart rate to 900 beats per minute (36% higher than the baseline frequency) could alter levels of anxiety in freely behaving mice. Hsueh *et al.* used two methods to assess anxiety – placing the animals in a maze or in an open field, both of which include safe and exposed areas. The authors found that optically induced tachycardia led to a greater avoidance of exposed areas in both assays, reflecting an increase in anxiety-related behaviour. This is an unequivocal demonstration that, at least in mice, heart rate can affect anxiety, and can probably influence

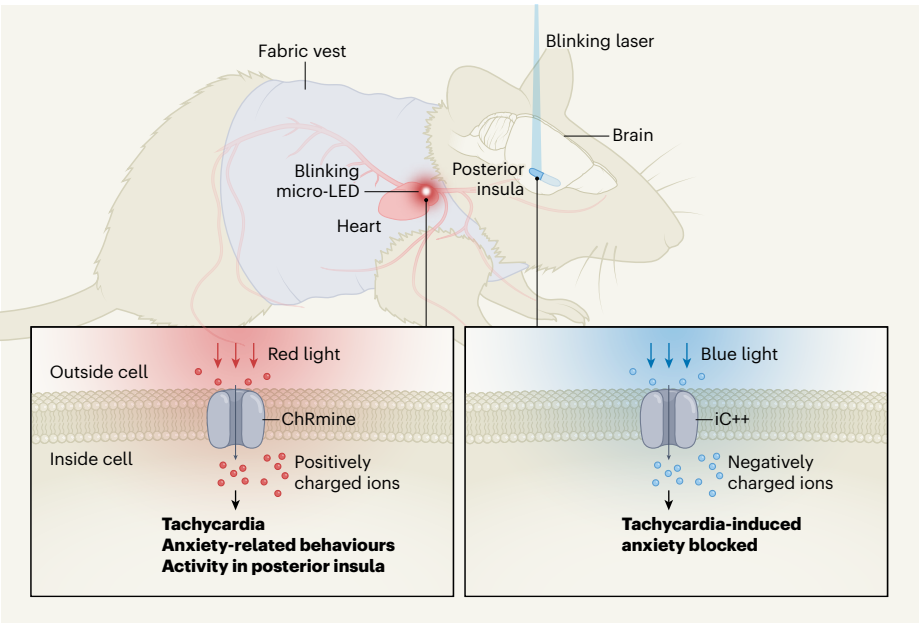

Figure 1 | Controlling anxiety with a non-invasive optical pacemaker. Hsueh *et al.*[3] studied the effects of a racing heart on anxiety-related behaviour in mice. They injected the animals with a heart-selective viral vector encoding a red-light-sensitive opsin protein called ChRmine. They dressed the mice in a vest containing a red micro-light-emitting diode (micro-LED), blinking at 900 beats per minute, that was fastened to the animals' chests over their hearts. The red light activated ChRmine in heart muscle cells, opening up the protein to let positively charged ions flow through it. This caused depolarization of the cells and an increase in the animals' heart rate (tachycardia). This, in turn, led to an increase in anxiety-related behaviours, and activated the posterior insula (among other brain regions). Hsueh *et al.* then inhibited the posterior insula using a blue-light-sensitive opsin, iC++, through which negatively charged ions flow. They activated iC++ using a blue laser, while optically increasing heart rate. This prevented tachycardia-induced anxiety.

other emotional behaviours, too.

To investigate the neurobiology that underlies this tachycardia-induced anxiety, Hsueh *et al.* performed a screen of brain activity after 15 minutes of optically induced tachycardia. Whole-brain mapping of neurons revealed changes in gene expression in response to tachycardia. The authors found that neurons in two regions — the posterior insular cortex (posterior insula) and the brainstem — were strongly activated. Electrophysiological recordings in live mice also showed an increase in the firing rate of neurons in the posterior insula during optically induced tachycardia.

The insular cortex is involved in both interoceptive processing and anxiety-related behaviours[8–10]. By this stage, the authors had discovered a correlative increase in the activity of the posterior insula after an increase in heart rate. But any involvement of this region in the tachycardia-induced anxiety remained to be determined. To investigate this, Hsueh *et al.* optogenetically inhibited posterior insula neurons using a different opsin — the blue-light-sensitive protein iC++.

In so doing, they made a second discovery: inhibition of the posterior insula during optical pacing reduced the anxiety behaviours induced by tachycardia. This indicates that the posterior insula relays information about heart rate to affect anxiety. The attenuation was specific to the posterior insula, and was not observed with optogenetic inhibition of a different region, the medial prefrontal cortex.

Overall, then, Hsueh *et al.* have found that increases in heart rate promote anxiety-related behaviours in mice, and that this is mediated through the activation of specific brain structures that include the posterior insula. The authors' comprehensive study raises new questions, and opens up areas for research. For example, the neural circuits and mechanisms that allow the posterior insula to be activated by tachycardia — as well as the circuits that induce anxiety behaviours — have yet to be identified.

Another unexplored aspect is the long-term effect of days (or weeks) of optically induced tachycardia — a question with substantial clinical implications. This study lays the foundation for testing whether chronic increases in heart rate induce long-term changes in the brain, which could underlie harmful levels of anxiety. Testing this hypothesis would raise technical challenges, because the micro-LED vest used here is not suitable for such long periods of stimulation.

Finally, from a translational and therapeutic perspective, it might be possible to design experiments to slightly decrease heart rate. Would this change reduce anxiety-related behaviours? Hsueh and colleagues' work has provided the means to investigate this prospect.

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Structural biology

MicroRNA uses a gym to get fit for Dicer enzyme

Gunter Meister

The enzyme Dicer cleaves a type of RNA called a pre-microRNA to make the mature functional RNA. Structural evidence now sheds light on the catalytic mechanism involved and the role of a newly found RNA sequence termed GYM. **See p.323 & p.331**

Gene expression can be silenced through targeted pathways that depend on small RNAs. On pages 323 and 331, Lee *et al.*[1,2] provide insights into a key step in the maturation of such RNAs that is mediated by the enzyme Dicer.

RNA-dependent gene-silencing pathways are found in almost all eukaryotes (organisms whose cells contain a nucleus). Many of these systems are fuelled by immature versions of RNA that are often in the form of double-stranded RNA (dsRNA). Such dsRNA serves as the origin of small regulatory RNAs that include microRNAs (miRNAs) and short-interfering RNAs (siRNAs). In both cases, the dsRNA precursors are cleaved by a particular class of enzyme that is characterized by having what is termed an RNase III domain[3].

The miRNAs are processed from stem-loop-structured precursors, also described as a hairpin, and they require the consecutive action of two RNase III enzymes. In animals, the enzyme Drosha conducts the first cut and Dicer the second. Both enzymes define the ends of a short double-stranded miRNA intermediate from which one miRNA strand is selected and incorporated into the protein complex RISC, in which the RNA directly binds to a member of the Argonaute protein family.

The siRNAs are typically processed from the ends of long dsRNAs by only one enzyme, Dicer[4]. This scenario, however, requires that Dicer moves along the dsRNA. Therefore, Dicer enzymes can generally be divided into what are called non-processive and processive enzymes, depending on whether they generate more than one small RNA from a dsRNA

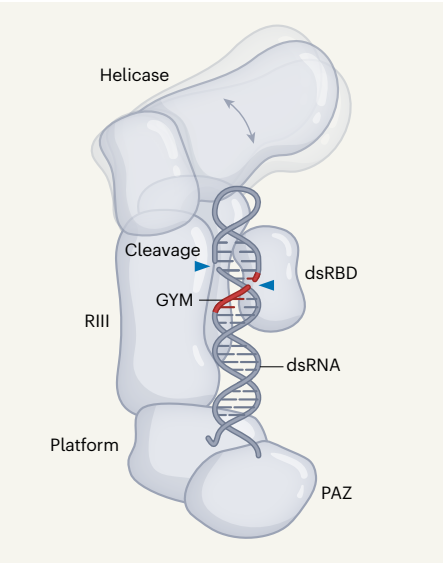

Figure 1 | Structural clues to how human Dicer enzyme functions. Some small RNAs, such as microRNAs, that function in gene silencing undergo a maturation step in which a double-stranded RNA (dsRNA) is cleaved by Dicer. Lee *et al.*[1] reveal that an RNA sequence that the authors term GYM has a role in facilitating this process. The authors' second paper[2] presents structural data obtained using cryo-electron microscopy, which captured the enzyme at the stage associated with RNA cleavage. This structure reveals the orientation of the dsRNA with respect to various domains of Dicer, a subset of which are shown here: helicase domain, RNase III (RIII) domain, dsRNA-binding domain (dsRBD), platform domain and PAZ domain. The helicase domain was not clearly visible in the structure, which suggests that it is in a flexible conformation at this step. (Adapted from Fig. 5 of ref. 2.)