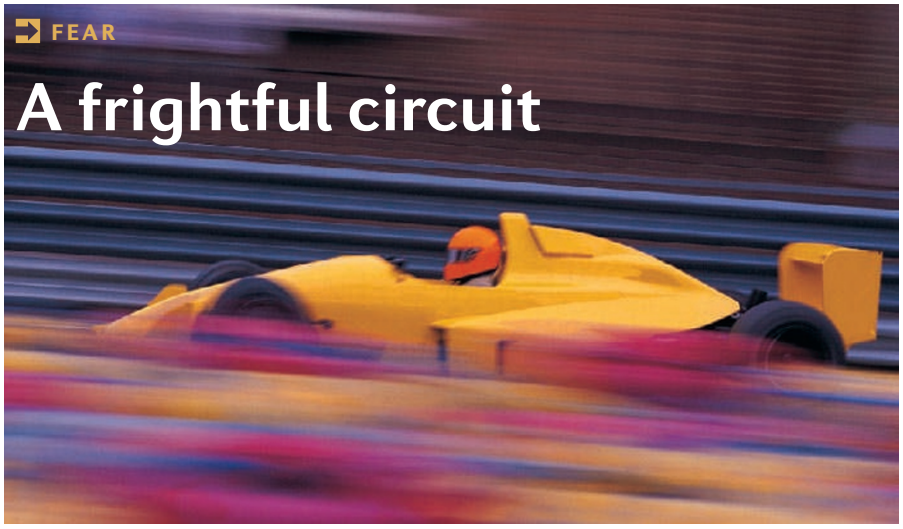
FEAR

A frightful circuit



DIGITAL VISION

The amygdala is central to the learning and expression of fear, but the circuits and neuron types within the amygdala that mediate these aspects of fear conditioning are poorly understood. In two new papers, Lüthi and colleagues and Anderson and colleagues use various pharmacological and cutting-edge molecular genetics techniques to identify an inhibitory microcircuit within the central nucleus of the amygdala, of which the lateral (CEl) and medial (CEm) subdivisions mediate the acquisition and expression of conditioned fear, respectively, in mice.

In the first paper, Ciocchi *et al.* showed that bilateral activation of CEm or bilateral inactivation of CEl induced spontaneous freezing behaviour, suggesting that CEm drives freezing behaviour and receives tonic inhibition from CEl. To assess the roles of these subdivisions in stimulus-induced freezing, the authors trained mice in a fear conditioning paradigm. Expression of conditioned fear could be reduced both by inactivation of CEl during conditioning and by inactivation of CEm immediately before testing, indicating that CEl and CEm mediate the acquisition and expression of conditioned fear, respectively.

How do neurons in CEl respond to the conditioned stimulus (CS+)? Ciocchi *et al.* showed that ~30% of CEl neurons increased firing upon CS+ exposure (CEl_{on}), whereas ~25% reduced firing (CEl_{off}). The shorter latency of CEl_{on} responses suggested that they might inhibit the CEl_{off} neurons upon CS+ exposure. The increases were still apparent 24 h later, indicative of CS+ induced cell-type specific plasticity of neuronal activity. In retrograde neuron labelling studies, Cioccha *et al.* showed that projections are mainly unidirectional, from CEl to CEm, and cross-correlating spiking activity in CEl and CEm neurons revealed that both CEl_{on} and CEl_{off} neurons contribute to these projections.

Haubensak *et al.*, the authors of the second paper, also focused on subpopulations of neurons in CEl. They found that about half of CEl GABA (γ-aminobutyric acid)-ergic neurons express protein kinase δ (PKCδ). Optogenetic activation of individual $PKC\delta^+$ neurons elicited inhibitory postsynaptic currents in $PKC\delta^-$ CEl neurons and in CEm output neurons. Viral tracing studies showed that CEl $PKC\delta^+$ neurons themselves receive inhibitory input from CEl $PKC\delta^-$ neurons

Because in both studies two distinct neuronal populations in CEl seemed to form a local inhibitory circuit and send inhibitory projections to CEm, it is possible that these populations are the same. Indeed, Haubensak *et al.* showed that silencing $PKC\delta^+$ neurons suppressed tonic activity of CEl_{off} neurons, did not affect CEl_{on} activity and increased activity of CEm neurons. Moreover, it enhanced conditional freezing. Taken together, this suggests that CEl_{off} neurons are $PKC\delta^+$ neurons.

The inhibitory nature of the connections in the central amygdala microcircuit raises the question of whether conditioned freezing results from activation or disinhibition of CEm neurons. Ciocchi *et al.* showed that CS+ exposure increased firing in 83% of CEm neurons in a biphasic manner. The first component had a very short latency (suggesting that CEm neurons receive direct CS+ input, presumably from the thalamus), and the second latency matched that of CEl_{off} neurons, suggesting these neurons disinhibit CEm output neurons.

Together, these two studies support a model in which a conditioned stimulus activates CEl_{on} neurons, which in turn inhibit CEl_{off} neurons and thereby remove the tonic inhibition on CEm output neurons, thus enabling the expression of conditioned fear. These papers increase our understanding of how highly organized microcircuits control behaviour. Further dissection of the circuits that mediate fear acquisition and expression may benefit the development of drugs used to treat anxiety disorders.

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ORIGINAL RESEARCH PAPERS Haubensak, W. *et al.* Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature* **468**, 270–276 (2010) | Ciocchi, S. *et al.* Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature* **468**, 277–282 (2010)