Neuronal circuits for fear and anxiety

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Abstract | Decades of research has identified the brain areas that are involved in fear, fear extinction, anxiety and related defensive behaviours. Newly developed genetic and viral tools, optogenetics and advanced *in vivo* imaging techniques have now made it possible to characterize the activity, connectivity and function of specific cell types within complex neuronal circuits. Recent findings that have been made using these tools and techniques have provided mechanistic insights into the exquisite organization of the circuitry underlying internal defensive states. This Review focuses on studies that have used circuit-based approaches to gain a more detailed, and also more comprehensive and integrated, view on how the brain governs fear and anxiety and how it orchestrates adaptive defensive behaviours.

Fear and anxiety elicit defensive behavioural responses that have evolved to enable the organism to avoid or reduce harm and thus ensure its survival. Behavioural correlates of fear and anxiety can be observed in many animal species, which reflects their importance as adaptations to a potentially dangerous environment. However, in humans, excessive fear and/or chronic anxiety are major burdens on both affected individuals and, because of their high prevalence, society in general. To develop novel strategies to alleviate these burdens, neuroscientists are studying the neural substrates and mechanisms that underlie fear and anxiety in animal models of normal and pathological brain function.

Conceptually, fear and anxiety can be regarded as brain states that are caused by external or internal stimuli and that underlie a specific set of measurable behavioural, physiological, hormonal and autonomic reactions (as previously reviewed in REFS 1–6). Past research has emphasized the role of particular brain areas in generating fear and anxiety, and the contribution of synaptic and neuromodulatory processes within identified brain areas to these internal states. However, recent evidence indicates that emotional states correspond to the functional states of defined neuronal circuits within and between various brain regions.

The classic neuroscientific methods for interfering with circuit activity — such as lesions, electrical stimulation and micro-injections — lack the spatial and temporal resolution to identify and to functionally characterize individual circuit elements and their interactions within larger-scale brain-wide networks. Owing to the development of optogenetic and pharmacogenetic tools in recent years, these limitations can now be overcome to allow the identification and targeting of individual cell types based on their molecular profile or connectivity (for reviews, see REFS 7,8). In addition, novel imaging techniques, together with improved activity sensors[9], allow visualization and functional analysis of cellular networks in the intact brains of behaving animals (reviewed in REF. 10).

The neural networks of fear are favourable targets for the application of these modern neuroscience techniques because there is substantial knowledge about the brain regions that are involved in fear and the cellular mechanisms that underlie fear-related behavioural readouts. Importantly, the experimental acquisition of fear responses serves as a powerful model system for studying associative learning and memory — processes of pivotal importance for the ability of an organism to adjust to a fluctuating environment.

Anxiety is less well understood than fear, and much of what constitutes this more complex emotion remains to be elucidated. Indeed, fear is elicited upon factual, acute sensory input, whereas anxiety can be evoked by potential, circumstantial and anticipated threats (for reviews, see REFS 4–6,11). The circuitry that is required to detect, evaluate and process anxiogenic stimuli is arguably more complex than the circuits that are dedicated to fearful stimuli. However, the brain areas and neuromodulatory systems that contribute to fear and anxiety exhibit great overlap, and the ultimate behavioural output circuits might be largely shared between fear and anxiety. Modern circuit-centred approaches have enabled us to investigate the divergence and convergence of fear and anxiety. Furthermore, recent studies have suggested that there is partial overlap between neuronal circuits that mediate

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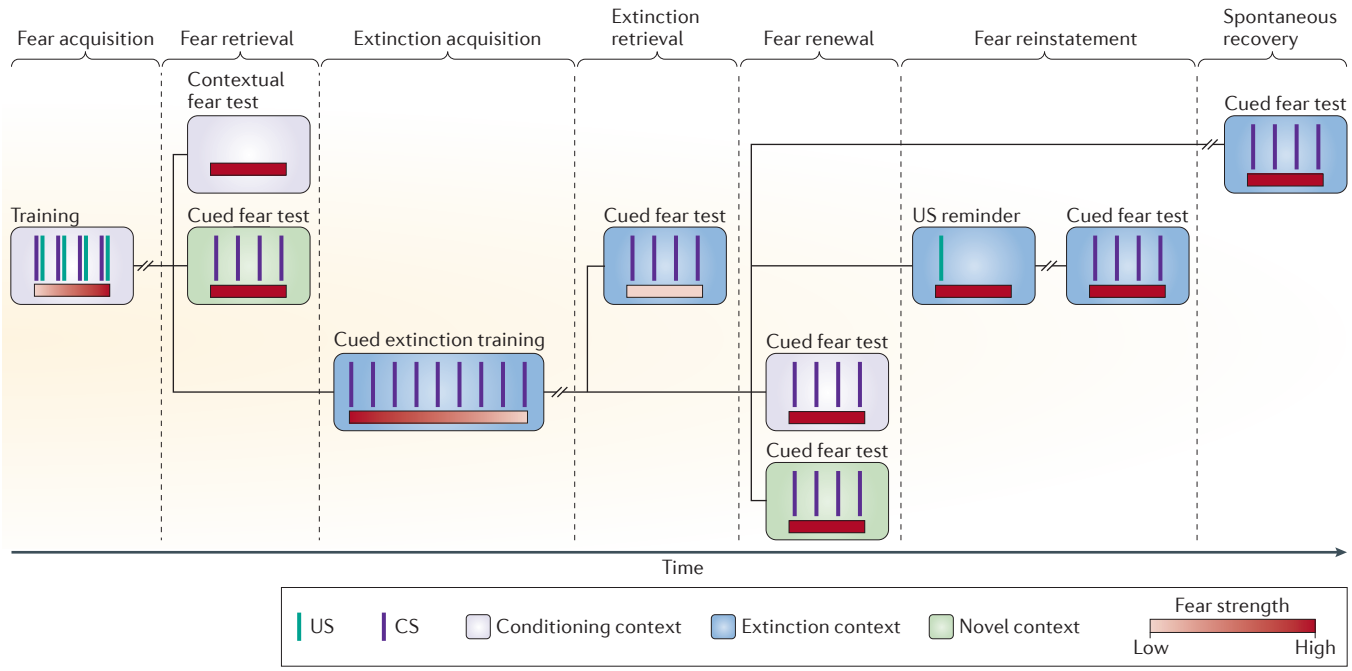
Box 1 | **Fear conditioning**

Fear can be evoked by innately fearful stimuli or by stimuli that acquire fearful properties through association with aversive events. In rodents, the most commonly used procedure for inducing learned fear is Pavlovian fear conditioning. In this paradigm, a normally innocuous stimulus, such as a particular context or a distinct cue (for example, a tone, a light or an odour), is presented together with an aversive event, such as a footshock. The aversive event induces fear responses, thus representing an unconditioned stimulus (US); the previously neutral stimulus acquires aversive properties through an associative learning process and thereby becomes the conditioned stimulus (CS). When presented alone, the CS will evoke fear responses, as measured by increased defensive behaviour, stress hormone release and activation of the sympathetic nervous system (for more detailed reviews, see REFS 2,19,22).

Both short-term and long-term fear-learning processes can be investigated using fear conditioning (see the figure). The fear acquisition phase is typically characterized by a gradual increase in the expression of the conditioned response when multiple CS–US pairings are presented during training. Fear memories are consolidated over time, and their retrieval can be induced and measured by presenting the CS alone in a novel context (cued fear test) or by re-exposure to the conditioning context (contextual fear test). Repeated presentations of the CS alone result in decrement of the conditioned response, which reflects a context-dependent learning process termed extinction. Extinction learning does not completely erase conditioned fear, because the fear memories can show spontaneous recovery over time when the CS is presented in the extinction context. In addition, fear of the CS can be reinstated by exposure to a single US alone or can be renewed by presentation of the CS in either the conditioning context or a novel context (for reviews, see REFS 52,115).



emotional states with a negative valence (for example, fear and anxiety) and those that mediate positive valence (for example, reward)[12–15].

Research into circuit organization and function in fear and anxiety holds promise to provide general insights into brain functions that turn sensory input into specific behavioural output. This Review presents an overview of recent research that, through investigation of local microcircuits and long-range projection-specific pathways, is starting to reveal the circuit basis underlying adaptive behavioural states.

Neuronal circuits for fear conditioning

Most of what we understand about learned fear stems from studies using Pavlovian fear conditioning. In this paradigm, an initially neutral stimulus (the conditioned stimulus, such as a tone) evokes fear through association with an aversive event (the unconditioned stimulus; for example, a footshock) (BOX 1). Owing to its simplicity and robust behavioural output (BOX 2), Pavlovian fear conditioning is a powerful model for studying the neuronal substrates of associative learning and the mechanisms of memory formation. Indeed, studies using this model have revealed that there is a distributed network of brain regions that are involved in learning and expressing fear responses. These include, but are not limited to, the amygdala, the medial prefrontal cortex (mPFC) and the hippocampus (FIG. 1).

Much research has been devoted to discovering the contribution of neuronal and synaptic plasticity mechanisms within these brain regions to the acquisition and expression of fear behaviour. However, the major questions of how plasticity is implemented within defined circuits and how plasticity is regulated by discrete components of local microcircuits remain unanswered. In addition, we are just starting to understand how individual components of a distributed, brain-wide network interact to influence local plasticity. The ultimate goal is to provide mechanistic insights into the plastic events that enable sensory input to drive the acquisition of defined behavioural outputs.

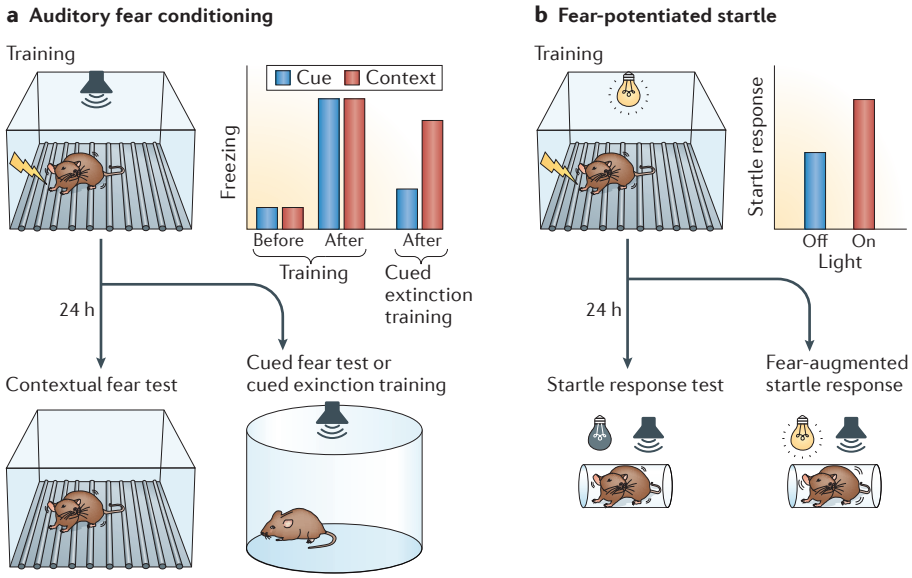
Valence
In the psychological or behavioural context, valence is used to describe the emotional value — positive or negative — that is associated with a distinct or situational stimulus.

Microcircuits
In contrast to long-range projection pathways between distinct brain regions, microcircuits consist of interconnected neurons within a specific brain region and often involve inhibitory interneurons.

Box 2 | **Measuring fear**

Auditory fear conditioning is a form of fear conditioning that uses precisely timed tone–footshock pairings during training. After training, exposure to the conditioning context or conditioned stimulus (CS)-only presentations induces conditioned fear, which is expressed as freezing[2,19,22] (see the figure, part **a**).

The startle reflex of a rodent to loud and sudden noise can also be used to assess fear levels (see the figure, part **b**). Presentation of the startle stimulus in the presence of an aversively conditioned light cue enhances the startle response when compared with responses under dark conditions. Similar augmentation of the startle reflex results if the startle stimulus is presented under continuous bright light conditions[19]. Startle potentiation by discrete and short-duration conditioned stimuli reflects internal fear states, whereas startle enhancement by diffuse and innately aversive stimuli is attributed to anxiety states[4,5,171].



Neuronal substrates
Used as an umbrella term to encompass multiple aspects of brain function, neuronal substrates include anatomical and cellular neuroarchitecture, electrical and neurochemical processes, and circuit mechanisms.

Plasticity
Often used to describe changes specifically in synaptic transmission, plasticity can also refer to different experience- or learning-induced changes within neuronal circuits — such as alteration of connectivity, morphology, and cellular and molecular composition — or to observed changes in stimulus- or context-driven neuronal activity patterns.

Projection neurons
(Also known as principal neurons). An excitatory glutamatergic or inhibitory GABAergic projection neuron projects to a brain area outside the region in which its cell body is located.

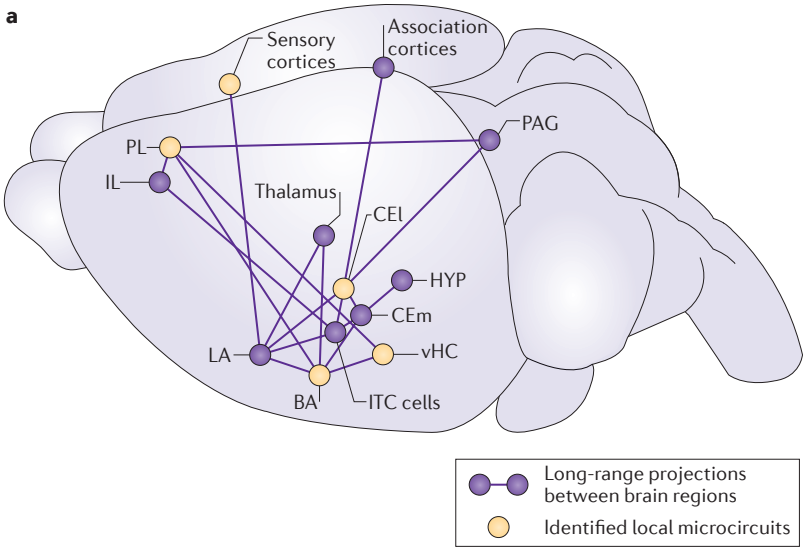
Interneurons
Mainly comprising inhibitory GABAergic cells, locally connected interneurons exhibit specific morphology, electrophysiological properties, molecular composition, projection targets and cellular functions to control activity of projection neurons or other interneurons.

Amygdala circuit plasticity underlies conditioned fear. Research from many laboratories has identified a collection of nuclei in the temporal lobe — termed the amygdala because of its almond-like shape — that are essential for the acquisition and expression of conditioned fear (for a comprehensive overview, see REFS 2,4,16–22). The amygdala nuclei involved in fear learning can be divided into two main sub-areas — the basolateral amygdala (BLA) and the central amygdala (CEA) — that fundamentally differ in terms of cell types and functional organization. The BLA is a cortex-like structure, approximately 80% of which consists of glutamatergic spiny projection neurons and approximately 20% of which consists of GABAergic interneurons[23–25]. The BLA can be subdivided into the lateral amygdala (LA), basal amygdala (BA) and basal medial amygdala (BMA) nuclei. By contrast, the CEA, which can be further subdivided into the lateral CEA (CEl) and the medial CEA (CEm)[26], is a striatum-like structure that is composed of GABAergic medium spiny neurons, many of which project to brain areas that are important for mediating defensive behaviours (for reviews, see REFS 20,27).

One of the fundamental principles of fear learning is the necessity for activity-dependent plasticity within the amygdala. Early studies showed that auditory fear conditioning increases the magnitude of evoked auditory neuronal responses in the LA and that this conditioning-induced enhancement occludes synaptic plasticity that is induced by electrical stimulation of sensory afferents[28–31]. Conditioning-induced plasticity in the LA precedes that in the cortex and thalamus, develops faster than the conditioned behavioural response and is therefore thought to drive conditioned fear behaviour[32,33]. Supporting this notion, optogenetic activation of LA projection neurons can substitute, at least in part, for the unconditioned stimulus during conditioning[34]. Moreover, in line with the necessity for plasticity in the LA, a recent study showed that conditioned fear memories can be reversibly inactivated through optogenetic depression of sensory afferents to the LA[35].

Sensory inputs of every modality terminate in the LA, including auditory, visual and somatosensory inputs that convey information about the conditioned and unconditioned stimuli. Of relevance to auditory conditioned stimuli, there are projections from auditory and multimodal areas of the thalamus to the LA[36–38]. These projections mediate short-latency auditory responses of LA projection neurons and exhibit broadly tuned auditory response properties[39]. A second pathway that conveys auditory information to the LA originates in the ventral auditory cortex[40–42]. It has been suggested that this pathway is particularly important for transmitting information about more complex auditory stimuli[32,43]. However, more detailed studies *in vivo* are required to better define and understand the role of these and additional pathways in conveying specific aspects of conditioned and unconditioned auditory stimuli to the amygdala and to understand the extent to which conditioned-stimulus- and unconditioned-stimulus-related information is associated outside the amygdala.

In contrast to the auditory pathways, much less is known about how the aversive unconditioned stimulus reaches the LA to induce associative plasticity upon convergence with the conditioned stimulus. However, it has been reported that LA neurons preferentially respond to an unexpected rather than expected unconditioned stimulus[14,44]. This raises important questions about what information is encoded by unconditioned-stimulus-related neuronal signals in the LA and where this information is coming from. In line with different accounts of learning theory, graded unconditioned stimulus responses can be regarded as prediction error signals that instruct neuronal plasticity depending on expectancy (reviewed in REFS 45,46). That is, when the occurrence



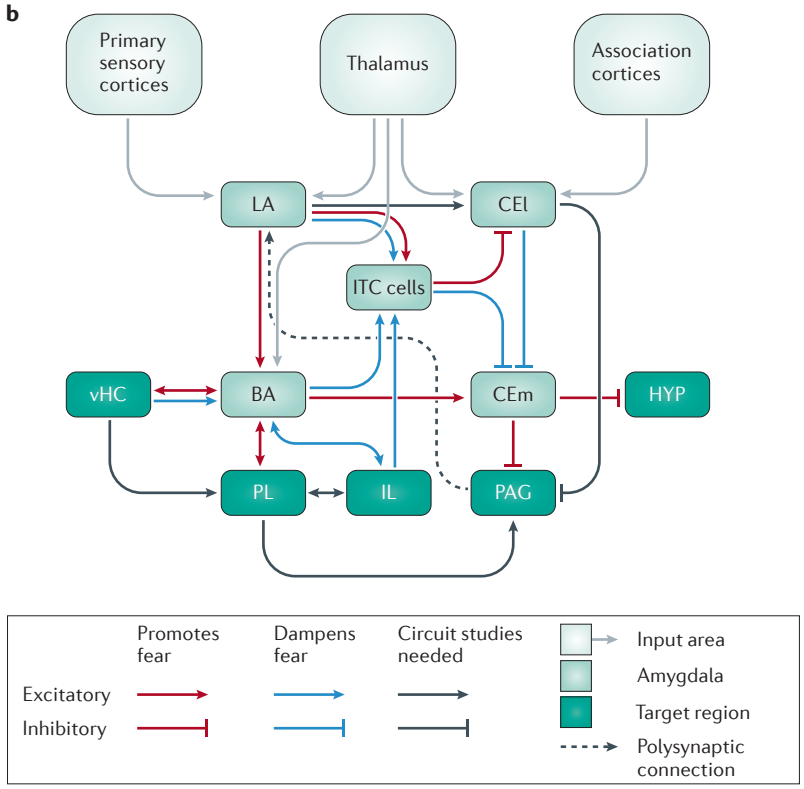


Figure 1 | **The fear and extinction network.** **a** | Fear states are mediated by long-range excitatory and inhibitory connections between multiple brain areas. **b** | Several amygdala nuclei receive sensory input from cortical and thalamic centres and are major sites of fear-related neuronal plasticity. This plasticity is modulated by reciprocal connections between the basal amygdala (BA) and the ventral hippocampus (vHC) as well as between the BA and the prelimbic cortex (PL). In turn, central nuclei of the amygdala project to hypothalamic and brainstem centres to promote fear behaviour. Extinction of fear is mediated by different circuit elements within the same structures. Input from the infralimbic cortex (IL) to the BA and to the intercalated (ITC) cells is instrumental in dampening fear output from lateral central amygdala (CEl) nuclei to the hypothalamus (HYP) and the periaqueductal grey (PAG). The identity, connectivity and function of important forebrain-to-brainstem fear pathways remain to be characterized by modern circuit-based approaches. CEm, medial central amygdala; LA, lateral amygdala.

or magnitude of the unconditioned stimulus is unexpected, strong teaching signals (large prediction errors) drive plasticity and learning in LA neurons. Vice versa, an expected unconditioned stimulus induces weak teaching signals (small prediction errors), and subsequent learning is unchanged. Accumulating evidence suggests that, in fear-conditioning experiments using noxious footshocks, these instructive, prediction error-modulated signals are indirectly introduced into LA circuits by a pathway involving the periaqueductal grey (PAG)[44], a midbrain structure that is a target of CEA output[47] and is instrumental to fear expression[48,49] but that also receives ascending pain signals through the spinomesencephalic tract (as reviewed in REF. 50). Importantly, understanding the function of this circuit may help to explain non-Hebbian phenomena in fear learning, such as the blocking effect[45]. However, the precise pathway and circuit mechanisms through which the PAG instructs the LA during fear learning remain to be determined.

A great diversity of synaptic plasticity mechanisms have been implicated in fear conditioning (for reviews, see REFS 18,51,52). Most of these mechanisms have been described in *in vitro* or *ex vivo* acute-slice preparations[53–56], which raises questions about how they relate to plasticity mechanisms *in vivo*. There is strong evidence to support a role for NMDA-type glutamate receptor (NMDAR)-dependent plasticity at sensory afferents to the LA. Pharmacological blockade of NMDARs abolishes not only fear conditioning at the behavioural level but also its physiological correlates in the LA[55–58]. Similar results have been obtained by interfering with NMDAR-dependent recruitment of synaptic AMPA-type glutamate receptors in the LA, which is a well-characterized mechanism underlying the expression of plasticity in other brain areas[59]. Nevertheless, given that NMDARs are expressed by many different cell types in the LA and neighbouring subnuclei, including interneurons (see below), additional and/or alternative mechanisms could also contribute.

Interestingly, the occurrence of plasticity has been demonstrated in a subset of LA cells that develop responsiveness to a conditioned stimulus during fear conditioning. *In vivo* extracellular recordings, neuronal silencing and immediate-early gene analyses have revealed that 10–40% of neurons in the LA become activated by fear conditioning, and the same or overlapping network is reactivated during fear expression[30,60–64]. In line with these data, a recent study showed that the reactivation of a sparse LA network that has previously been recruited to the memory trace is sufficient to induce fear behaviour, even in novel contexts[65]. Interestingly, a similar phenomenon has also been observed in the dorsal hippocampus, where reactivation of the network that encodes a contextual fear memory induces freezing in a novel context[66]. In addition to modelling studies, these experiments support the idea that there is a competitive process that recruits a limited set of neurons to the fear memory trace[67,68]. The number of plastic cells that are recruited may be constrained by cell-intrinsic and circuit mechanisms, which could possibly involve interactions with interneurons, as discussed below.

Defensive behaviours
Expressed in response to threatening stimuli or situations, defensive behaviours serve to avoid or reduce harm and are highly conserved across mammals.

Fear memory trace
Often used to emphasize its physical location inside the brain, a fear memory trace refers to the neuronal substrates that underlie the formation, storage and recall of the internal representation of a fearful event.

Inhibitory and disinhibitory control in fear circuits. To identify the molecular and cellular plasticity mechanisms underlying fear conditioning *in vivo*, it will be important to address the constraints imposed by control elements in the circuit. Local circuit inhibitory interneurons represent one type of regulatory control element. Accumulating evidence indicates that the neuronal activity and plasticity of BLA projection neurons are tightly controlled by GABAergic inhibition[69–76]. Early *in vitro* studies showed that induction of synaptic plasticity at glutamatergic afferents requires feedforward inhibitory control to be temporally alleviated[71,74]. More recently, an *in vivo* study explored how the intricate organization and function of interneurons in the BLA control the sensory-evoked activity of BLA projection neurons during learning[77] (FIG. 2). In this study, an optogenetic approach was used to identify and manipulate soma-targeting, parvalbumin-expressing (PV^+) interneurons or dendrite-targeting, somatostatin-expressing (SOM^+) interneurons, which are the two main interneuron subtypes in the BLA[25,78–80]. Combined data from *in vivo* and *in vitro* recordings revealed that PV^+ interneurons were excited by the conditioned stimulus and thereby inhibited SOM^+ interneurons, which in turn led to dendritic disinhibition of projection neurons (FIG. 3a). By contrast, the unconditioned stimulus (in this case, footshocks) was found to inhibit both PV^+ and SOM^+ interneurons, which in turn led to disinhibition along the entire somatodendritic axis. Thus, conditioned stimulus–unconditioned stimulus associations during fear conditioning involve a stimulus-specific shift in inhibition along the somatodendritic axis of BLA projection neurons that is mediated by the interaction

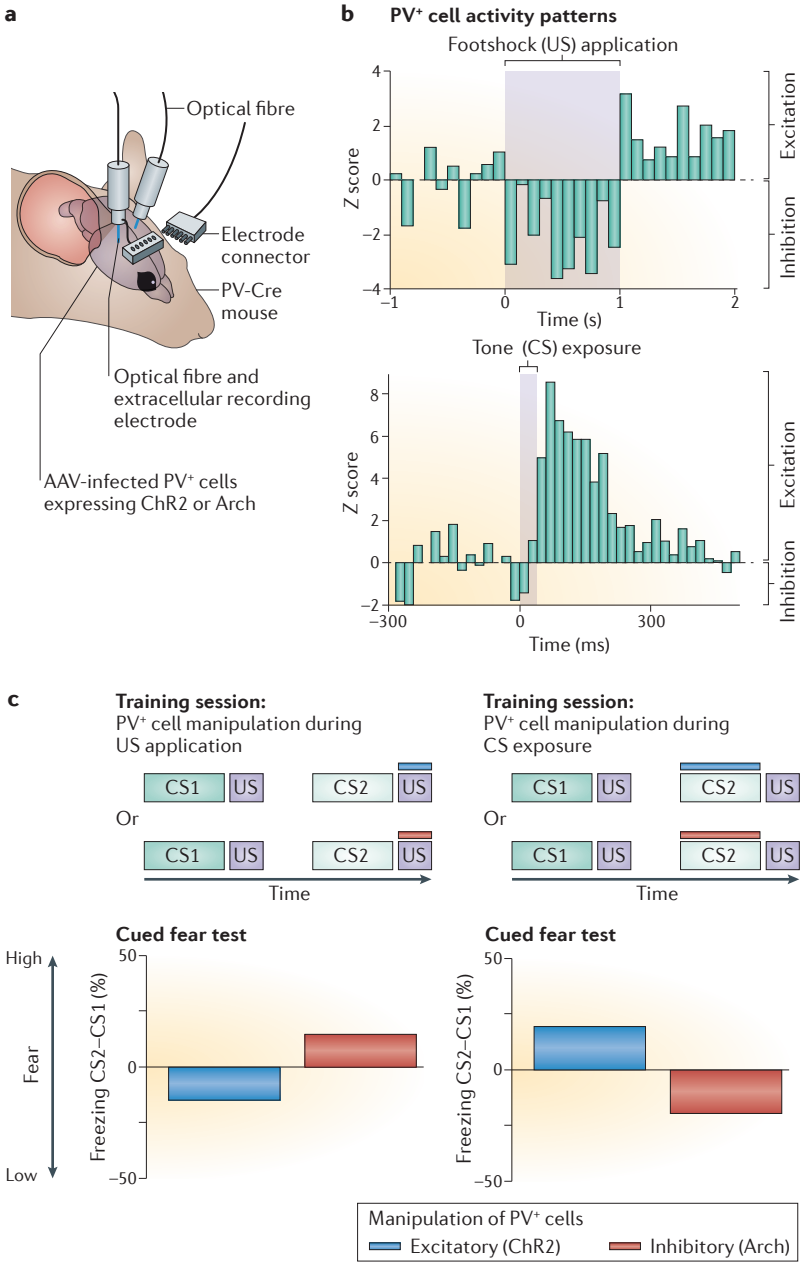


Figure 2 | **Using optogenetics in auditory fear conditioning.** **a** | Cre-conditional viruses (such as modified adeno-associated virus (AAV)) that express light-sensitive opsins (such as channelrhodopsin 2 (ChR2) or archaerhodopsin (Arch)) can be injected locally into the brain of mutant mouse lines in which Cre recombinase expression is controlled by the promoter of a specific genetic marker, such as parvalbumin (PV). Only the infected cells of defined genetic identity will then develop light sensitivity and can be optically activated or inhibited. In addition, individual neurons of a specific neuronal subtype can be identified using combined optical stimulation and extracellular recordings in freely moving mice. **b** | Stimulus-specific activity patterns of optically identified cells can be measured during auditory fear conditioning. For example, PV-expressing (PV^+) cells in the basolateral amygdala (BLA) are inhibited by the footshock (the unconditioned stimulus (US)) but are excited by the tone (the conditioned stimulus (CS)). Such physiological activity profiles can instruct precisely timed optogenetic interventions during stimulus presentations. **c** | Fast and temporally precise optogenetic manipulation of neuronal activity presents a powerful tool with which to dissect the circuits underlying conditioned fear. Activity of defined neuronal subpopulations (such as PV^+ cells) can be differentially manipulated during CS or US presentations, thus revealing timing-specific and stimulus-specific roles of individual circuit elements in the acquisition of conditioned fear. Specific effects of optical manipulations can be controlled for by a within-subject experimental design: several CS–US pairings with concomitant light exposure are compared with CS–US pairings without light exposure using a tone of a different frequency (in the top panels, differences in frequency are denoted by the different shades of green). Optogenetic augmentation of the natural activity profile of BLA PV^+ cells enhances fear learning. That is, when BLA PV^+ cells are inhibited during US application in the training session, fear responses to the CS, expressed as freezing behaviour during the cued fear test, are enhanced (lower left panel). Vice versa, activation of BLA PV^+ cells during US impairs fear learning and results in diminished fear responses during the cued fear test. By contrast, optogenetic training manipulations during CS exposure result in the opposite effects (lower right panel). Figure is from REF. 77, Nature Publishing Group.

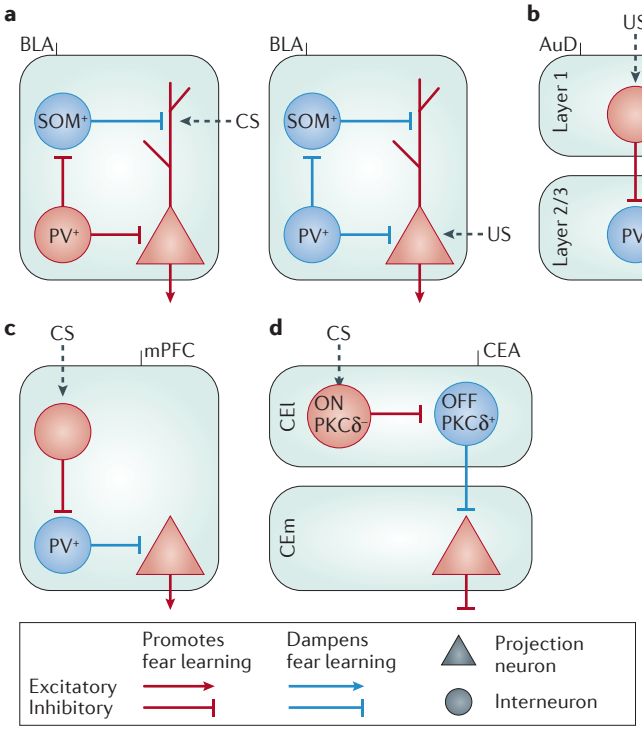


Figure 3 | **Disinhibitory microcircuits in fear learning.** **a** | Projection neurons in the basolateral amygdala (BLA) are under inhibitory control by parvalbumin-expressing (PV^+) interneurons[77], which target the cell body, and by dendrite-targeting, somatostatin-expressing (SOM^+) interneurons, which in turn are under the inhibitory control of PV^+ interneurons. During exposure to the conditioned stimulus (CS), increased inhibition of PV^+ interneurons onto SOM^+ cells results in disinhibition of projection neuron dendrites, thus increasing CS-evoked projection neuron activity (left panel) and enhancing acquisition of fear memory. Application of the unconditioned stimulus (US) causes disinhibition of projection neurons along the entire somatodendritic axis, thus promoting fear learning (right panel). **b** | In the auditory cortex (AuD), a footshock (the US) excites layer 1 inhibitory cells that project onto layer 2/3 PV^+ interneurons, thereby disinhibiting output cells and promoting fear learning[43]. **c** | In the medial prefrontal cortex (mPFC), output cells are under inhibitory control of PV^+ cells, which themselves become inhibited by CS-induced excitation of presynaptic inhibitory neurons. CS-mediated disinhibition of output cells plays a major part in fear learning[87]. **d** | CS-induced excitation of inhibitory ON cells in the lateral central amygdala (CEl) increases inhibition onto inhibitory OFF cells expressing protein kinase Cδ (PKCδ) that project to the medial central amygdala (CEm) output neurons. Disinhibition of these output cells by the CS results in enhanced fear expression. CEA, central amygdala.

between defined interneuron subtypes. This mechanism could enable the association of a conditioned-stimulus-induced dendritic signal with a more widespread activity signal that is induced by the unconditioned stimulus. Identifying the physiological and molecular nature of these signals may reveal the core mechanisms of synaptic and cellular plasticity underlying fear conditioning. Moreover, as interneurons are an important target of different neuromodulatory systems[74,81–85], regulation of disinhibitory circuits might represent a fundamental mechanism by which neuromodulators control circuit states and behaviour.

Indeed, disinhibitory microcircuits are necessary for the acquisition and expression of conditioned fear responses throughout the brain, as has now been observed in multiple areas of the cortex, in the hippocampus and in the CEA. For example, in the auditory cortex, a disinhibitory circuit that involves layer 1 interneurons and PV^+ cells in deeper layers gates the acquisition of conditioned fear responses[43] (FIG. 3b). Conversely, SOM^+ interneuron-mediated dendritic inhibition of hippocampal projection neurons during the unconditioned stimulus has recently been identified as a circuit mechanism that supports contextual fear learning[86]. Similarly, a recent study showed that local disinhibitory microcircuits in the prelimbic cortex (PL; a region in the mPFC) control the expression of fear behaviour[87] (FIG. 3c). Conditioned fear responses result from the disinhibition and synchronized firing of PL projection neurons, a process caused by the release of local PV^+ interneuron-mediated inhibition. Together, these studies add to a growing body of literature characterizing the intricate organization of interneuron ensembles in both cortical and subcortical areas[88–91], and they identify disinhibitory microcircuits as key features of the neuronal networks that mediate learning and memory.

CEA microcircuits mediate fear. Rather than being a passive relay station in the fear pathway, the CEA may play a major part in the acquisition of conditioned fear[92]. In recent years, circuit-based approaches have further refined our view of the role of the CEA in fear acquisition and expression by elucidating the function of distinct cell types within the CEA in fear learning. One study investigated the differential contribution of the CEl versus that of the CEm in conditioned fear[93]. The authors showed that pharmacological inactivation of the CEl or optogenetic activation of the CEm induces unconditioned freezing, suggesting that, under baseline conditions, the CEm is under tonic inhibitory control by the CEl. Moreover, they found that, during conditioning, neuronal activity in the CEl (but not in the CEm) is required for acquisition, whereas activity in CEm is required for the expression of conditioned fear responses.

Extracellular *in vivo* recordings revealed that the CEl has distinct subpopulations of inhibitory cells, which undergo functional plasticity as a result of fear conditioning. One cell type, CEl^{ON}, is excited by the conditioned stimulus, whereas the other, CEl^{OFF}, is inhibited[92]. The authors further showed that CEl^{OFF} cells are inhibited by CEl^{ON} cells, that CEl^{OFF} cells project to the CEm and that inhibition of CEl^{OFF} cells was associated with disinhibition of CEm output neurons (FIG. 3d). A complementary study demonstrated that CEl^{OFF} cells express protein kinase Cδ (PKCδ) and that pharmacogenetic silencing of PKCδ-expressing ($PKC\delta^+$) cells can enhance conditioned fear responses[94]. Interestingly, many $PKC\delta^+$ cells co-express the oxytocin receptor, and it has recently been demonstrated that release of endogenous oxytocin into the CEl increases inhibitory currents in CEm output neurons and attenuates conditioned freezing[95].

A study using cell-specific optogenetic manipulations and recordings discovered an additional component of the CEl microcircuit[96]. Using *in vitro* recordings, the authors showed that excitatory input from the LA onto SOM^+ cells in the CEl is potentiated following

Disinhibition
Describing a neuronal mechanism prevalent in fear learning, disinhibition results in enhanced activity of a postsynaptic neuron by way of inhibiting an inhibitory presynaptic input.

fear conditioning and that synaptic potentiation onto SOM$^+$ cells in the CEl is necessary for fear acquisition. Furthermore, SOM$^+$ cells provide potent inhibition to other CEl cells but not to the CEm. Because synaptic input onto SOM$^-$ cells is weakened by fear conditioning, it is possible that fear conditioning biases the competition between cell types. Interestingly, optogenetic activation of SOM$^+$ cells leads to unconditioned freezing, and inhibition of SOM$^+$ cells can reduce conditioned freezing. Furthermore, pharmacogenetic inhibition of the SOM$^+$ network during conditioning impairs the acquisition of conditioned fear. It remains to be investigated how CEl cell populations that are defined by functional readouts (for example, CEl^{OFF} and CEl^{ON} neurons) map onto genetically defined cell types and how they interact within the local microcircuitry to generate activity patterns that are associated with specific behavioural outputs.

There is evidence that discrete output pathways from the CEA mediate distinct fear-related behaviours. CEm neurons send projections to many brain regions that directly regulate fear responses (reviewed in REFS 2,20; see also REF. 97). Many CEm output neurons increase their firing in response to the conditioned stimulus, and optogenetically increasing CEm neuronal activity leads to unconditioned fear responses, whereas decreasing CEm output attenuates conditioned fear[93,98]. These effects could be mediated by the GABAergic projection from the CEm to the ventrolateral PAG (vlPAG)[47], as lesions or neuronal blockade of the vlPAG impair conditioned freezing[48,49]; however, the specific targets within the vlPAG and the circuit mechanisms that mediate freezing remain unclear. There are diverging CEm output pathways for regulating behavioural versus autonomic aspects of the fear response[81]. These pathways have different physiological properties, and only a small number of CEm neurons project to multiple targets, such as the vlPAG and the dorsal vagal complex[81]. These data suggest that at least some of the physiological responses to fearful stimuli may be mediated by distinct neuronal pathways. However, it has also been shown that individual CEA projection neurons serially target downstream brain regions, raising the alternative possibility that some pathways are able to simultaneously orchestrate multiple fear reactions[99]. Interestingly, the CEl also sends projections to brain regions that are involved in defensive behaviours[100–102], and the function of these distinct pathways has yet to be explored.

Distributed networks of conditioned fear. Although the acquisition and expression of conditioned fear depend on associative plasticity in the LA, fear responses are, in fact, mediated by a distributed, highly interconnected network of forebrain regions (FIG. 1). Fear conditioning-induced changes in conditioned stimulus responses are not only limited to the amygdala but have also been observed in auditory and multimodal nuclei of the thalamus[103], auditory cortex[32,43], mPFC[87,104] and hippocampus[105]. Recently, inputs from the paraventricular nucleus of the thalamus to the CEA have been implicated in fear expression[106–108]. Given that these brain regions are reciprocally connected, either through direct projections or through polysynaptic pathways, future studies need to address how these brain regions contribute to the acquisition and/or expression of conditioned fear — either by feeding back onto amygdala circuits or by bypassing the amygdala through projections to downstream areas such as the PAG.

Fear expression, even in response to simple auditory conditioned stimuli, depends on the PL[109]. The PL receives direct input from a population of BA neurons that is active during states of high fear[110]. Consistent with this notion, BA inputs are important for the gating of fear responses in the PL[111], and reciprocal interactions between the BA and the mPFC may underlie the entrainment of theta rhythms that are associated with successful stimulus discrimination after fear conditioning[112]. Likewise, theta-rhythm entrainment during fear expression also occurs between the LA and the hippocampus[113]. Further details about hippocampus–amygdala interactions were provided by a study showing that calbindin-expressing interneurons in the BLA target the dendrites of BLA projection neurons and provide inhibition in phase with the hippocampal theta rhythm[78]. Because fear responses can be context specific (see below), and because the hippocampus is known to encode contextual information, it is conceivable that hippocampus–amygdala interactions are important for contextual modulation of fear. Along those lines, the BLA projection to the entorhinal cortex, which in turn provides major input to the hippocampal formation, has recently been demonstrated to contribute to contextual fear conditioning[114].

The cellular identities of these long-range interactions are not yet fully understood. The BLA contains subpopulations of neurons that are active during states of high or low fear[33,61,62]. Interestingly, these BLA 'fear neurons' and 'extinction neurons', respectively, exhibit differential functional interactions with the hippocampus and with distinct subdivisions of the mPFC[61,110]. Thus, to understand how acquisition, expression and contextual modulation of fear are encoded within these brain-wide networks, it will be important to investigate these interactions at the cellular level.

Taken together, recent advances in circuit-based research have contributed to the emerging view that learned fear is mediated by coordinated activity among distributed cue- and context-specific networks of specialized neuronal subpopulations in multiple brain regions.

Neuronal circuits for fear extinction

Extinction is largely regarded as a new type of learning in which extinction networks inhibit fear networks[115] (BOX 1). The distributed network that controls fear extinction involves many of the same brain areas that are important for fear conditioning, including the amygdala, the mPFC and the hippocampus (FIG. 1).

Circuit balance in extinction learning. As with fear acquisition and expression, manipulations that inhibit neuronal activity or disrupt synaptic plasticity in the BLA impair extinction[116–119]. Extinction reduces

Theta rhythms
A specific type of oscillatory neuronal activity in the 4–10 Hz range. Theta rhythms have been strongly implicated in fear learning and expression.

conditioned-stimulus-evoked activity in the LA in a context-specific manner, and the extent of the reduction in activity correlates with a decrease in behavioural measurements of fear[33]. Furthermore, during the formation of stable long-term extinction memories, a switch in the balance of activity between BLA fear neurons and extinction neurons occurs[61], which is consistent with the idea that NMDAR-dependent cellular plasticity in the BLA is an important process for the formation of long-term extinction memories[119,120]. A recent study targeted subpopulations of BA neurons based on their projection target in the mPFC[110]. The authors showed that fear neurons project specifically to the PL, whereas extinction neurons project to the infralimbic cortex (IL; a region in the mPFC). Moreover, they found that the balance of activity between the BA–PL and BA–IL projection pathways determines the relative expression of fear and extinction memories upon extinction retrieval. The local circuit mechanisms underlying the switch in activity between different output pathways, as well as their precise cellular targets in downstream structures, remain important topics for future research.

The presence of fear neurons and extinction neurons in the BA, and their differential long-range connectivity, suggests that discrete circuits might mediate fear extinction. Extinction pathways could directly inhibit fear pathways locally within the amygdala, and/or there could be competition between fear pathways and extinction pathways in the way that they impinge on brainstem-targeting output pathways of the CEA or on subdivisions of the mPFC. Interestingly, consistent with the notion that extinction is a new form of learning and does not erase fear memories, another population of cells in the BLA ('extinction-resistant neurons') maintains an increased responsiveness to the conditioned stimulus, even after extinction[33,61,62].

The finding that extinction reduces the activity of fear neurons suggests that amygdala fear networks that are recruited to the memory trace are inhibited by an extinction-specific network as a result of extinction training. This very likely involves the recruitment of local interneurons and may also involve the coordinated activity of the inhibitory intercalated cell masses (ITC cell masses)[75,121–123]. In the BLA, the role of inhibitory cellular mechanisms during extinction has attracted increasing interest, and several lines of evidence suggest that fear extinction learning induces plasticity and remodelling of inhibitory circuits and synapses[124,125]. In particular, perisomatic inhibition that is mediated by PV^+ and cholecystokinin-expressing basket cells exhibits differential plasticity during extinction[126]. The plasticity of cholecystokinin-expressing interneurons, which express presynaptic type 1 cannabinoid receptors[127], may account for the extinction deficit that is observed in the absence of functional type 1 cannabinoid receptors[128]. Future research will have to address whether remodelling and functional plasticity of BLA inhibitory circuits contribute to the selection of distinct BLA output pathways that support fear or extinction behaviour and, if so, how this might occur.

Intercalated cell masses (ITC cell masses). As specialized clusters of mostly inhibitory neurons nestled in the fibre bundles surrounding the amygdala, ITC cell masses are thought to gate information flow in the amygdala.

Distributed networks of fear extinction. The IL is vital for fear extinction[121,129,130], and IL neurons show increases in conditioned-stimulus-induced firing during extinction retrieval but not during extinction training[130]. Importantly, extinction training induces NMDAR-dependent plasticity in IL neurons[130,131]. Extinction also causes increased burst firing in IL neurons, which stabilizes fear extinction memory[131]. Although it is not yet clear which cell types are involved in IL extinction-learning circuits, it is conceivable that circuit motifs similar to those described in the BLA and PL mediate plasticity in the IL during extinction.

Consistent with the observation that IL neurons exhibit conditioned-stimulus-evoked responses during extinction retrieval[130], electrical stimulation of the IL leads to inhibition of the CEm, where most of the brainstem-targeting output neurons are located[132]. The pathways through which the IL inhibits CEA output neurons may include several elements of amygdala circuitry; there is converging evidence that this inhibitory effect involves the ITC cells. The medial ITC (mITC) cells are situated between the BLA and the CEA, and they act to gate information flow between these regions[75,123]. Indeed, activation of mITC cells leads to inhibition of CEA targets, which provides a direct pathway that could dampen fear responses[75]. Extinction causes increased expression of immediate-early genes in mITC cells, and lesioning mITC cells after the acquisition of extinction results in the spontaneous recovery of the conditioned fear response[122]. Additionally, BA inputs onto mITC cells are potentiated as a result of extinction training, causing feedforward inhibition of the CEm[121]. In addition to these mITC cells, other pathways, including projections from the IL to the CEl or to the BA, may also contribute. Consistent with this notion, a recent study found that extinction leads to decreased synaptic efficacy in the mPFC–BA pathway[133]. Future studies will have to address the relative importance of distinct pathways that connect the amygdala and the mPFC in specific aspects of fear extinction.

One important feature of fear extinction is that it is heavily dependent on context. This aspect is also of high clinical relevance because context-dependent relapse of pathological fear and anxiety is often observed during therapy of anxiety disorders[134–136]. The contextual input that is necessary for the acquisition and retrieval of extinction involves the hippocampus[137,138]. The mechanism by which the hippocampus interacts with the amygdala to regulate conditioning-related firing and contextual modulation of amygdala outputs is currently not well understood. The ventral hippocampus sends strong projections to the BA and the mPFC[61,139,140] as well as weaker projections to other circuit components, such as the CEA[140]. Indeed, pharmacological inactivation of the ventral hippocampus prevents context-dependent fear renewal[141] and interferes with context-dependent changes in conditioned-stimulus-driven firing in the mPFC and the LA[142,143]. Together, these studies have started to shed light on the neuronal circuits underlying context-dependent fear extinction and indicate that extinction involves intricate functional changes in defined long-range circuits that link the amygdala, the mPFC and the hippocampus.

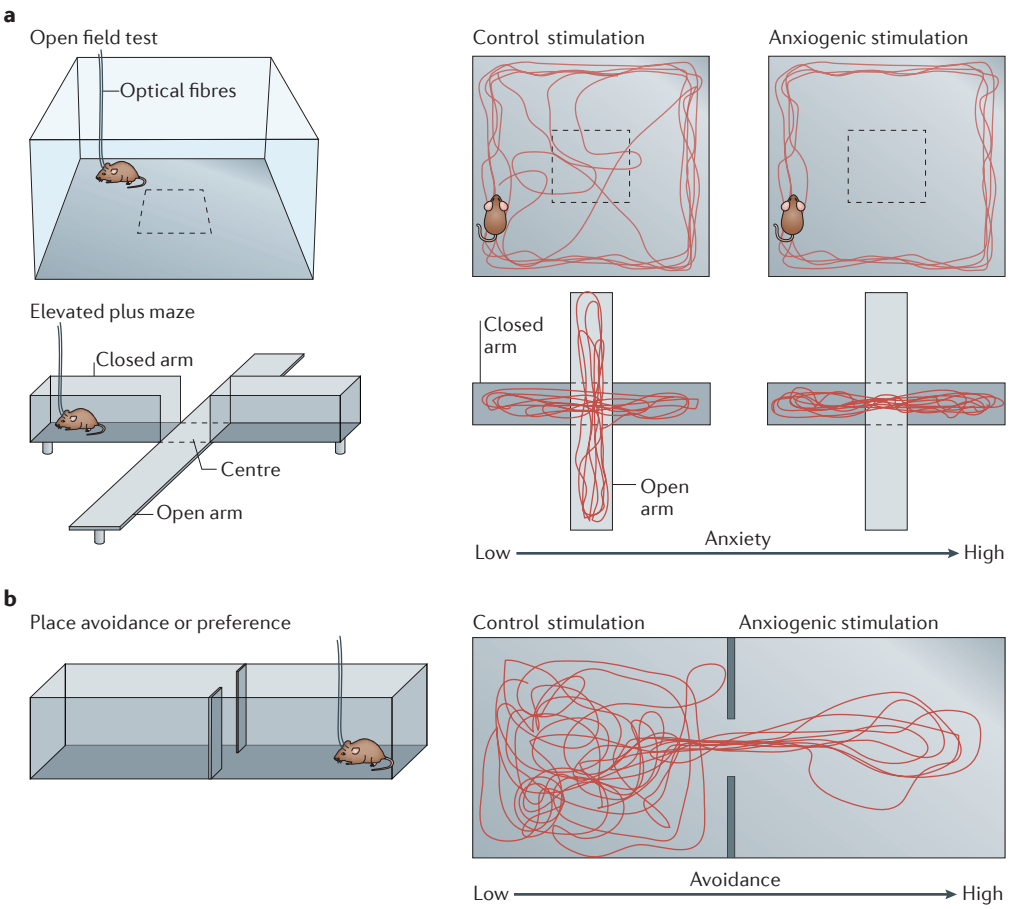
Neuronal circuits for anxiety

Whereas fear is evoked by discrete and acutely threatening stimuli, anxiety can be operationalized as an emotional response to vague, potential threats (for detailed reviews, see REFS 5,6,11,144). Anxiety is characterized by sustained arousal, vigilance and apprehension, and it results in specific patterns of defensive behaviours (BOX 3) and concomitant autonomic responses depending on the nature of the threat and the situational context. A large body of evidence suggests that the central mechanisms underlying fear and anxiety states are similar in both animals and humans and that fear and anxiety processes are mediated by partially overlapping neuronal substrates (as reviewed in REFS 4,5); however, the precise circuits underlying anxiety behaviour have not been investigated as much. Recent studies, which used optogenetic targeting of neuronal subpopulations, have revealed novel aspects of specific circuits underlying anxiety-like behaviour — and, by inference, anxiety states — in rodents. By adding insights into the circuit mechanisms underlying anxiety states, these studies provide biological entry points to a behavioural phenomenon that was previously only diffusely defined in terms of its neuronal components.

Box 3 | **Measuring anxiety and avoidance**

The open field test (see the figure, part **a**, upper panel) and the elevated plus maze (see the figure, part **a**, lower panel) are widely used and pharmacologically validated procedures for assessing anxiety in rodents (for comprehensive reviews of methodology, see REFS 6,173). In the open field test, rodents are placed into a relatively large, brightly lit novel context of circular or rectangular shape. Anxiety-like behaviour is assessed by measuring the extent to which the animal avoids the centre of the arena and stays close to the walls compared with the control animal. In the elevated plus maze, rodents are placed on an elevated cross-shaped maze with two open arms and two arms enclosed by walls. Rodents generally make fewer entries onto the open arms of the maze, and anxious animals avoid the open arms even more. The open field test and elevated plus maze can easily be used in combination with optogenetic stimulation and/or electrical recordings[146,151] (see the figure, part **a**, left panel).

Although freezing is the dominant defensive behaviour observed in small and closed contexts, rodents exhibit fear-induced or anxiety-induced avoidance behaviour in aversive contexts in which an escape route is available[6]. In place avoidance or preference tests, animals can move between different compartments of a context, and optogenetic stimulation can be paired with a specific compartment (see the figure, part **b**). Avoidance or preference of a specific location can be measured either as a real-time outcome of optogenetic stimulation or as a learned behaviour after stimulation. Strong avoidance behaviour has been interpreted to reveal functional roles of the targeted circuits in aversion[13,147,174].



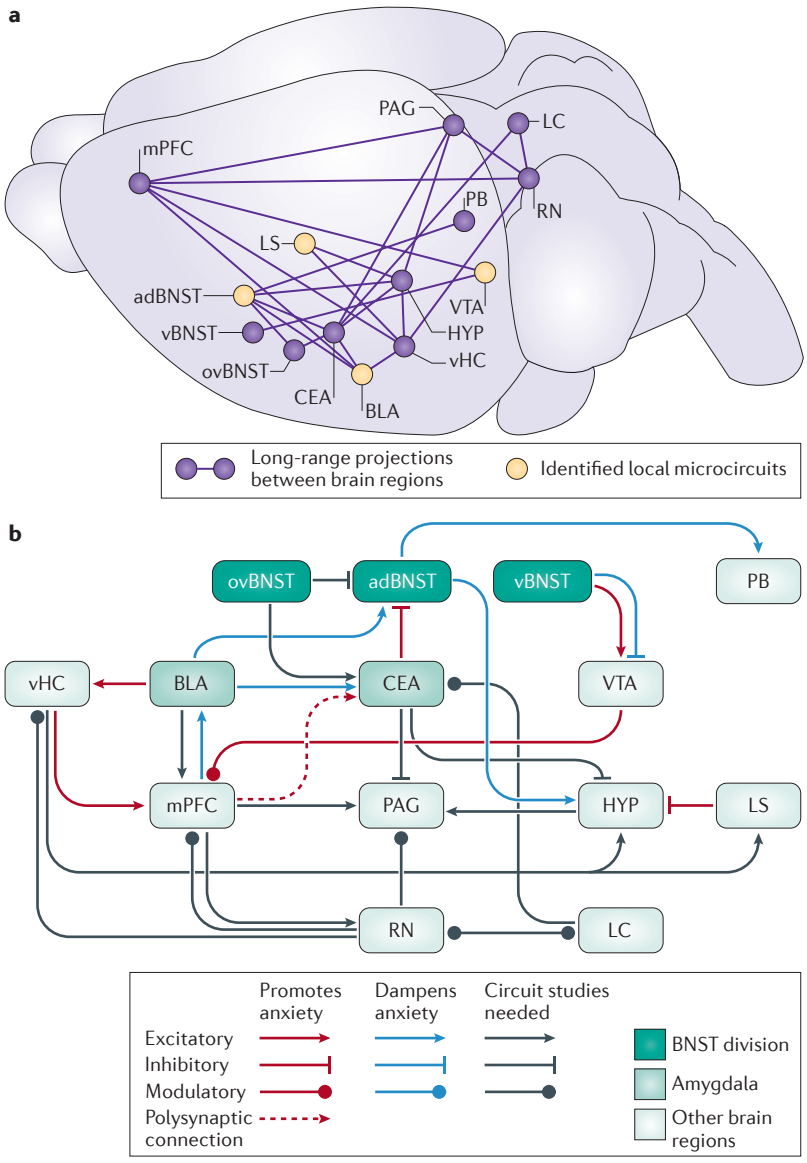


Figure 4 | **The anxiety network.** **a** | Anxiety states are mediated by local and long-range connections between multiple brain areas. **b** | Some regions that have major roles in anxiety, such as the basolateral amydala (BLA) and the anterodorsal bed nucleus of the stria terminalis (adBNST), mediate both anxiogenic and anxiolytic behavioural effects. This indicates the presence of distinct neuronal circuits in anxiety, the functions of which are determined by their target-specific and/or cell-specific connections. For example, activation of the BLA-to-ventral hippocampus (vHC) pathway is anxiogenic[152], whereas activation of the BLA-to-central amygdala (CEA) projection is anxiolytic[151]. By contrast, two parallel ventral BNST (vBNST)-to-ventral tegmental area (VTA) pathways mediate either anxiogenic or anxiolytic behavioural outcomes[147]. Large parts of the anxiety network remain to be characterized in terms of cellular identity and functions as well as precise local and long-range connectivity using modern circuit-based approaches. HYP, hypothalamus; LC, locus coeruleus; LS, lateral septum; mPFC, medial prefrontal cortex; ovBNST, oval BNST; PAG, periaqueductal grey; PB, parabrachial nucleus; RN, raphe nuclei.

BNST circuits have opposing roles in anxiety. Based on lesion and pharmacological studies, it has long been hypothesized that the bed nucleus of the stria terminalis (BNST), which is a major target of projections from the BLA and CEA and is part of the so-called extended amygdala, has an important role in mediating anxiety (reviewed in REF. 5; see also REF. 145). A recent study using optogenetic targeting of different BNST subregions and output pathways refined this view by showing surprising, opposing roles for the oval BNST (ovBNST) and anterodorsal BNST (adBNST) in anxiety as well as functional segregation of the adBNST output to the lateral hypothalamus, the ventral tegmental area (VTA) and the parabrachial nucleus[146] (FIG. 4). Optical inhibition of BNST somata had an anxiolytic behavioural effect, and similar effects were obtained through selective inhibition of the ovBNST. By contrast, inhibition of BLA fibre terminals that target the adBNST increased anxiety-like behaviour. Conversely, activation of BLA–adBNST projections led to behaviours that were indicative of anxiolysis. Furthermore, independent features of this anxiolytic effect were mediated by different projection targets: although activation of adBNST fibre terminals in the lateral hypothalamus recapitulated the anxiolytic behavioural effects, adBNST projections to the parabrachial nucleus mediated the autonomic anxiety response. Thus, a complex state such as anxiety is parsed into discrete behavioural components (for example, increased avoidance) and autonomic components (for example, increased respiration and heart rate) at the level of defined circuits and pathways. It remains to be tested how, and through which projection pathway, the predominately GABAergic ovBNST mediates anxiety.

Localized just ventrally of the adBNST and the anterior commissure, the ventral BNST (vBNST) sends both excitatory glutamatergic and inhibitory GABAergic projections to non-dopaminergic cells in the VTA. Activation of glutamatergic vBNST inputs to the VTA induced avoidance and enhanced anxiety, whereas activation of GABAergic inputs produced rewarding and anxiolytic effects[142]. Importantly, this correlation of cellular and behavioural function was also reflected in the neuronal activity of optically identified glutamatergic or GABAergic vBNST cells. Specifically, glutamatergic cells showed enhanced activity during an aversive footshock session, whereas GABAergic cells were strongly inhibited[147]. This suggests opposite involvement of these distinct BNST–VTA pathways in the generation of anxiety states.

Distinct amygdala circuits promote or dampen anxiety. Evidence from human studies suggests that the amygdala has an important role in anxiety (for reviews, see REFS 11,148). However, both enlarged[149] and reduced[150] amygdala volumes have been associated with human anxiety disorders. These discrepancies are unsurprising in light of the findings discussed above, which demonstrate that the amygdala consists of several functionally distinct nuclei. It is therefore conceivable that the circuitry within and among amygdala subnuclei, and the various long-range projections from the amygdala, may have different, potentially opposing functions in anxiety (FIG. 4). Along these lines, several studies using murine models of anxiety have targeted specific intra-amygdala circuits[151] and long-range projections[152] to study their role in anxiety-like behaviour. Strikingly, whereas the somatic activation of BLA projection neurons resulted in enhanced anxiety-like behaviour, the selective activation of excitatory BLA

axonal projections that terminate in the CEl was anxiolytic[151]. This points to a functional heterogeneity at the level of BLA cell types or projections, similar to what has been found in BLA fear circuits. Thus, it is conceivable that CEA microcircuits that are important for anxiety overlap with those that are necessary for fear.

A recent study provided the first mechanistic evidence for this hypothesis: an increase in the tonic activity of CEl^{OFF} cells after fear conditioning was stronger in mice that exhibited fear responses not only to the conditioned stimulus but also to a second tone that was not paired with a footshock[93]. Because this type of generalized fear response is considered to be a hallmark of anxiety[6], these findings implicate changes in tonic activity within CEA fear circuits in anxiety. A recent study also found evidence for the involvement of phasic LA neuronal activity in fear generalization. In rats exhibiting generalized fear, more cells responded to conditioned stimuli that were not paired with a footshock[63]. Long-lasting changes in the responsivity of these microcircuits could be attributable to changes in inhibitory control, as interference with GABAergic signalling in the amygdala has been shown to affect anxiety[153,154]. Further research is needed to clarify the role of individual circuit elements — such as the $PKC\delta^{+}$ CEl^{OFF} cell population and their projection targets — in generalized fear responses and anxiety-like behaviour.

Because activation of the BLA produced a net anxiogenic effect[151], a BLA projection pathway other than that to the CEl could be responsible for promoting anxiety-like behaviour. The ventral hippocampus has been implicated in anxiety[155–159] and is reciprocally connected to the BLA[160]. In addition, synchronization of rhythmic activity between the BLA and the hippocampus correlates with fear behaviour[113]. A recent study showed that activation of a monosynaptic glutamatergic projection from the BLA to the ventral hippocampus mediates anxiety-like behaviour[152]. Thus, akin to the BLA projections to the PL and IL, which support high and low fear states, respectively, distinct output pathways from the BLA can induce or suppress anxiety-like behaviour following extinction.

Cortico–hippocampal inputs to the amygdala mediate anxiety. Several input pathways to the amygdala have been suggested to have a role in anxiety. Lesioning and pharmacological interventions have provided strong evidence for a major role specifically of the ventral portion of the hippocampus in anxiety (as reviewed in REFS 161,162); however, the precise circuit mechanisms for this remain to be elucidated. Of high interest is the question through which projections — to the BLA, to hypothalamic nuclei or to the lateral septum — the ventral hippocampus contributes to different aspects of anxiety. The mPFC projection to the BLA has a major role in fear extinction (see above). In addition, rhythmic mPFC firing may entrain BLA cells to signal safety and reduce anxiety[112]. A recent study implicated an input pathway from the PFC of monkeys and humans to the CEA in heightened anxiety[163]. Although this study lacked circuit specificity, together with another study[164], it suggested that an anxiogenic phenotype is caused in part by disinhibition of the CEA owing to reduced functional connectivity between prefrontal areas and the CEA. Because the PFC sends only weak projections to the CEA[140], it is likely that polysynaptic circuit mechanisms underlie this phenotype. To address this question, especially in light of the contrasting roles of the IL and PL mPFC subregions in conditioned fear, optogenetic studies are required.

Whereas activity of excitatory BLA inputs to the PL is associated with high conditioned fear states[110], another input pathway to the mPFC from the ventral hippocampus has been suggested to play a part in anxiety. Studies using *in vivo* extracellular recordings in mice showed increased synchrony between the ventral hippocampus and the mPFC during anxiety-like behaviour, particularly in mPFC neurons that encoded the anxiogenic features of the context[155,156]. The functional significance of the ventral hippocampus–mPFC pathway in anxiety has not yet been addressed, as these compelling findings have yet to be complemented by optogenetic experiments aimed at dissecting this circuit using projection targeting within the mPFC (that is, the IL or PL).

A refined role for the septohippocampal system in anxiety. The septohippocampal system has long been hypothesized to play a major part in stress-induced anxiety[165] in that it detects conflict and uncertainty that are evoked in anxiogenic contexts, and it serves to promote arousal, attention and behavioural inhibition[159]. Although there is strong evidence that the ventral hippocampus mediates these functions, both anxiolytic and anxiogenic functions have been reported for the septum[166–168]. Recent findings on specific circuit elements of the septohippocampal system can now reconcile contradictory findings on the role of the lateral septum (LS), particularly for stress-induced anxiety[169]. This study demonstrated that a specific subset of LS projection neurons, which is characterized by the expression of corticotropin-releasing factor receptor 2 and targets the anterior hypothalamus, promotes rather than suppresses stress-induced anxiety. Future research can be expected to reveal additional functional specificity of intra-septohippocampal connectivity as well as its output pathways in anxiety. Furthermore, how those distinct anxiety circuits interact with the fear network remains an open research question.

Although previous studies have emphasized important functional differences between the brain regions underlying fear and anxiety, such as the BNST (sustained fear or anxiety), the CEA (conditioned fear), the ventral hippocampus (contextual fear) and the LS (stress-induced anxiety), novel findings suggest that specific circuits within, and distinct pathways among, these structures mediate the observable range of learned and innate defensive behaviours. This organizing principle will probably be applicable to other brain areas that have previously been implicated in anxiety but on which modern circuit-based research has not been conducted. These include the midbrain serotonergic raphe nucleus (for a review, see REF. 170); the corticotropin-releasing factor system that originates in the paraventricular thalamic nucleus, the CEA and the BNST[5,171]; and the noradrenergic locus coeruleus[172] (FIG. 4).

Box 4 | **Interactions between valence networks**

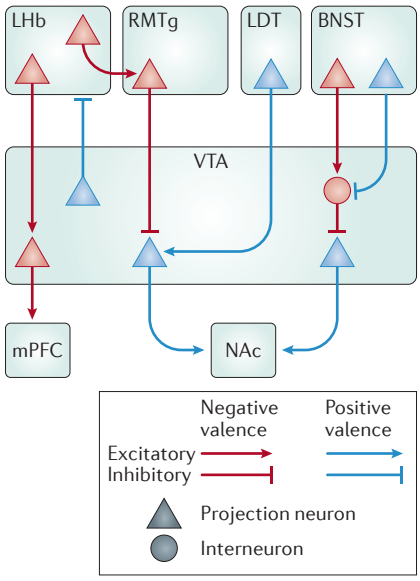
Fear and anxiety can elicit avoidance behaviour in response to negatively valenced contexts or stimuli. By contrast, rewarding stimuli elicit positively valenced emotions, which promote adaptive behaviours such as approach. Emerging evidence suggests that circuits encoding negatively valenced information closely interact, and possibly overlap, with those encoding positive valence (see the figure).

In addition to its long-known role in encoding positive valence and reward (see REF. 175 for a review), recent studies suggest novel roles for the ventral tegmental area (VTA) dopaminergic system in signalling negative valence[176–179]. This functional heterogeneity could arise from the known specificity in VTA dopaminergic projection populations bearing distinct physiological and molecular properties[12,180,181]. Indeed, recent studies have confirmed the presence of multiple valence-encoding circuits in the VTA. Three distinct dopaminergic VTA projections were identified based on synaptic modifications after rewarding or aversive stimulation[12]. One population of dopaminergic cells responds with synaptic modulation to both appetitive and aversive stimulation. By contrast, dopaminergic cells projecting to the nucleus accumbens (NAc) display synaptic plasticity only after the rewarding stimulus, whereas dopaminergic cells targeting the medial prefrontal cortex (mPFC) are sensitive only to aversive stimulation. The latter finding is consistent with results from another recent optogenetic stimulation study that demonstrated the anxiogenic nature of the VTA-to-mPFC pathway[182].

In addition, VTA dopaminergic circuits were found to have input-specific functional roles in signalling positive or negative valence[13]. Reward is mediated by NAc-projecting dopaminergic cells receiving excitatory input from the laterodorsal tegmental nucleus (LDT), whereas avoidance is caused by stimulation of lateral habenula (LHb) pathways to the GABAergic rostromedial tegmentum (RMTg), which inhibits VTA dopaminergic cells. Avoidance is also induced by stimulation of the LHb pathway projecting to VTA cells that target the mPFC. These results demonstrate that pathway-specific dopaminergic output of the VTA is intricately regulated by long-range inputs to drive reward or aversion. In addition, this work adds to a growing body of research defining the LHb as an important locus of negative valence signalling, via its projections to the RMTg and VTA[183–187]. Furthermore, GABAergic VTA cells are activated in response to aversive footshocks, and optogenetic activation of these same neurons results in conditioned place avoidance[174]. Taken together, these results demonstrate that dopaminergic output of the VTA is intricately regulated by intra-VTA microcircuits that receive specific long-range inputs to drive reward or aversion.

One of these inputs to the VTA is provided by the anterodorsal bed nucleus of the stria terminalis (adBNST). Optogenetic activation of this pathway produces conditioned place preference, suggesting a role for this projection in signalling positive valence[146]. The ventral BNST sends both excitatory and inhibitory projections to non-dopaminergic cells in the VTA. Activation of excitatory inputs induces avoidance, whereas activation of GABAergic inputs produces rewarding effects[147]. Furthermore, glutamatergic cells show enhanced activity during an aversive footshock session, whereas GABAergic cells are strongly inhibited. It remains to be determined whether this cell type-specific dichotomy has a role in rewarding states, as suggested by the results obtained through optogenetic interference[147].

Accumulating evidence suggests that the amygdala also signals multiple valences[15,188–191]. Recently, optogenetic approaches were used to address the specific organization of functional circuits within the amygdala[151] and the role of long-range amygdala projections to brain areas that are classically implicated in reward[10,192]. In the future, more detailed circuit-based research needs to address cell type and pathway specificity of these functions.



Furthermore, depending on specific input and output pathways, structures such as the BLA and the BNST have now been shown to mediate both anxiogenic and anxiolytic effects[146,151]. Although this provides entry points into studying the neuronal circuitry of anxiety — a hitherto only vaguely defined behavioural state in terms of its neuronal substrates — it also raises important questions. Because the different pathways associated with anxiogenic and anxiolytic function, respectively, must ultimately converge onto the same motor output to affect behaviour, it remains to be determined to which extent, and precisely where in the brain, these circuits are segregated, where information is integrated and where decisions are made to execute appropriate behaviours.

Conclusions and future directions

Novel circuit-based approaches addressing the role of both local microcircuits and long-range projection-specific pathways have helped to advance our view of how the brain produces fear and anxiety states and the resulting adaptive defensive behaviours. In the future, even more refined intersectional optogenetic approaches that allow projection-specific as well as cell type-specific targeting of circuit elements will be required to reveal additional details about circuit function in fear and anxiety. An important next step will be to investigate how long-range projections interact with local microcircuits. Eventually, this will only be possible if different levels of analysis are integrated; that is, studies should aim to characterize cellular and molecular mechanisms within defined functional networks.

Questions that remain include: how do BLA cells integrate converging sensory inputs, and which plastic changes occur within the BLA microcircuits that are important for gating the acquisition and expression of conditioned fear responses? What are the teaching signals underlying fear learning, and where are they generated? What are the molecular mechanisms underlying cellular and synaptic plasticity in defined CEA neurons, and which CEA output pathways and circuit mechanisms underlie the switch from passive to active fear behaviour? In which ways do BLA and VTA circuits for different valences (BOX 4) interact to produce the appropriate balance of avoidance and approach behaviour in social situations?

An additional challenge for future studies will be to go beyond a functional and anatomical analysis of these circuits and address the computations they carry out. To tackle this challenge, future research needs to address how stimulus representations, associations and behavioural output programmes are encoded at the level of larger-scale neuronal populations that are organized in defined circuits. This will require implementation of electrophysiological and/or optical ensemble recordings in deep brain regions, similar to approaches that have been successfully used to further our understanding of cortical function.

Characterizing the distributed and highly organized neuronal circuits underlying the acquisition and expression of defensive behaviours will not only lead to a better understanding of fear, fear extinction and

anxiety processes but also has the potential to reveal general principles of brain organization and function. Going beyond behaviouristic, outcome-based explanations of fear and anxiety, we are now able to identify specific inputs that cause these internal emotional states and to characterize outputs leading to specific behavioural states. This is important from a translational perspective, because it enables us to study the basic processes of emotions in animal models even in the absence of complex human behaviours. Ultimately, this knowledge will enable us to selectively target and to more effectively treat psychiatric conditions caused by dysregulation within circuits for fear and anxiety.

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Competing interests statement
The authors declare no competing interests.