NEURONAL SIGNALLING OF FEAR MEMORY

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Abstract | The learning and remembering of fearful events depends on the integrity of the amygdala, but how are fear memories represented in the activity of amygdala neurons? Here, we review recent electrophysiological studies indicating that neurons in the lateral amygdala encode aversive memories during the acquisition and extinction of Pavlovian fear conditioning. Studies that combine unit recording with brain lesions and pharmacological inactivation provide evidence that the lateral amygdala is a crucial locus of fear memory. Extinction of fear memory reduces associative plasticity in the lateral amygdala and involves the hippocampus and prefrontal cortex. Understanding the signalling of aversive memory by amygdala neurons opens new avenues for research into the neural systems that support fear behaviour.

If there is one central tenet of the neurobiology of learning and memory, it is that plasticity in the CNS is essential for the representation of new information. Experience-dependent plasticity in the brain might take many forms, ranging from the synthesis and insertion of synaptic proteins to whole-brain synchronization of neuronal activity. An important challenge is to understand how these various forms of experience-dependent plasticity are reflected in the activity of neuronal populations that support behaviour. Donald Hebb referred to these populations as cell assemblies, and this concept has had important heuristic value in empirical studies of the neurobiology of memory[1]. With the advent of modern electrophysiological recording techniques, Hebb's concept of the cell assembly is now amenable to experimental study in awake, freely behaving animals. Using parallel recording techniques, multiple extracellular electrodes can be used to 'listen' to the action-potential dialogue between several neurons at once[2,3] (BOX 1).
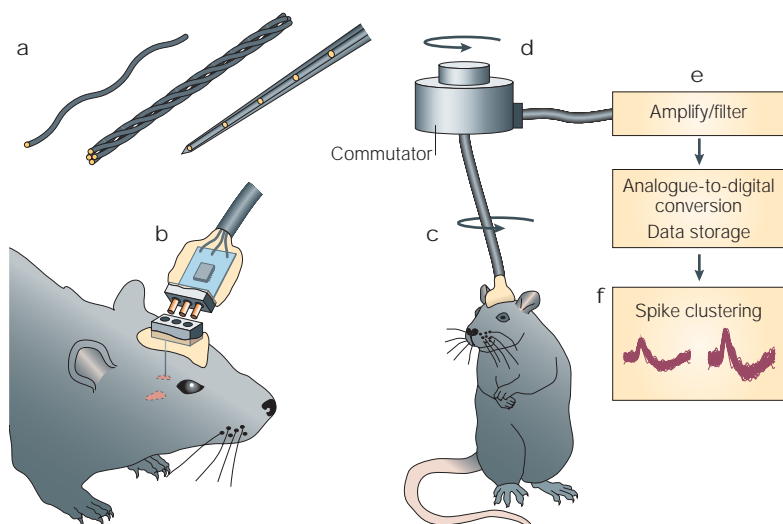
In this article, we review recent single-unit recording studies that have provided considerable insight into the neuronal mechanisms of learning and memory, focusing particularly on Pavlovian fear conditioning. In this form of learning, a neutral stimulus, such as an acoustic tone (the conditional stimulus, or CS) is paired with a noxious unconditional stimulus (US), such as a foot-shock. After only a few conditioning trials, the CS comes to evoke a learned fear response (conditional response, or CR). Pavlovian fear conditioning is particularly amenable to electrophysiological analysis because it is acquired rapidly and yields long-lasting memories. Moreover, the behavioural principles and neural circuits that underlie this form of learning are well characterized, allowing an unprecedented analysis of the relationship between neuronal activity and learned behaviour.

Neuronal correlates of aversive memory

The search for the neurophysiological mechanisms of aversive memory began in the early 1960s with the observation that an auditory stimulus that was paired with an electric shock modified auditory-evoked field potentials in cats and rats[4,5]. Because cortical field potentials are generated by large populations of neurons, changes in early components of the field potentials (reflecting processing in ascending auditory tracts) were variable and poorly localized. Other investigators observed changes in late components of cortical potentials that were attributed to a general state of 'fear'[6], but these changes were not associative (that is, they did not reflect a specific CS–US association) because they occurred in response to both the CS and a novel stimulus. Therefore, it became clear that field-potential recordings would not be sufficient to identify loci of fear memory.

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Box 1 | Single-unit recording methods

Parallel advances in computing hardware (for example, data storage capacity and processor speed), software (for example, neuronal data acquisition and spike sorting) and electrode technology have coalesced to yield powerful multichannel single-unit recording systems for behaving animals. In a typical system, recording electrodes consist of bundles of single wires, multi-wire stereotrodes or TETRODES, or thin-film silicon arrays (**a**). Electrode assemblies are either chronically implanted in brain tissue or affixed to moveable microdrives, some of which have been engineered to independently drive up to 16 tetrodes (64 channels) (**b**). Voltages recorded on each electrode are typically passed through integrated circuits in source-follower configurations that are mounted near the animal's head (a headstage) to convert neuronal signals into low-impedance signals that are less sensitive to cable and line noise (**c**). Signals are then fed from the headstage through a commutator to allow free movement of the animal and cable assembly (**d**). Neuronal signals are amplified, band-pass filtered and digitized (**e**). Once digitized, spike waveforms on each electrode channel are sorted into single units using sophisticated clustering algorithms (**f**). The isolation of single units using such methodology varies widely and depends on several parameters. Most importantly, multichannel electrodes, such as tetrodes, seem to yield the most reliable single-unit isolation. Several commercial packages are available to acquire neuronal signals from high-density recording systems, although most electrophysiologists use a combination of home-made technology and commercial products.

TETRODE
An extracellular electrode that comprises four juxtaposed recording channels, which can be used to disambiguate the signals emitted by individual point sources. Because each neuron occupies a unique position in space, its spikes are 'seen' slightly differently by each electrode, providing a unique signature. This technique allows the identification of many more neurons than there are sampling electrodes.

Subsequent single-unit recording studies in cats and monkeys showed conditioning-induced changes in evoked spike activity in several brain areas, including the midbrain, thalamus and cortex[7–9]. These changes seemed to be associative because they were not observed during pseudo-conditioning, in which the CS and US were unpaired. In addition, sensitizing effects of the shock were ruled out with discriminative models, in which responses to a CS that was paired with the US (CS+) were compared with responses to a CS that was never paired with the US (CS−)[10,11]. However, from these studies it was not possible to determine whether structures that showed increased neuronal responsiveness after conditioning were primary sites of plasticity or were downstream from other plastic sites.

To address this issue, Olds and colleagues[12] assessed the latency of conditioned single-unit responses in various brain areas in an appetitive auditory conditioning task. They reasoned that structures showing the earliest increases in auditory responses (in terms of milliseconds after CS onset) were probably primary sites of plasticity, whereas those showing longer-latency changes were probably downstream sites that were involved in the expression of learned responses. Short-latency plastic responses (within 40 ms of tone onset) were observed in the posterior thalamus, medial geniculate nucleus and auditory cortex, indicating that these areas might be primary sites of plasticity. Although this approach was criticized for not taking into account descending modulation from the cortex[13], subsequent work by Disterhoft and colleagues showed that thalamic neurons were able to learn in fewer trials than cortical neurons[14,15], confirming that thalamic plasticity preceded cortical plasticity, in terms of both latency and trials.

Therefore, plasticity in subcortical structures could occur independently of the cortex, and indeed, learning-related plasticity might not even require the forebrain under some circumstances. In the most systematic neurobiological analysis of Pavlovian learning so far, Thompson and colleagues found that although hippocampal neurons show considerable plasticity during eyeblink conditioning, hippocampal plasticity is not essential for this form of learning. In fact, neuronal plasticity in the cerebellum is crucial for the acquisition and expression of eyeblink conditioning[16,17].

Fear-related plasticity in the lateral amygdala

Notably absent from these early studies of conditioning was any mention of the amygdala. The thalamus and cortex were thought to be the sites that most probably encode emotional associations (but see REF. 18), and the amygdala was suspected to have a role in modulating memory storage in these areas[19]. However, an influential study by Kapp and co-workers showed that lesions of the central nucleus of the amygdala prevented heart-rate conditioning in rabbits[20], consistent with central nucleus modulation of fear-expression centres in the midbrain and hypothalamus[21,22]. Subsequent single-unit recording studies of the central nucleus revealed associative plasticity[23,24], indicating that the amygdala might be a site of plasticity in fear conditioning.

Converging on a similar conclusion, LeDoux and co-workers discovered direct projections from the auditory thalamus to the amygdala in rats, and determined this projection to be vital for auditory fear conditioning[25–27]. Specifically, the lateral nucleus of the amygdala (LA) receives direct projections from the medial subdivision of the medial geniculate nucleus and the adjacent thalamic posterior intralaminar nucleus (MGm/PIN), and it relays this information by way of the basal amygdaloid nuclei to the central nucleus[28–31] (FIG. 1). Small lesions of the LA or the MGm/PIN prevent fear conditioning, whereas large lesions of the auditory cortex or striatum do not[32,33], indicating that thalamo–amygdala inputs are sufficient for conditioned fear responses. This finding galvanized interest in the LA as a potential site of plasticity in fear conditioning, and set the stage for the next 15 years of work on the role of the amygdala in this form of learning. Indeed, considerable research now indicates that the amygdala is necessary for both the acquisition and expression of Pavlovian fear memories[34], but not for all forms of aversive memory[35,36].
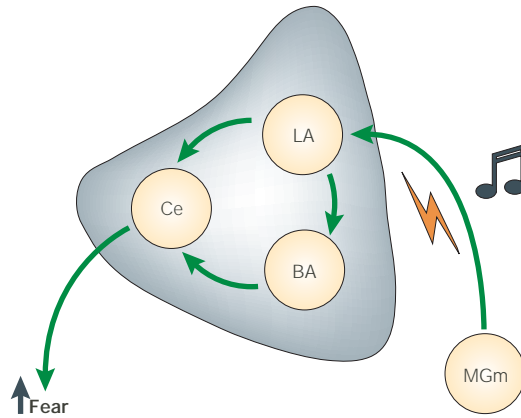
Figure 1 | **Neural circuits that are necessary for auditory fear conditioning.** Tone and shock inputs from the medial subdivision of the medial geniculate nucleus (MGm) converge in the lateral amygdala (LA), resulting in potentiation of auditory responses of LA neurons. The LA projects to the central nucleus of the amygdala (Ce), both directly and indirectly by way of the basal amygdala (BA). Descending outputs of the Ce to brainstem and hypothalamic structures trigger fear responses.

Because the LAd projects to ventral parts of the LA, which in turn project to basolateral and central nuclei, plasticity downstream from the LAd could be passively fed forward from the LAd. To address this issue, Quirk and colleagues recorded LAd neurons in behaving rats, and observed robust increases in tone responses during fear conditioning compared with a sensitization control phase[39] (FIG. 2; BOX 1). Most of the conditioned increases in spike firing occurred within 15 ms of tone onset, corresponding to the latency of thalamic (12 ms) rather than cortical (>20 ms) activation of LA neurons[40]. Maren subsequently confirmed this extremely short-latency plasticity in LAd, and showed that it persisted at these latencies through extensive overtraining[41]. Parallel work has revealed that LA neurons show synaptic LONG-TERM POTENTIATION (LTP)[42–44], and that fear conditioning is associated with LTP-like changes in thalamo–amygdala synaptic transmission[45–47]. Together with evidence of converging auditory and somatosensory inputs onto LA neurons from the thalamus[48,49], this indicated that the LAd might be a site of long-term memory in fear conditioning (BOX 2).

Although these findings are consistent with a primary locus of conditioning-related plasticity in the LAd, it is necessary to show that LAd plasticity is not passively fed forward from either the auditory thalamus or the auditory cortex. Indeed, short-latency plastic responses in fear conditioning have been observed in both the MGm/PIN[50] and the auditory cortex[51]. To determine the contribution of the cortical pathway, Quirk and colleagues compared conditioned unit responses of LAd neurons with those in

An important question is whether neurons in the LA show associative plasticity during fear conditioning. Although previous work implied that this was the case [37,38], nobody had recorded from the dorsal subdivision of the LA (LAd), which is the primary target of MGm/PIN inputs and a site of CS and US convergence.

LONG-TERM POTENTIATION
(LTP) An enduring increase in the amplitude of excitatory postsynaptic potentials as a result of high-frequency (tetanic) stimulation of afferent pathways. It is measured both as the amplitude of excitatory postsynaptic potentials and as the magnitude of the postsynaptic cell-population spike. LTP is most frequently studied in the hippocampus and is often considered to be the cellular basis of learning and memory in vertebrates.
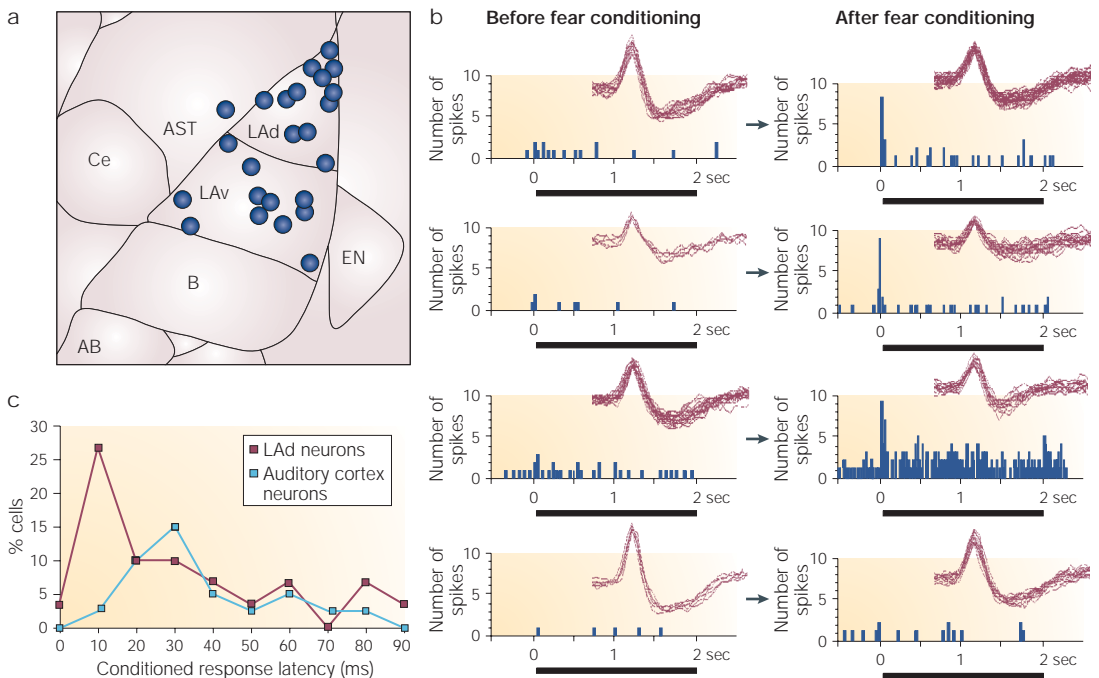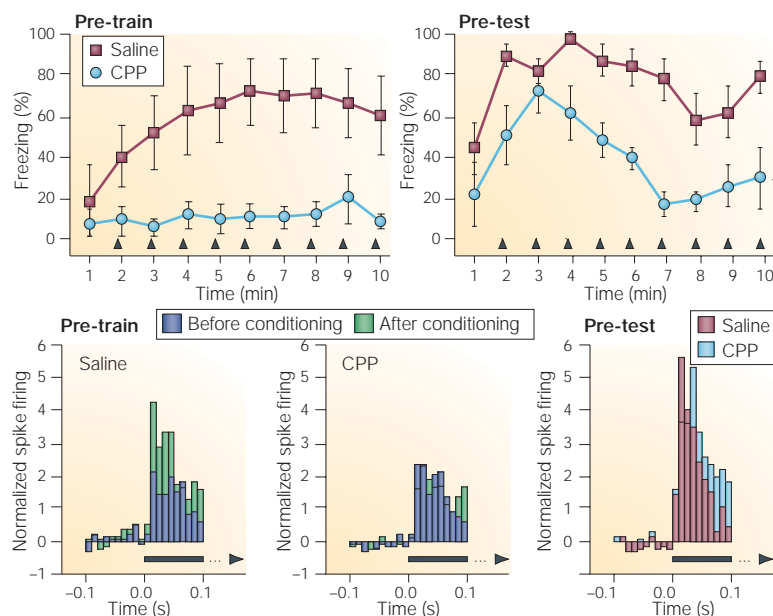

Figure 2 | **Effects of fear conditioning on lateral amygdala neurons.** Fear conditioning induces increases in conditional stimulus (CS)-evoked spike firing in lateral amygdala (LA) neurons. **a** | Electrode placements in the dorsal (LAd) and ventral (LAv) divisions of the lateral amygdala. AB, accessory basal nucleus; AST, amygdalo-striatal transition zone; B, basolateral nucleus; Ce, central nucleus of the amygdala; EN, endopiriform nucleus. **b** | Peri-event time histograms from eight simultaneously recorded single units in the LA. Each histogram represents the sum of ten CS presentations (black bar) before or after fear conditioning. Representative spike waveforms for each unit are shown as pink lines in the insets. **c** | Neurons in the LAd show conditioned increases in spike firing at shorter latencies (from CS onset) than do auditory cortical neurons. Adapted, with permission, from REF. 52 © (1997) Cell Press.

Box 2 | NMDA receptors and associative plasticity in the amygdala

There is considerable evidence that long-term synaptic plasticity in the lateral amygdala (LA) mediates the acquisition of fear memory (see REFS 98–100 for reviews). There is strong evidence that the NMDA (*N*-methyl-D-aspartate) subclass of glutamate receptors is involved in both the acquisition of fear memory and the induction of long-term potentiation (LTP) in the amygdala[44,101], and although there is debate concerning the role of NMDA receptors in the expression of learned fear responses[102,103], recent work indicates that NMDA receptors might be selectively involved in fear-memory acquisition under some conditions[104]. A recent experiment by Maren and colleagues (see figure) examined whether NMDA receptors are also involved in the acquisition of associative neuronal activity in the LA during fear conditioning[105]. In this experiment, CPP (3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid), a competitive NMDA-receptor antagonist, was administered either before training (pre-train) or before retention testing (pre-test) to examine the influence of NMDA-receptor blockade on the acquisition and expression, respectively, of conditional freezing and LA unit activity. Systemic administration of CPP impaired both the acquisition of auditory fear conditioning (as indexed by conditional freezing; arrowheads indicate conditional stimulus (CS) presentations) and conditioning-related increases in CS-elicited spike firing (pre-train panels; first 100 ms of the 2-second CS is indicated by the black bar and arrow). Although CPP completely eliminated the acquisition of conditional fear and associative spike firing in the LA, it had only a mild effect on the expression of these responses (pre-test panels). That is, CPP administered before a retention test in previously conditioned animals moderately attenuated conditional freezing, but did not reduce the magnitude of conditional spike firing in the LA. These data are consistent with models of fear conditioning that posit a role for NMDA-receptor-dependent synaptic plasticity in the formation of fear memory, and reveal that similar neurochemical mechanisms underlie the induction of amygdaloid LTP, conditioning-related increases in spike firing and conditional fear behaviour. Modified, with permission, from REF. 105 © (2004) Blackwell Publishing.

cortical area Te3 during auditory fear conditioning in rats[52]. Te3 is the auditory association area that projects to the LAd[53,54]. They observed that conditioned plasticity in Te3 neurons occurred later than in the LAd (30–50 ms versus 10–20 ms; FIG. 2c). Also, LAd neurons developed conditioned responses within the first three trials of fear conditioning, whereas Te3 neurons required between six and nine conditioning trials to show conditioned responses. Therefore, plasticity in the LAd is not likely to be fed forward passively from Te3, because it precedes Te3 both within and across trials.

It remains possible that LA plasticity is passively fed forward from the MGm/PIN. However, this seems unlikely, because inactivation of the BASOLATERAL AMYGDALA (BLA) with the GABA$_A$ (γ-aminobutyric acid, type A) receptor agonist muscimol prevents the acquisition of fear conditioning, as well as the expression of fear memory, 24 hours after training when rats are tested drug-free[55–57]. Therefore, the primary site of plasticity in fear conditioning is unlikely to be the MGm/PIN, although an effect of muscimol on brainstem projections that regulate ascending modulation of the thalamus cannot be ruled out.

An alternative explanation is that plasticity in thalamic or cortical neurons depends on the amygdala. To address this issue, Maren and colleagues used muscimol to inactivate the BLA while recording single-unit activity in the MGm/PIN[58]. In addition to preventing the development of conditioned fear, muscimol in the amygdala prevented the development of unit plasticity in the MGm/PIN. A similar observation was made for INSTRUMENTAL AVOIDANCE LEARNING in rabbits[59]. In a related experiment, Armony and co-workers recorded single-unit activity from cortical area Te3 in rats that had first received BLA lesions[60]. Although short-latency plastic responses were still observed in amygdala-lesioned rats, long-latency responses anticipating the onset of foot-shock were lost. Because muscimol inactivation of the BLA prevents the development of conditioned fear responses[57,58], amygdala-independent short-latency plasticity in Te3 does not seem to be sufficient to drive fear behaviour, and might represent associative learning at a more cognitive level[61]. By contrast, the loss of shock-anticipatory responses in Te3 neurons indicates that ascending projections from the amygdala might 'interrupt' cortical processing when danger is imminent[62].

Rather than mirroring thalamic or cortical plasticity, it seems that conditioning-related spike firing in the amygdala is independent of — and in some cases essential for — plasticity in the MGm/PIN and Te3. In fact, the LAd seems to be the first site in the auditory pathway to show associative plasticity that is not fed forward passively from upstream sites, is not dependent on downstream sites and is crucial for conditioned fear behaviour. Furthermore, LA neurons seem to drive plasticity at both thalamic and cortical levels, indicating that the amygdala facilitates memory storage in widespread areas, as shown by McGaugh and co-workers for inhibitory avoidance[63–65]. However, several important issues need to be resolved before we can conclude that the LA is a primary site of plasticity in fear conditioning, such as how LA spike firing relates to behaviour and the frequency specificity of LA plasticity in auditory fear conditioning (BOX 3).

Associative coding in the amygdala

For any conditioning-induced change in neuronal activity, it is essential to determine whether the change is related to the associative learning that encodes the CS–US contingency or whether it represents a non-associative process (a form of learning that does not depend on a CS–US association) that is consequent to
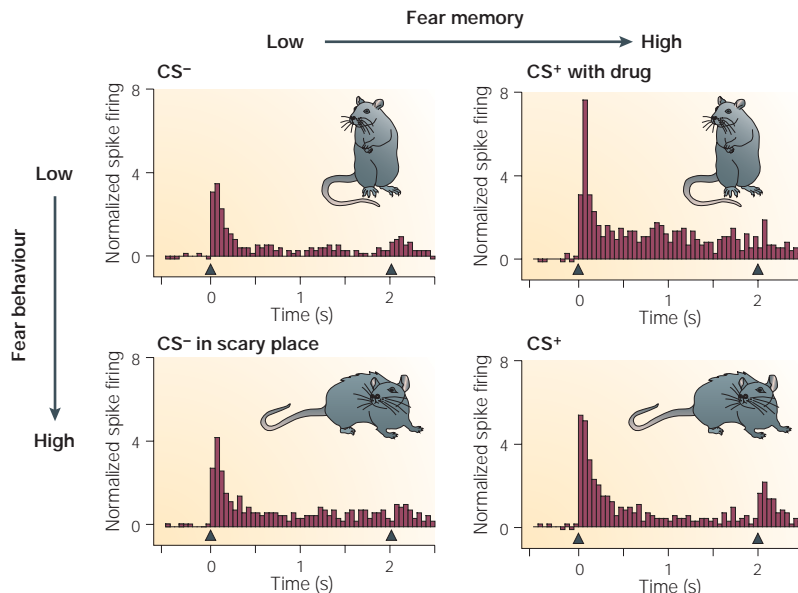
Figure 3 | **Lateral amygdala neurons encode fear memory independently of fear behaviour.** Each panel shows population averages for single units recorded in the lateral amygdala (LA) during presentations of an auditory cue paired with a footshock (CS+) or an auditory cue that has never been paired with a shock (CS−). Onset and offset of the auditory CSs are indicated by arrowheads. Fear conditioning increases both CS-evoked spike firing and freezing behaviour to the CS+ (bottom right), but not to the CS− (top left). This typical correlation between the associative history of the CS and freezing behaviour can be broken by testing a CS− in a context that has been paired with unsignalled shock (CS− in scary place; bottom left) or by testing a CS+ after inactivating the central nucleus of the amygdala (CS+ after drug; top right). In these cases, the CS− is presented against a background of high fear behaviour, or the CS+ is presented to animals that are not capable of showing conditioned fear responses. Nonetheless, LA neurons continue to show activity patterns that are consistent with the associative history of the CS− and CS+; that is, LA neurons represent fear memory, and are not biased by the performance of fear responses. Adapted, with permission, from REF. 73 © (2003) Cell Press.

either CS or US exposure. It is possible, for example, that increases in the responsiveness of LA neurons to auditory CSs are due to non-associative learning processes such as sensitization or pseudo-conditioning. Moreover, changes in behaviour and arousal that accompany learned fear might alter sensory processing in the brain in a way that mirrors associative learning but is not itself the substance of memory[6].

Quirk and colleagues[39] showed that CS-elicited firing in the LA was greater after CS–US pairings than with an earlier phase of unpaired CS and US presentations. This implies that LA firing is regulated by the associative contingency between the CS and the US. However, it is also possible that shock exposure during conditioning promoted further non-associative sensitization of spike firing to the CS. If so, changes in CS-evoked spike firing after conditioning might have resulted from nonspecific changes in the responsivity of amygdala neurons to any auditory stimulus, rather than an associative change to the specific CS paired with the US.

To assess this possibility, Paré and colleagues used a discriminative fear-conditioning procedure in conscious cats to determine the specificity of LA plasticity for the auditory CS paired with the US[66]. In this procedure, there were two distinct auditory cues: a CS+ that was paired with a US, and a CS− that was not. In such a design, differential behaviour to the two CSs is taken as an index of

associative learning, and changes in behaviour to the CS− relative to the pre-conditioning baseline are taken as an index of non-associative sensitization. Of course, the CSs must be chosen carefully to avoid generalization between the cues, which would mask the different associative strengths of the CSs.

Collins and Paré[66] found that discriminative fear conditioning produced CS-specific changes in fear behaviour, single units and local field potentials in the LA; that is, after fear conditioning, the CS+ (a 5- or 10-kHz pure tone) evoked a larger LA field potential and more spike firing than it did before conditioning. Conversely, fear conditioning decreased the field potentials and spike firing that were elicited by the CS−. These changes in CS-elicited neural activity also showed EXTINCTION, returning to baseline levels after several presentations of each CS without the US. Therefore, the increased spike firing in the LA after fear conditioning is CS-specific and cannot be explained by a nonspecific sensitization of spike firing to auditory stimuli or to pseudo-conditioning. It should be noted, however, that a complete frequency RECEPTIVE FIELD analysis[61] has not yet been carried out in the LA.

Conditioning-related changes in LA activity are closely correlated with the expression of fear responses. Presentations of CSs that have been paired with a footshock evoke behavioural responses, such as freezing or an increased state of arousal associated with fear[67–69]. In many cases, these fear responses outlast the stimuli that produce them, and might therefore affect the processing of subsequent CSs. For example, LA neurons in cats that have undergone auditory fear conditioning show increased responsiveness not only to the auditory CS, but also to electrical activation of cortical inputs[70]. Because the cortical stimulation was never explicitly paired with the shock US in these animals, the potentiation of these responses might reflect nonspecific increases in LA excitability. A similar change in the intrinsic excitability of LA neurons has been observed after olfactory conditioning in rats[71].

Therefore, it is necessary to determine whether associative plasticity of CS-elicited LA spike firing is a cause of learned fear responses or a consequence of the behavioural changes that are engendered by the fear state. One approach to this question is to examine the development of neuronal plasticity over the course of conditioning[12]. If LA firing codes for fear associations, learning-related activity in the LA should occur before (or coincident with) the emergence of fear CRs. Repa and colleagues addressed this question by examining spike firing in the LA during the gradual acquisition of CONDITIONED LEVER-PRESS SUPPRESSION[72]. Interestingly, most of the neurons that were recorded in the LA showed increases in CS-elicited spike firing on or before the trial in which the first significant behavioural CR appeared. There were also neurons that increased their firing to the CS after this point. Moreover, some LA neurons maintained their conditioning-related increase in spike firing after extinction of the fear response, indicating that the expression of fear behaviour is not driving LA responsiveness.

Fear conditioning increases the responses of single lateral amygdala (LA) neurons to the conditional stimulus (CS). However, this observation alone is not sufficient to imply that LA neurons signal fear memory. Additional criteria (all of which are met by the LA) are as follows:

Is plasticity in the LA associative?
Yes. LA neurons increase their tone responses during conditioning in contrast to pseudo-conditioning (unpaired tones and shocks). Increases are specific to stimuli that are paired with a shock (CS+), and are not seen with unpaired stimuli (CS−).

Does plasticity in the LA depend on plasticity in the auditory cortex?
No. Plasticity in the LA precedes plasticity in the auditory cortex, both within and across training trials.

Does plasticity in the LA depend on plasticity in the auditory thalamus?
Probably not. Inactivation of the LA with the GABA$_A$ (γ-aminobutyric acid, type A) agonist muscimol prevents the development of plasticity in medial geniculate inputs to the LA. Therefore, plasticity in the medial geniculate nucleus seems to depend on plasticity in the LA.

Do LA neurons learn as fast as the rat learns?
Yes. Across trials, plasticity in the LA develops as fast as — or faster than — conditioned fear responses.

Is plasticity in the LA caused by fear behaviour?
No. Plasticity in LA neurons can be dissociated from freezing behaviour, implying that LA neurons signal the strength of the conditional–unconditional stimulus association rather than fear *per se*.

CONDITIONED LEVER-PRESS SUPPRESSION
The reduction in pressing for food reward in the presence of a fear-conditioned stimulus.

THETA OSCILLATIONS
Rhythmic neural activity with a frequency of 4–8 Hz.

In a more direct examination of this issue, Goosens and colleagues recently asked whether increases in LA spike firing are caused by the expression of conditional freezing behaviour[73] (FIG. 3). In one experiment, rats received discriminative fear conditioning using distinct auditory CSs. Separate groups of animals were then tested to each CS in either a neutral context (control group) or in a context that they had come to fear through contextual fear conditioning (experimental group). In this way, it could be determined whether fear *per se* was sufficient to alter LA spike firing to a cue (CS−) that was not paired with a footshock. In fact, the expression of fear behaviour did not alter LA spike firing, and the degree of neuronal discrimination between the control and experimental rats was nearly identical. In a follow-up experiment, the influence of inhibiting the expression of conditional freezing on LA plasticity was explored[72]. Reversible inhibition of the central nucleus of the amygdala eliminated conditional freezing behaviour but not associative increases in CS-elicited spike firing in the LA.

Together, these experiments show that the expression of fear is neither sufficient nor necessary for the expression of associative plasticity in the LA, supporting the view that LA neurons encode fear memories. The essence of this mnemonic code seems to be contained in the rate at which LA neurons fire action potentials in response to auditory CSs. In addition to this rate code, however, the LA might also signal fear associations by the timing of spikes within a CS-evoked spike train: a rhythm code. Fear conditioning has been shown to increase synchrony in LA neurons[39,70], and THETA OSCILLATIONS become more frequent in the LA after

fear conditioning[70,74]. It has been suggested that increased synchrony after fear conditioning could increase the impact of the LA on neocortical targets that consolidate and store emotional memories[75].

Fear not: amygdala inhibition after extinction
Fear memories enable us to anticipate and respond to dangers in our environments. However, when signals for aversive events no longer predict those events, fear to those signals subsides. This inhibitory learning process, known as extinction, has important clinical relevance as a treatment for anxiety disorders, such as panic disorder[76] and post-traumatic stress[77]. Importantly, the inhibitory memories that are learned during extinction compete with the excitatory memories that are formed during conditioning, thereby suppressing fear responses[78]. Although fear subsides after extinction, the fear memory is not erased. In fact, the inhibitory memories of extinction are relatively short-lived and context-dependent. This means that extinction is expressed only in the context in which extinction was given, and even in that context, fear responses will spontaneously recover over time[79]. This transience and context dependence of extinction implies that biology has deemed it better to fear than not to fear.

There is considerable interest in understanding the neurobiological mechanisms of fear extinction, and substantial progress has been made in recent years[80,81]. As for fear conditioning, the amygdala seems to have a vital role in the extinction of learned fear. Pharmacological manipulations that inhibit neuronal activity or disrupt the cellular processes that underlie synaptic plasticity in the amygdala impair extinction[82,83]. The mediation of extinction by the amygdala is also manifested in the firing of LA neurons. Presenting the CS in the absence of the US reduces the expression of both behavioural CRs and CS-evoked spike firing in most LA neurons[39,72]. However, not all LA neurons reduce their firing after extinction[72], and even neurons that do reduce their firing continue to show the synchrony that is fostered by conditioning[39]. This implies that even after extinction, residual traces of conditioning persist in the activity patterns of LA neurons.

The reduction in CS-evoked spike firing in the LA that accompanies extinction correlates with the attenuation of fear CRs to the extinguished CS. However, as described earlier, fear extinction is context-dependent and is primarily expressed only in the extinction context. This raises the question of whether the suppression in LA spike firing after extinction is also context-dependent. To address this question, Hobin and colleagues used an elegant within-subjects behavioural design to observe the activity that is elicited in LA neurons by extinguished CSs that are presented either within or outside their extinction context[84]. Rats were conditioned to fear two distinct auditory CSs, then they received extinction training to each CS in a different context. Neurophysiological recordings were taken in a series of four test sessions, in which each CS was tested in each context. This design eliminated the possibility that any particular CS, context or CS/context combination

a Prefrontal cortex (safety memory)

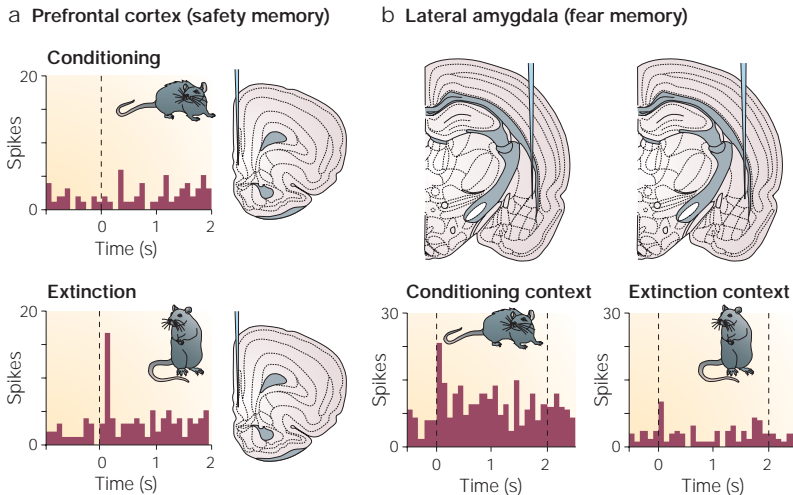**b** Lateral amygdala (fear memory)

Figure 4 | **Neuronal signalling of extinction in the prefrontal cortex and lateral amygdala.** Panels show a representative single unit recorded from the infralimbic region of the medial prefrontal cortex (PFC; **a**) and the lateral amygdala (LA; **b**). **a** | Unlike neurons in the LA, PFC neurons are initially silent during conditional stimulus (CS) presentations after fear conditioning (conditioning), but greatly increase their CS-elicited firing after extinction training (extinction). **b** | Although spike firing is inhibited in the LA by extinction training (extinction context), it can be renewed by a change in context (conditioning context). These data reveal that neurons in both the PFC and LA respond to extinction contingencies, although they respond in opposite directions under these conditions. Adapted, with permission, from **REF. 84** © (2003) Society for Neuroscience, and from **REF. 88** © (2002) Macmillan Magazines Ltd.

PREFRONTAL CORTEX
(PFC) The non-motor sectors of the frontal lobe that receive input from the dorsomedial thalamic nucleus and subserve working memory, complex attentional processes and executive functions such as planning, behavioural inhibition, logical reasoning, action monitoring and social cognition.

might itself affect LA spike firing independently of the extinction history of the CS and context. Interestingly, most single units in the LA modulated their firing rates to extinguished CSs according to the context in which the CS was presented. When a CS was presented in the extinction context, spike firing to that CS was typically lower than when the CS was presented outside its extinction context; a small number of neurons showed the opposite pattern of modulation. However, the
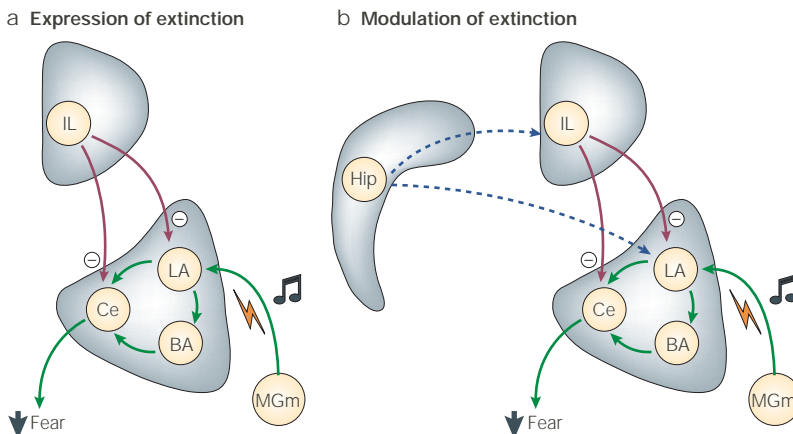
population average mirrored the behavioural expression of fear, indicating that the context dependence of extinguished fear is modulated at the level of the LA (FIG. 4).

It is of considerable interest to understand how LA activity and fear expression are modulated after extinction. Recent data indicate an important role for the medial PREFRONTAL CORTEX (mPFC). Rats with mPFC lesions can learn to extinguish fear CRs, but have difficulty recalling the extinction memory 24 hours after training[85–87]. This is precisely the time when mPFC neurons show robust increases in CS-elicited firing[88,89], consistent with a role in inhibition of fear after extinction (FIG. 4). mPFC neurons show an inhibitory influence on both the LA[90] and the central nucleus[91], the main output regions of the amygdala. Furthermore, pairing CSs with electrical stimulation of the mPFC mimics extinction behaviour[88,92]. Electrical stimulation of the mPFC inhibits both lateral and central amygdaloid neurons, presumably through a rich network of inhibitory interneurons embedded in the amygdala[93,94] (FIG. 5).

If the inhibitory signal for extinction originates in the mPFC, then it is probably modulated by context. One possible modulator of the mPFC is the hippocampus. A recent study indicates that the hippocampus modulates the expression of extinction memories[95]. Temporary inactivation of the dorsal hippocampus with muscimol eliminated renewal of fear to an extinguished CS; extinction performance prevailed under conditions in which it would normally be weak. This implies that although the hippocampus is not the repository for extinction memories, it is involved in regulating when and where extinction memories are expressed. The mechanism by which the hippocampus interacts with the amygdala to regulate CS-evoked spike firing is not clear, and could involve either a direct projection from the hippocampal formation to the LA[44,96] or an indirect projection through the prefrontal cortex[97] (FIG. 5).

Conclusions

Numerous studies have revealed electrophysiological correlates of memory in neuronal activity patterns of behaving animals, but few of these studies have established causality between learning-induced changes in neuronal activity and behaviour. Recent work in fear conditioning renews the promise of localizing memory in neuronal activity patterns in the mammalian brain. LA neurons seem to be the origin of associative plasticity that is relevant for both learned behavioural responses and physiological plasticity in other brain regions after aversive conditioning. Moreover, modulation of the fear-memory code in the LA is involved in the suppression and renewal of fear responses after extinction.

This research opens up new avenues to investigate how the hippocampus, prefrontal cortex and amygdala interact during the acquisition, storage and retrieval of fear memories, and how cellular and synaptic mechanisms encode inhibitory extinction memories together with excitatory fear memories. The central role for amygdala neurons in both processes reveals a common target for clinical interventions for anxiety disorders.

a Expression of extinction

b Modulation of extinction

Figure 5 | **Cortical modulation of amygdala fear memories in extinction. a** | Following extinction, neurons in the infralimbic region of the medial prefrontal cortex (IL) increase their responses to tones. The IL exerts feed-forward inhibition of neurons in the lateral amygdala (LA) and the central nucleus of the amygdala (Ce), thereby decreasing the expression of fear memories. **b** | Extinction is expressed only in the context in which it occurred. Contextual modulation of extinction requires the involvement of the hippocampus (Hip), which could modulate fear responses either at the level of the LA or the IL. BA, basal amygdala; MGm, medial subdivision of the medial geniculate nucleus.

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