Neuroscience

An altered state of consciousness illuminated

Ken Solt & Oluwaseun Akeju

The rhythmic activity of a single layer of neurons has now been shown to cause dissociation — an experience involving a feeling of disconnection from the surrounding world. **See p.87**

The state of dissociation is commonly described as feeling detached from reality or having an 'out of body' experience. This altered state of consciousness is often reported by people who have psychiatric disorders arising from devastating trauma or abuse. It is also evoked by a class of anaesthetic drug, and can occur in epilepsy. The neurological basis of dissociation has been a mystery, but on page 87, Vesuna *et al.*[1] describe a localized brain rhythm that underlies this state. Their findings will have far-reaching implications for neuroscience.

The authors first recorded brain-wide neuronal activity in mice using a technique called widefield calcium imaging. They studied changes in these brain rhythms in response to a range of drugs that have sedative, anaesthetic or hallucinogenic properties, including three that induce dissociation — ketamine, phencyclidine (PCP) and dizocilpine (MK801).

Only the dissociative drugs produced robust oscillations in neuronal activity in a brain region called the retrosplenial cortex. This region is essential for various cognitive functions, including episodic memory and navigation[2]. The oscillations occurred at a low frequency, of about 1–3 hertz. By contrast, non-dissociative drugs such as the anaesthetic propofol and the hallucinogen lysergic acid diethylamide (LSD) did not trigger this rhythmic retrosplenial activity.

Vesuna *et al.* examined the active cells in more detail using a high-resolution approach called two-photon imaging. This analysis revealed that the oscillations were restricted to cells in layer 5 of the retrosplenial cortex. The authors then recorded neuronal activity across multiple brain regions. Normally, other parts of the cortex and subcortex are functionally connected to neuronal activity in the retrosplenial cortex; however, ketamine caused a disconnect, such that many of these brain regions no longer communicated with the retrosplenial cortex.

The researchers next asked whether inducing the retrosplenial rhythm could cause dissociation. They made use of mice in which layer-5 cells were modified to simultaneously express two ion-channel proteins that are sensitive to light. The first, channelrhodopsin-2, elicits neuronal excitation in response to blue light. The second, eNpHR3.0, silences neurons in response to yellow light. Illuminating the cells with alternating blue and yellow light to induce an artificial 2-Hz rhythm produced behaviours indicative of a dissociated state, analogous to those caused by ketamine (Fig. 1a). For example, the animals did not jump or rear away from threats and did not try to escape when suspended by their tails, but responded normally to pain induced by a hotplate. Although sensation remained intact, the blunted responses to threats suggest dissociation from the surrounding environment.

The authors then deleted two genes that encode ion-channel proteins in the retrosplenial cortex. The first gene encodes a channel activated by the neurotransmitter molecule glutamate. The second encodes hyperpolarization-activated cyclic nucleotide-gated 1 (HCN1), a channel activated by cations that is sometimes called a pacemaker, because of its ability to produce rhythmic activity in the heart and neurons. Vesuna *et al.* found that the ketamine-induced rhythm was reduced in mice lacking either gene. However, only the HCN1 channel was needed for ketamine to elicit dissociation-like behaviours.

Do these findings translate to humans? Vesuna and colleagues recorded electrical activity from several brain regions in a person with epilepsy, who had previously had electrodes implanted in their cranium to locate seizure activity. The individual experienced dissociation before the onset of seizures. The authors found that this dissociation correlated with a 3-Hz rhythm in the deep posteromedial cortex — a human brain region analogous to the mouse retrosplenial cortex. When the team electrically stimulated the deep posteromedial cortex during a brain-mapping procedure, the person again experienced dissociation (Fig. 1b).

It is premature to draw definitive conclusions from a single individual. However, Vesuna and colleagues' work provides compelling evidence that a low-frequency rhythm in the deep posteromedial cortex is an evolutionarily conserved mechanism that underlies dissociation across species.

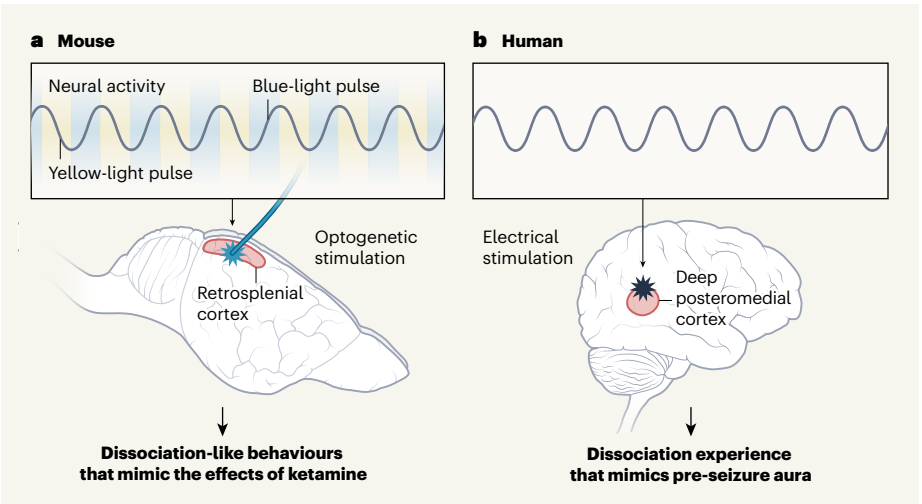

Figure 1 | Inducing a dissociative state. Dissociation is an altered state of consciousness in which people feel detached from reality. It can be triggered by the drug ketamine, and can occur before a seizure in epilepsy. **a**, Optogenetic techniques can modulate neuronal activity in response to light. Vesuna *et al.*[1] modulated neurons in a single layer of the retrosplenial cortex — a region inside the mouse brain. The group used blue light to stimulate activity and yellow light to repress it. This generated low-frequency neuronal oscillations, similar to those seen in mice that receive ketamine. The oscillations triggered behaviours indicative of dissociation. **b**, The authors show that the same oscillations occur in the equivalent brain region (called the deep posteromedial cortex) in a person who has epilepsy, before a seizure. Electrical stimulation of this brain region triggered the same oscillations and dissociative experience. Together, these experiments indicate that low-frequency oscillations in a small brain region trigger dissociation across species.

Much of the success of Vesuna and colleagues' study relies on the reversible dissociative effects of ketamine. At subanaesthetic doses, this fascinating drug elicits dissociation and pain relief (analgesia), and has antidepressant and anti-suicidal properties. At these doses, electroencephalograms (EEGs, which detect neuronal activity at the surface of the brain) show that ketamine broadly dampens 8–12-Hz oscillations[3]. At higher doses that induce unconsciousness, EEGs reveal a rhythm in the brain's frontal lobe in humans that alternates between low (1–4 Hz) and high (27–40 Hz) frequencies[4]. Given that these changes occur over large areas of the brain's surface, it is striking that a small layer of deep cells is specifically responsible for dissociation. To our knowledge, the oscillations described by Vesuna *et al.* have not been reported previously for ketamine. This is probably because surface EEG recordings cannot detect localized rhythms generated deep in the cortex.

Rapid technological advances are producing increasingly sophisticated techniques to manipulate neural circuits with precision and high temporal resolution. Vesuna and colleagues' work exemplifies how these advances are enabling investigators to probe the nature of consciousness itself. They are also revolutionizing the science of anaesthesiology[5] — allowing investigators to better understand how anaesthetics produce unconsciousness[6], how these mechanisms overlap with natural sleep[7], and how people recover consciousness after anaesthesia[8]. Research into consciousness and anaesthesia overlaps, too, because anaesthetics provide a powerful, reliable means of eliciting reversible states of altered consciousness. Understanding the neural mechanisms of these altered states might lead to fresh approaches to modulate consciousness and control pain without the undesirable side effects of currently available drugs, which include changes in heart rate and blood pressure, cessation of breathing, delirium and nausea.

The complex state of dissociation can be fully described only by humans, who can report their experience. For example, a study in humans was needed to prove that the dissociative and analgesic properties of ketamine are independent[9]. Going forward, studies that use dissociative drugs in people will continue to be of great interest — for instance, to reveal the connection (if any) between the brain rhythm reported by Vesuna *et al.* and the various desirable properties of ketamine. Such studies should also include medicines, such as benzodiazepines and lamotrigine, that attenuate ketamine-induced dissociation. An improved understanding of how ketamine alters brain rhythms and associated behavioural states could eventually lead to therapeutics for people experiencing chronic pain, depression and perhaps dissociative disorders.

These analyses will be highly challenging to perform, because studying deep cortical rhythms requires people in whom intracranial electrodes have been implanted. For ethical reasons, only individuals who require electrodes for therapeutic purposes can participate in such studies. We owe them a debt of gratitude for allowing us to better understand the inner workings of the human brain.

Ken Solt and **Oluwaseun Akeju** are in the Department of Anaesthesia, Harvard Medical School, Boston, Massachusetts 02115, USA, and the Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Boston.
e-mail: ksolt@mgh.harvard.edu

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Drug discovery

Modular synthesis enables molecular ju-jitsu

Daniel J. Blair & Martin D. Burke

An ancient resistance mechanism poses a problem when using streptogramin antibiotics. A modular approach to drug synthesis exploits this same mechanism to generate an antibiotic that avoids the emergence of resistance. **See p.145**

The development of resistance to antibiotics by microorganisms is a problem that has been billions of years in the making, but we don't have as long as that to solve it[1,2]. One way to speed up the search for solutions is to harness human creativity and modern science to rationally design resistance-evasive variants of naturally occurring antibiotic molecules[3,4]. Unfortunately, such molecules often have to be synthesized from scratch using long, highly customized sequences of reactions that are prohibitively slow and impractical at large scales. Li *et al.*[5] report on page 145 how a modular synthesis of the structurally complex antibacterial compound virginiamycin M2 (VM2), based on easily interchangeable molecular building blocks, has provided access to VM2 derivatives that could not previously have been prepared — and has thereby enabled the rational development of a variant that evades an ancient resistance mechanism.

"Replacement of a methyl group with a larger group yielded a compound with potent activity against a series of bacterial strains."

Virginiamycin M2 belongs to the streptogramin family of antibiotics, which is subdivided into groups A and B. The two groups work synergistically to inhibit bacterial protein synthesis by binding to complementary sites in the catalytic centre of the bacterial ribosome (the molecular machinery that coordinates protein synthesis). Group A streptogramins, such as VM2, bind to part of the ribosome called the peptidyl transferase centre (PTC), and promote the binding of group B streptogramins to the adjacent tunnel region, through which nascent proteins exit.

A key mechanism of bacterial resistance to this powerful antibiotic 'one–two punch' probably evolved in parallel with the streptogramins, in the form of acetyltransferase enzymes of the Vat family (VatA enzymes). These enzymes deactivate group A streptogramins by transferring an acetyl group ($-COCH_3$) to an alcohol group ($-OH$) attached to a specific site in the antibiotics, dubbed the C14 position[6]. The addition of an acetyl group produces a molecular bump that clashes with ribosome-bound RNA in the PTC, and thus blocks antibiotic activity (Fig. 1).

There have been several attempts to prepare derivatives of group A streptogramins that could avoid this deactivation mechanism[7]. But, in each case, the derivatives were limited to those that could be prepared from the natural product itself, through a process called semi-synthesis, and were found not to be resistant to VatA-mediated deactivation. Researchers from the same group as Li and colleagues previously developed[8] a highly modular synthesis