the existence of a further similarity between Earth and Titan — the presence of open bodies of liquid on its surface.

The idea that liquid bodies exist on Titan's surface is not new. After the discovery that Titan's atmosphere contains a substantial amount of methane, the question of its origin and evolution was seen as a key to understanding Titan's mysteries. A combination of light from the Sun and high-energy electrons from Saturn's magnetosphere would have destroyed all methane in the atmosphere within a few tens of millions of years, implying that the methane was being replenished by a source on, or in, Titan. The main volatile organic product of the light-induced breakdown of methane is ethane, and it was proposed that an ocean consisting of liquid ethane and methane with dissolved N_2 covered Titan[2].

The Voyager probes were unable to get a peek at Titan's surface, as it was masked by hazy layers in the atmosphere. But with the Cassini-Huygens mission, this veil has finally been lifted. Several instruments on the Cassini spacecraft have been observing the moon in regions of the electromagnetic spectrum in which the atmosphere is transparent.

These observations quickly ruled out the possibility of a global ocean[3], but radar data strongly supported the possibility of smaller seas and lakes, mainly in the colder northern regions[4]. Furthermore, camera images taken in mid-2005 showed a dark surface feature near the south pole as big as North America's Lake Ontario, speculatively named Ontario Lacus. Brown *et al.*[1] have now identified the characteristic spectrum of liquid ethane in infrared spectra of Ontario Lacus.

The Cassini-Huygens mission has demonstrated the existence of a complex cycle of methane in Titan's geochemistry similar to the water cycle on Earth (Fig. 1). Large stores of methane seem to exist within Titan in the form of methane hydrates (clathrates) trapped during the formation of the satellite[5]. Methane may also be produced through the reaction of water with igneous rocks under high pressures, which would also form hydrogen gas. Ethane, formed by photodissociation of methane in the atmosphere, accumulates on Titan's surface, replenishing the surface lakes — which are therefore one possible ethane reservoir, as are haze particles in the atmosphere into which ethane can be sequestrated[6].

Titan's lakes are probably a liquid ethane–methane mixture together with dissolved nitrogen, as previously proposed for the speculative oceans[2]. Also dissolved in them will be a variety of solutes, mainly organic compounds produced in the atmosphere and rained down in aerosol particles consisting of a nucleus of macromolecular materials coated with volatile hydrocarbons and nitriles[7]. Such solutes will be much more concentrated in the lakes than in the atmosphere[8]. Despite the low temperatures, the action of high-energy cosmic rays reaching the satellite's surface may produce additional organic compounds in this exotic chemical reactor.

It has been suggested that cold liquids such as those found in the lakes of Titan could contain life[9], but they also have less speculative interests for astrobiologists[10]. Although composed of a low-temperature, nonpolar solvent very different from water, they provide an analogue to Earth's oceans and are a potential chemical reactor. It is to be hoped that these organic lakes are made a priority target for future exploration missions, such as the Titan/Saturn System Mission (TSSM), a joint venture between NASA and the European Space Agency currently undergoing feasibility studies. ■

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BEHAVIOURAL NEUROSCIENCE

The circuit of fear

Pankaj Sah and R. Frederick Westbrook

Do you find it difficult to overcome an irrational fear? Blame it on the specific neural circuits hardwired in the brain that control fear recognition, and fear renewal even when fear has seemingly been overcome.

Learning to predict danger allows animals to defend themselves against harm and is crucial for survival. The neural mechanisms that subserve these functions are evolutionarily old, and their dysfunction is thought to underlie a host of anxiety disorders in humans, including post-traumatic stress and panic disorder[1]. Two papers in this issue, by Herry *et al.*[2] (page 600) and Likhtik *et al.*[3] (page 642), pinpoint the neural circuits that mediate the bidirectional transition between a defensive behaviour — fear — and the default exploratory behaviour.

To study the neural mechanisms that mediate fear responses, fear conditioning is widely used. In a typical procedure, animals are exposed to a normally harmless stimulus (such as a sound or light) before a brief exposure to an aversive stimulus — typically a foot shock. A few such rounds of pairing the harmless (conditioned) stimulus with the aversive (unconditioned) stimulus create an association between them in the animals' minds. So, on subsequent exposure to only the conditioned stimulus, they remember the association and express behavioural and physiological responses that correspond to fear in humans.

It is important to study how fear is first learned. However, the pertinent question for clinicians is how fear can be eliminated or reduced. Extinction of fear occurs when the association between the conditioned and the unconditioned stimuli is broken by repeated presentations of the conditioned stimulus only[4]. But does the association get completely erased from the memory? The answer is no. Although the conditioned stimulus eventually fails to elicit fear responses, much, if not all, of the original learned fear survives extinction[5]. So when the extinguished conditioned stimulus is tested either during, or shortly after, exposure to a dangerous context again, the conditioned fear is renewed spontaneously. Extinction therefore involves new learning, and its activation by situational cues inhibits the expression of fear responses to the conditioned stimulus.

Three brain regions implicated in fear conditioning and extinction are the amygdala, the medial prefrontal cortex and the hippocampus. The amygdala is the central structure involved in both fear conditioning and extinction[1]. It is divided into two main compartments: the basolateral amygdala, which receives sensory information about the conditioned and the unconditioned stimuli; and the central nucleus, which receives information processed in the basolateral amygdala. Neuronal projections from the central nucleus to the hypothalamus and brainstem then evoke the behavioural and physiological responses that constitute fear[6] (Fig. 1a, overleaf).

The learning of fear involves the co-occurrence of weak (conditioned stimulus) and strong (unconditioned) inputs to single neurons and leads to enhancement of neuronal activity known as excitatory postsynaptic potentials, which are elicited by the conditioned stimulus and mediated by NMDA glutamate receptors. This synaptic plasticity results in the conditioned stimulus evoking an increased response in basolateral-amygdala neurons that project to fear-output circuits in the central nucleus. Extinction results from NMDA-receptor-mediated changes in the amygdala[7] that block the effects of this long-term potentiation, possibly by activating local neurons that secrete the inhibitory neurotransmitter GABA. The medial prefrontal cortex mediates consolidation of extinction,

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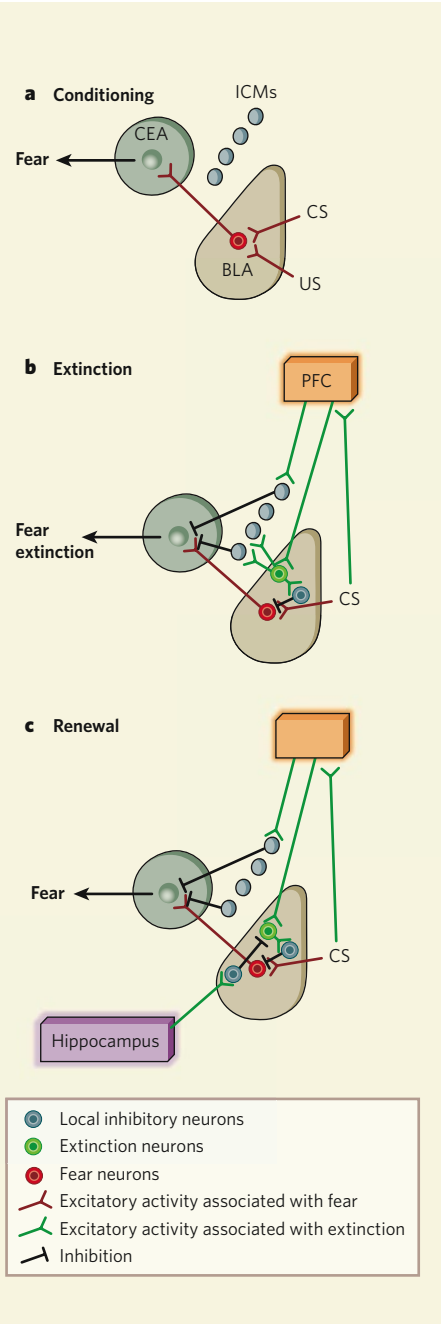

Figure 1 | Neural circuits that mediate fear. **a**, During fear conditioning, convergence of inputs from the conditioned stimulus (CS) and unconditioned stimulus (US) to fear neurons in the basolateral amygdala (BLA) leads to potentiation of the conditioned input and activation by the CS of neurons in the central nucleus (CEA) that initiates physiological and behavioural responses characteristic of fear. **b**, During extinction, inputs from the medial prefrontal cortex (PFC) activate neurons in the intercalated cell masses (ICMs) — either directly or through activation of extinction neurons in the basolateral amygdala — which then inhibit the activity of fear output neurons in the CEA. **c**, During fear renewal, inputs from the hippocampus, which evaluates the current context, activate inhibitory interneurons in the basolateral amygdala that silence extinction neurons, thus restoring fear responses.

as well as the subsequent activation of these GABA-secreting neurons by the conditioned stimulus. The hippocampus probably regulates the situational control over this activation, and so over extinction. But how fear-conditioning and extinction memories are stored, and how they are translated into behavioural outcomes, is poorly understood.

Herry *et al.*[2] set out to examine the role of the basolateral amygdala in fear learning and extinction in mice. In agreement with previous work[8,9], they found that the pairing of conditioned and unconditioned stimuli results in enhanced firing of a population of basolateral-amygdala neurons — fear neurons — when animals are subsequently exposed to the conditioned stimulus alone. After extinction, these neurons no longer fire in response to the conditioned stimulus. But, intriguingly, another population of neurons — extinction neurons — emerged, which selectively respond to the conditioned stimulus undergoing extinction. Moreover, moving the mice from the extinction context to the conditioning context not only renews fear responses but also leads to reactivation of fear neurons and deactivation of the extinction neurons.

The authors show that the change from fear-neuron firing to extinction-neuron firing precedes the behavioural shift from a fear to a no-fear response, suggesting that activities of these neurons control the observed behaviour. In addition, infusion of the GABA-receptor agonist muscimol into the basolateral amygdala to inactivate neurons there impaired both extinction learning and fear renewal, indicating that these neurons' activity is crucial for the inhibition that underlies extinction.

To examine connectivity of fear neurons and extinction neurons, Herry *et al.* next stimulated the medial prefrontal cortex and the hippocampus. Their findings suggest that fear neurons receive inputs from the hippocampus and project to the medial prefrontal cortex. Extinction neurons, by contrast, are connected to the medial prefrontal cortex in both directions.

How does the extinction neurons' activity produce the shift from fear to safety? Between the basolateral amygdala and the central nucleus are clusters of inhibitory neurons known as the intercalated cell masses (ICMs). These neurons are innervated by input neurons from the basolateral amygdala, and their activity inhibits output neurons in the central amygdala by shunting dendritic excitatory inputs[10]. Neurons in the medial prefrontal cortex also densely innervate ICM neurons[11]. It has been suggested[12,13], therefore, that activation of ICM neurons by these cortical neurons inhibits the amygdala's output during extinction, but conclusive evidence has been lacking.

Likhtik *et al.*[3] used an innovative technique to selectively silence ICM neurons. Following fear conditioning and its extinction in rats, the authors delivered a toxin called saporin specifically to the animals' ICM neurons, to block protein synthesis and cause ICM cell death. They find that, with a decrease in ICM neurons, extinguished fear responses can once again be reactivated, suggesting that these neurons are required for the expression of learned extinction (Fig. 1).

These authors' work, together with that of Herry *et al.*[2], clearly defines some of the neural circuits that mediate the extinction of conditioned fear. The amygdala, together with the hippocampus and the medial prefrontal cortex, uses situational information to evaluate an extinguished fear-conditioned stimulus and acts as a switching circuit that guides the appropriate behavioural response (Fig. 1). But, as with all interesting studies, these observations also raise questions.

The existence of fear and extinction neurons in the basolateral amygdala has been inferred from changes in the activity in this region after learning fear and extinction. These neurons are yet to be identified. Do extinction neurons activate ICM cells directly or through projections to the medial prefrontal cortex? Inhibitory neurons in the basolateral amygdala strongly inhibit the activity of local pyramidal neurons[14].

Does inhibition of fear neurons during extinction result from activation of such local feedback interneurons in the basolateral amygdala? Herry and colleagues' observations suggest that long-term memories of both learned fear and extinction are not ultimately stored in the amygdala. So another question is how these learned behaviours are 'gated' after consolidation.

Answers to these questions will require exploration of the local circuitry of the amygdala, identifying fear and extinction neurons and uncovering the final targets of these cells. Neuronal receptors in these circuits — such as those targeted with saporin in Likhtik and colleagues' study — are likely to become targets for the development of specific treatments for many anxiety disorders. ■

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