

# Fear conditioning induces associative long-term potentiation in the amygdala

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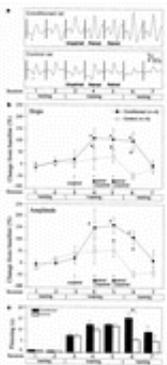
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Long-term potentiation (LTP) is an experience-dependent form of neural plasticity believed to involve mechanisms that underlie memory formation<sup>1–3</sup>. LTP has been studied most extensively in the hippocampus, but the relation between hippocampal LTP and memory has been difficult to establish<sup>4–6</sup>. Here we explore the relation between LTP and memory in fear conditioning, an amygdala-dependent form of learning in which an innocuous conditioned stimulus (CS) elicits fear responses after being associatively paired with an aversive unconditioned stimulus (US). We have previously shown that LTP induction in pathways that transmit auditory CS information to the lateral nucleus of the amygdala (LA) increases auditory-evoked field potentials in this nucleus<sup>7</sup>. Now we show that fear conditioning alters auditory CS-evoked responses in LA in the same way as LTP induction. The changes parallel the acquisition of CS-elicited fear behaviour, are enduring, and do not occur if the CS and US remain unpaired. LTP-like associative processes thus occur during fear conditioning, and these may underlie the long-term associative plasticity that constitutes memory of the conditioning experience.

To determine whether fear conditioning results in learning-related changes in CS processing that are similar to the effect of LTP induction in auditory CS pathways, we concurrently measured auditory CS-evoked field potentials in LA and CS-evoked fear behaviour, before, during and after fear conditioning in freely behaving rats. The rats were randomly assigned to groups that underwent either fear conditioning (in which the CS and US were paired) or a non-associative control procedure (in which the CS and US were explicitly unpaired). The CS was a 20-s series of acoustic tones (1 kHz, 50 ms, 72 dB) delivered at 1 Hz. The onset of each tone in the series triggered the acquisition of an evoked waveform from the electrode in LA, so that each 20-s CS produced 20 evoked responses. The 100 evoked waveforms from each session (5 CS per session; mean inter-CS interval, 170 ms, range 140–200 ms) were averaged to yield a mean CS-evoked field potential (CS-EP) for that session. The use of this 'one tone per second' 20-s CS allowed the sampling of CS-evoked activity at 20 points within a single CS, greatly increasing the signal-to-noise ratio of the field potentials under study over that obtainable with the continuous-tone CS typically used in conditioning studies<sup>8–10</sup>.

The CS-EPs were quantified by measuring the latency, slope and amplitude of the negative-going potential occurring 15–30 ms after the onset of the tone stimulus, as per our previous study of auditory evoked field potentials in LA<sup>7</sup>. Anatomical and physiological<sup>6</sup> evidence indicates that these field potentials are generated in the LA<sup>6</sup>. A set of CS-EPs for two rats, one from the 'conditioned' group and one from the 'control' group, over the seven sessions of testing and training is shown in Fig. 1a. As previously reported<sup>7</sup>, before training the CS elicited a negative-going field potential with a latency of about 18 ms ( $18.52 \pm 3.58$  ms across animals). The raw (not normalized) slope and amplitude of these potentials did not differ between the two groups in the baseline tests before training (slope: conditioned group,  $-1.649 \pm .425$   $\mu\text{V ms}^{-1}$ , control group,  $-2.329 \pm .346$   $\mu\text{V ms}^{-1}$ ;  $t$ -test,  $P > 0.05$ , conditioned group,  $14.186 \pm 4.103$   $\mu\text{V}$ ; control group,  $18.116 \pm 4.214$ ;  $t$ -test,  $P > 0.05$ ). As seen in the examples shown (Fig. 1a), paired training led to an increase in the slope and amplitude of the CS-EPs, whereas unpaired training did not. Mean group data of slope and amplitude of CS-EPs, normalized as a

percentage of mean baseline measures, are shown in [Fig. 1b](#). For both groups, slope and amplitude were stable for the first two sessions (testing), in which only the CS was presented. Responses in these sessions were used as a baseline from which to measure changes due to training. For the conditioned group, slope and amplitude were unchanged by unpaired presentations of the CS and US in session 3, but increased significantly above baseline in sessions 4 and 5 when the CS was paired with the US (statistics in [Fig. 1b](#)). Both measures remained elevated in session 6, in which only the CS was presented, and fell towards baseline in the last session, reflecting the weakening of the CS-US relation by presentations of the CS without the US (extinction trials). The slope and amplitude of the CS-EPs remained statistically unchanged throughout the course of training and testing for the control group (statistics in [Fig. 1b](#)). Slope and amplitude did not differ between the groups until pairing occurred, and remained different until the last session (statistics in [Fig. 1b](#)). The fact that the two groups received an equal number of CS and US presentations during training, and that unpaired training was not accompanied by increases in CS-EPs in either group, indicates that the effect of paired training on the field potentials in the conditioned group is due to the associative relation of the CS and US and not to nonspecific arousal elicited by either stimulus alone<sup>11</sup>.

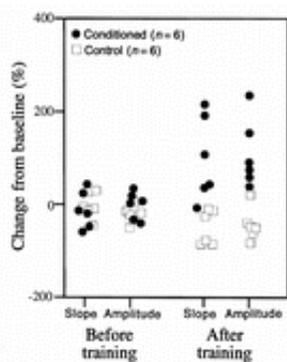


**Figure 1** The effect of paired and unpaired training on CS-evoked field potentials and behaviour.

[Full legend](#)

[High resolution image and legend](#) (130k)

The differential effects of training on CS-EPs for each member of the control and conditioned groups is shown in [Fig. 2](#). This scattergram demonstrates the consistency with which the control group was unaffected by training and the reliability of the increases in slope and amplitude of the CS-EPs in the conditioned group.



**Figure 2** Scattergram of slope and amplitude values for each of the control and conditioned animals, before and after training.

[Full legend](#)

[High resolution image and legend](#) (60k)

The acquisition of conditioned fear behaviour was evaluated by measuring 'freezing', a characteristic defensive posture expressed in the presence of stimuli that predict danger<sup>12-15</sup>. The amount of time accounted for by freezing was measured during the 20-s CS and also during the 20-s immediately before CS onset (pre-CS period). The latter is a measure of the acquisition of aversive conditioning to the experimental context in which the US is delivered (such as the conditioning chamber); freezing to the experimental context is independent of the presence or absence of an explicit CS, and is typically seen with both paired and unpaired training<sup>8,10</sup>. In this experiment and pilot studies, the pattern of behaviour exhibited during the 'one tone per second' 20-s CS was in all respects similar to the behaviour exhibited by animals trained with a 20-s continuous tone CS; for example, rats did not respond to the individual tones that made up the CS, but rather behaved as though the 20-s CS period was a continuous tone.

Analyses of variance and post hoc tests of the behavioural data showed the expected result from paired and unpaired training. Thus there was a significant interaction between group and session, owing to the higher level of freezing in the conditioned group in session 6, the first test session after training (statistics in [Fig. 1c](#)). This was also the session in which the CS-EP measures differed most between the groups ([Fig. 1b](#)). By the next session, freezing responses, like field-potential measures, no longer differed between the groups, showing that the CS-US relation had extinguished. Although both groups froze extensively during training (sessions 3–5), freezing measured in sessions with US presentations is not generally useful as an index of CS-related learning, owing to the confounding effects of the US on freezing behaviour<sup>8</sup>.

To further investigate the differential effect of paired versus unpaired training we analysed freezing before the CS and during the CS in the two groups (data not shown). Pre-CS freezing, which reflects conditioning to contextual stimuli<sup>8</sup>, did not differ between the conditioned and control groups at any point in the course of training ( $F(6, 60) = 0.42, P > 0.1$ ). Conditioned animals showed more freezing during the CS than during the pre-CS period ( $F(6, 60) = 5.2, P > 0.01$ ), whereas freezing did not differ during the pre-CS and CS periods in the control group ( $F(6, 60) = 0.67, P > 0.1$ ). The elevated freezing to the CS relative to the pre-CS period in the conditioned group and the equivalence of freezing in the CS and pre-CS periods for the control group leads to two conclusions. First, freezing during the CS in the control group after training is caused by the experimental context and continues into but is not evoked by the CS. Second, freezing during the CS in the conditioned group is, at least in part, specifically related to the occurrence of the CS and its association with the US.

In previous studies of freely behaving rats, changes in hippocampal field potentials measured in the course of learning have been shown to be attributable in part to modulation of brain temperature by task-related changes in locomotor activity<sup>11, 16</sup>. Also, hippocampal field potentials are generally susceptible to modulation by behavioural state at the time of evoked potential sampling<sup>11, 17-19</sup>. The dramatic acquisition of freezing behaviour in the course of fear conditioning therefore raises the question of whether this learning-induced change in behaviour may produce the observed changes in CS-EPs in the LA through tonic effects related to brain temperature or other behaviourally related factors that merely coincide with field-potential measurements.

Despite a greater than 10-fold increase in freezing behaviour by both groups in the course of training compared with pre-training testing, and the corresponding increase in the proportion of freezing-coincident sampling of evoked potentials during the CS (from approximately 1 of 20 freezing-coincident samples for both groups in sessions 1 and 2, to approximately 12 of 20 freezing-coincident samples for both groups in session 5), only conditioned animals showed increases in CS-EPs during training with respect to baseline levels, and only in sessions with paired training (sessions 4 and 5); control group CS-EPs showed no significant change during any session, relative to baseline testing.

These data indicate that our measures of CS-EPs are not modulated by freezing expressed at the time of field-potential sampling, or by possible behavioural modulation of brain temperature during the CS. The increases in slope and amplitude of CS-EPs measured in this experiment do not simply correlate with freezing behaviour, rather, they correlate with the presence of contingency information that identifies the CS as a danger signal, and with the degree to which the conditioned group makes use of this information after training.

As in our previous study of LTP and auditory evoked field potentials in the LA, the latency of CS-EPs measured in the present study varied between rats, but always fell within the latency range (15–30ms) that invariably corresponded to histologically confirmed electrode placement within the LA<sup>7</sup>. The mean latency of CS-EPs across all rats ( $18.52 \pm 3.58$ ms) matched that measured in the LTP study ( $18.50 \pm 2.65$ ms), which used similar auditory stimulation parameters<sup>7</sup>, and these latencies were not altered by either LTP induction or fear conditioning. This indicates that the potentials recorded in the two studies reflect similar stimulus-locked responses from the same general population of cells. As noted above, anatomical and physiological evidence identified these field potentials as being locally generated in the LA. Further, the coincidence of the latency of the peak negativity of the evoked potentials with the latency of single neuron activity concurrently elicited by the auditory stimulus suggested that the negative-going component of the potentials reflects extracellular currents arising from local postsynaptic activation<sup>7</sup>. LTP induction in anaesthetized animals produced effects of similar magnitude on both auditory evoked field potentials (change in slope over baseline,  $+129.59 \pm 6.88\%$ ) and on

electrical single-pulse stimulation (the typical test stimulus for LTP studies; change in slope over baseline,  $+108.2 \pm 10.93\%$ )<sup>7</sup>. Fear conditioning produced effects of similar magnitude on CS-EPs (change in slope over baseline,  $+98.5 \pm 36.94\%$ ). Fear conditioning also alters single unit responses in the LA<sup>20</sup>, and the conditioned changes in unit activity occur at latencies consistent with the changes we found in CS-EPs.

Our data indicate that CS-EPs in the LA reflect processes relevant to conditioned fear. In particular, to the extent that the negative-going slope can be interpreted as a measure of synaptic activation, we can conclude that fear conditioning, like LTP induction in CS pathways, potentiates synaptic currents. Because the same treatment potentiated both synaptic currents and conditioned fear behaviour over the same general time course, it is plausible that the enhancement of the field potentials reflects synaptic mechanisms that are responsible for the conditioning of fear behaviour. Processes mechanistically similar to LTP may therefore underlie the learning process which results from temporal association of the CS with the US, through which the CS comes to elicit conditioned fear responses.

Several previous studies have attempted to show that natural learning induces LTP-like changes in the hippocampus. In some of these studies, learning altered hippocampal physiology, but because the hippocampus is not required for the learned behaviour, the changes cannot account for learning<sup>21,22</sup>. Other studies have used behavioural tasks that are dependent on the hippocampus<sup>23</sup>, but interpretation of these data is limited by the poor understanding of the flow of task-relevant information in specific synaptic circuits within the hippocampus and the contribution of these circuits to the behaviour under study<sup>5,6</sup>. In contrast, the well-defined and easily controlled sensory components of fear conditioning, and their tight coupling to mechanisms controlling the expression of learned fear responses, make this system well suited for such an analysis. We previously induced LTP in circuits known to be involved in fear conditioning<sup>7</sup> and have now shown that fear conditioning alters neural activity in these circuits in the same way as LTP induction. Furthermore, we measured both artificial LTP and fear conditioning using an auditory test stimulus, which in the fear-conditioning experiment was the environmental cue that the animals learned to fear.

Other similarities exist between fear conditioning and the classic form of hippocampal LTP, which depends on glutamatergic mechanisms, particularly processes mediated by the NMDA (*N*-methyl-D-aspartate) receptor<sup>1,2</sup>. CS processing in LA involves glutamatergic transmission<sup>24-26</sup>, and the blockade of NMDA receptors in LA and adjacent regions interferes with fear conditioning<sup>27-29</sup>. Also, facilitation of AMPA/NMDA receptor function modulates fear conditioning and hippocampal LTP in much the same way: both fear conditioning and LTP induction occur at an accelerated rate, but with no change in the final level of acquired conditioned fear or ceiling of potentiation<sup>9</sup>. Thus the LTP-like mechanisms engaged by fear conditioning may share mechanistic features with the more thoroughly studied, NMDA-dependent mechanisms known to be involved in hippocampal LTP, but which have been difficult to relate to hippocampal-dependent learning processes. It remains to be determined whether changes in synaptic strength produced in the amygdala by LTP induction and those produced by fear conditioning are both NMDA dependent. Such a demonstration would help to provide a mechanistic link between LTP and at least one form of memory.

## Methods

**Surgery.** Rats were anaesthetized and implanted with a stainless-steel recording electrode ( $0.6\text{M}\Omega$ ) in the LA, and a ground electrode in the skull, under aseptic surgical conditions. The electrodes were mounted to the skull using dental cement. The wound was sutured and analgesics administered, and animals recovered for at least 5 days before the experiment.

**Apparatus.** The conditioning chamber was constructed of stainless-steel bars, acoustically transparent to the CS frequency. The chamber was kept within a ventilated and temperature-regulated acoustic isolation box lined with anechoic panels. Stimulus delivery and data acquisition were controlled by a custom-made Matlab application, using a Cambridge Electronics Devices 1401+. The isolation box was equipped with a video camera and VCR for recording of behaviour.

**Conditioning protocol.** The CS frequency was chosen so that the rat's head would be acoustically transparent to the CS, reducing the effect of head position on CS intensity at the tympani. The US ( $0.3\text{mA}$ ,  $500\text{ms}$ ) was

delivered through the floor of the conditioning chamber. In paired sessions, the US occurred immediately after the end of each CS. In unpaired sessions, the US occurred during the inter-CS interval (5 US per session; mean interval between CS and US, 78 ms; range, 60–120 ms). The sequence of testing and training sessions over 6 days is shown in [Fig. 1](#).

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## References

1. Malenka, R. C. & Nicoll, R. A. NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms. *Trends Neurosci.* **16**, 521-527 (1993). [PubMed](#) [ISI](#)
2. Bliss, T. V. P. & Collingridge, G. L. Asynaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31-39 (1993). [PubMed](#) [ISI](#)
3. Brown, T. H. & Chattarji, S. in *Models of Neural Networks II* (eds Domany, E., Van Hemmen, J. L. & Schulten, K.) 287-314 (Springer-Verlag, New York, (1994)).
4. Stäubli, U. V. in *Brain and Memory: Modulation and Mediation of Neuroplasticity* (eds McGaugh, J. L., Weinberger, N. M. & Lynch, G.) 303-318 (Oxford Univ. Press, New York, (1995)).
5. Barnes, C. A. Involvement of LTP in memory: Are we "searching under the streetlight?". *Neuron* **15**, 751-754 (1995).
6. Eichenbaum, H. The LTP-memory connection. *Nature* **378**, 131-132 (1995). [PubMed](#) [ISI](#)
7. Rogan, M. T. & LeDoux, J. E. LTP is accompanied by commensurate enhancement of auditory-evoked responses in a fear conditioning circuit. *Neuron* **15**, 127-136 (1995). [PubMed](#) [ISI](#)
8. Phillips, R. G. & LeDoux, J. E. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav. Neurosci.* **106**, 274-285 (1992). [Article](#) [PubMed](#) [ISI](#)
9. Rogan, M. T., Stäubli, U. V. & LeDoux, J. E. AMPA-receptor facilitation accelerates fear learning without altering the level of conditioned fear acquired. *J. Neurosci.* **17**, 5928-5935 (1997). [PubMed](#) [ISI](#)
10. Kim, J. J. & Fanselow, M. S. Modality-specific retrograde amnesia of fear. *Science* **256**, 675-677 (1992). [PubMed](#) [ISI](#)
11. Moser, E. I., Moser, M.-B. & Andersen, P. Potentiation of dentate synapses initiated by exploratory learning in rats: dissociation from brain temperature, motor activity, and arousal. *Learn. Memory* **1**, 55-73 (1994). [PubMed](#) [ISI](#)
12. Blanchard, R. J. & Blanchard, D. C. Passive and active reactions to fear-eliciting stimuli. *J. Comp. Physiol. Psychol.* **68**, 129-135 (1969). [PubMed](#) [ISI](#)
13. Blanchard, R. J. & Blanchard, D. C. Crouching as an index of fear. *J. Comp. Physiol. Psychol.* **67**, 370-375 (1969). [PubMed](#) [ISI](#)
14. Bouton, M. E. & Bolles, R. C. Conditioned fear assessed by freezing and by the suppression of three different baselines. *Anim. Learn. Behav.* **8**, 429-434 (1980). [ISI](#)
15. Bolles, R. C. & Fanselow, M. S. A perceptual-defensive-recuperative model of fear and pain. *Behav. Brain Sci.* **3**, 291-323 (1980). [ISI](#)
16. Moser, E., Mathiesen, I. & Anderson, P. Association between brain temperature and dentate field potentials in exploring and swimming rats. *Science* **259**, 1324-1326 (1993). [PubMed](#) [ISI](#)
17. Winson, J. & Absug, C. Neuronal transmission through hippocampal pathways dependent on behavior. *J. Neurophysiol.* **41**, 716-732 (1978). [PubMed](#) [ISI](#)
18. Leung, S. Behavior-dependent evoked potentials in the hippocampal CA1 region of the rat. I. Correlation with behavior and EEG. *Brain Res.* **198**, 95-117 (1980). [PubMed](#)
19. Buzsaki, G., Grastyan, E., Czopf, J., Kellenyi, L. & Prohaska, O. Changes in neuronal transmission in the rat hippocampus during behavior. *Brain Res.* **225**, 235-247 (1981). [PubMed](#) [ISI](#)
20. Quirk, G. J., Reppas, J. C. & LeDoux, J. E. Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. *Neuron* **15**, 1029-1039 (1995). [PubMed](#) [ISI](#)
21. Skelton, R. W., Scarth, A. S., Wilkie, D. M., Miller, J. J. & Phillips, G. Long-term increases in dentate granule cell responsiveness accompany operant conditioning. *J. Neurosci.* **7**, 3081-3087 (1987). [PubMed](#) [ISI](#)
22. Deadwyler, S. A., West, M. O., Christian, E., Hampson, R. E. & Foster, T. C. Sequence-related changes in sensory-evoked potentials in the dentate gyrus: as mechanism for item-specific short-term information storage in the hippocampus. *Behav. Neural Biol.* **44**, 201-212 (1985). [PubMed](#) [ISI](#)
23. Jeffrey, K. J. LTP and spatial learning -- where to next? *Hippocampus* **7**, 95-110 (1997). [Article](#) [PubMed](#) [ISI](#)
24. Farb, C. R. & LeDoux, J. E. NMDA and AMPA receptors in the lateral nucleus of the amygdala are postsynaptic to auditory thalamic afferents. *Synapse* **27**, 106-121 (1997). [Article](#) [PubMed](#) [ISI](#)
25. Li, X., Phillips, R. G. & LeDoux, J. E. NMDA and non-NMDA receptors contribute to synaptic transmission between the medial geniculate body and the lateral nucleus of the amygdala. *Exp. Brain Res.* **105**, 87-100 (1995). [PubMed](#) [ISI](#)
26. Li, X. F., Stutzmann, G. E. & LeDoux, J. L. Convergent but temporally separated inputs to lateral amygdala neurons

- from the auditory thalamus and auditory cortex use different postsynaptic receptors: *in vivo* intracellular and extracellular recordings in fear conditioning pathways. *Learn. Memory* **3**, 229-242 (1996). [PubMed](#) [ISI](#)
27. Miserendino, M. J. D., Sananes, C. B., Melia, K. R. & Davis, M. Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. *Nature* **345**, 716-718 (1990). [PubMed](#) [ISI](#)
  28. Maren, S., Aharonov, G., Stote, D. L. & Fanselow, M. S. N-Methyl-D-Aspartate receptors in the basolateral amygdala are required for both acquisition and expression of the conditional fear in rats. *Behav. Neurosci.* **110**, 1365-1374 (1996). [Article](#) [PubMed](#) [ISI](#)
  29. Gewirtz, J. C. & Davis, M. Second-order fear conditioning prevented by blocking NMDA receptors in amygdala. *Nature* **388**, 471-473 (1997). [Article](#) [PubMed](#) [ISI](#)
  30. Rogan, M. T. & LeDoux, J. E. Intra-amygdala infusion of APV blocks both auditory evoked potentials in the lateral amygdala and thalamo-amygdala transmission, but spares cortico-amygdala transmission. *Soc. Neurosci. Abstr.* **21**, 1930 (1995).

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