Note added in proof: Similar conclusions were reached by Lin *et al.*[29] studying the association of APL fusion proteins and SMRT. □

Methods

Mutants, constructs and cell lines. PML–RARα and PLZF–RARα AHT mutants[19] were generated by the polymerase chain reaction (PCR) and subcloned in the pGMT-SV-NEO and pCDNA3 eukaryotic expression vectors. Stable U937 transfectants of PML–RARα, PML–RARα AHT and PLZF–RARα cloned into pGMT-SV-NEO were obtained by electroporation. The used PLZF mutants were generated by PCR and cloned within the pCDNA3 vector.

***In vitro* binding assays with GST fusion proteins.** The GST–N-CoR constructs 4–12 have been described previously[25]. Constructs 1–3 were generated by PCR and cloned in the pGex4T-1 vector. GST beads containing the fusion proteins (10 μg) were incubated in E1A buffer (50 mM HEPES, pH 7.8, 50 mM NaCl, 5 mM EDTA, 1 mM DTT, 0.1% NP40) with 5 μl of *in vitro*-translated polypeptides. After washing, bound proteins were eluted by boiling in SDS–PAGE loading buffer, resolved by electrophoresis, and visualized by autoradiography.

Co-immunoprecipitation experiments and western blotting analysis. Nuclear extracts were prepared as described[26]. Where indicated, RA (20 μM) was added 1 h before incubation of the nuclear extracts with the appropriate antibodies or controls. Immunocomplexes were recovered by protein A-Agarose beads and resolved by electrophoresis. Anti-N-CoR antiserum was raised against the GST–N-CoR 1–224.

Histone deacetylase assay. Assays for histone deacetylase activity were performed as described[27]. Immunoprecipitated complexes on protein A-agarose beads were incubated at 30 °C for 1 h with [^{3}H]acetate-labelled chicken erythrocyte histones. The reaction was stopped by addition of 0.7-vol of 1M HCl–0.4 M acetate. Released [^{3}H] acetic acid was extracted with ethyl acetate and quantified by liquid scintillation analysis. As a positive control, we measured histone deacetylase activity in anti-histone deacetylase-1 immunocomplexes.

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correction

The cerebellar leucine-rich acidic nuclear protein interacts with ataxin-1

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Nature **389,** 974–978 (1997)

At the time of submission of this Letter, it escaped our attention that murine and human LANP sequences had previously been reported under different names in the following papers:

1. Vaesen, M. *et al*. Purification and characterization of two putative HLA class II associated proteins: PHAPI and PHAPII. *Biol. Chem. Hoppe-Seyler* **375,** 113–126 (1994).
2. Li, M., Makkinje, A. & Damuni, Z. Molecular identification of I1PP2A, a novel potent heat-stable inhibitor of protein phosphatase 2A. *Biochemistry* **35,** 6998–7002 (1996).
3. Chen, T.-H. *et al*. Structure of pp32, an acidic nuclear protein which inhibits oncogene-induced formation of transformed foci. *Mol. Biol. Cell.* **7,** 2045–2056 (1996).
4. Ulitzur, N., Humbert, M. & Pfeffer, S. R. Mapmodulin: A possible modulator of the interaction of microtubule-associated proteins with microtubules. *Proc. Natl Acad. Sci. USA* **94,** 5084–5089 (1997).

Although the gene products reported in these papers were isolated from peripheral tissues, the levels of LANP in cerebellar RNA are significantly higher than in peripheral tissues. Hence the central conclusion of our paper is unchanged. □

erratum

Fear conditioning induces associative long-term potentiation in the amygdala

Michael T. Rogan, Ursula V. Stäubli & Joseph E. LeDoux

Nature **390,** 604–607 (1997)

In Fig. 1a of this Letter, the labelling for the control rat (bottom panel) was erroneously relettered as 'Unpaired/Paired/Paired', whereas it should have read 'Unpaired/Unpaired/Unpaired'.

Also, the report of raw (not normalized) values of slope and amplitudes before training (third paragraph) should read 'slope: conditioned group, $-1.649 \pm 0.425\,\mu V\,ms^{-1}$, control group, $-2.329 \pm 0.346\,\mu V\,ms^{-1}$; *t*-test, $P > 0.05$; amplitude: conditioned group, $14.186 \pm 4.103\,\mu V$, control group, $18.116 \pm 4.214\,\mu V$; *t*-test, $P > 0.05$)'. Finally, on the third line of page 606, $P < 0.01$ (not >0.01 as published). □

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stimuli similar to that used for the psychophysical experiment. Pairs of textures either containing or not containing an embedded contour were generated and processed using the model. The longest blob was calculated for both images, and the image producing the longer of the two was selected as the one containing the contour. Performance of the model was determined as a function of path angle (the orientational difference between successive elements of the contour).

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Fear conditioning induces associative long-term potentiation in the amygdala

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Long-term potentiation (LTP) is an experience-dependent form of neural plasticity believed to involve mechanisms that underlie memory formation[1–3]. LTP has been studied most extensively in the hippocampus, but the relation between hippocampal LTP and memory has been difficult to establish[4–6]. 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[7]. 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 s, range 140–200 s) 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[8–10].

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[7]. Anatomical and physiological evidence indicates that these field potentials are generated in the LA[6]. 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[7], before training the CS elicited a negative-going field potential with a latency of about 18 ms (18.52 ± 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, $-1.649 \pm .425\ \mu V\ ms^{-1}$, control group, $-2.329 \pm .346\ \mu V\ ms^{-1}$; *t*-test, $P > 0.05$, conditioned group, $14.186 \pm 4.103\ \mu V$; control group, 18.116 ± 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[11].

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.

The acquisition of conditioned fear behaviour was evaluated by measuring 'freezing', a characteristic defensive posture expressed in the presence of stimuli that predict danger[12–15]. 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

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independent of the presence or absence of an explicit CS, and is typically seen with both paired and unpaired training[8,10]. 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[8].

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[8], did not differ between the conditioned and control groups at any point in the course of

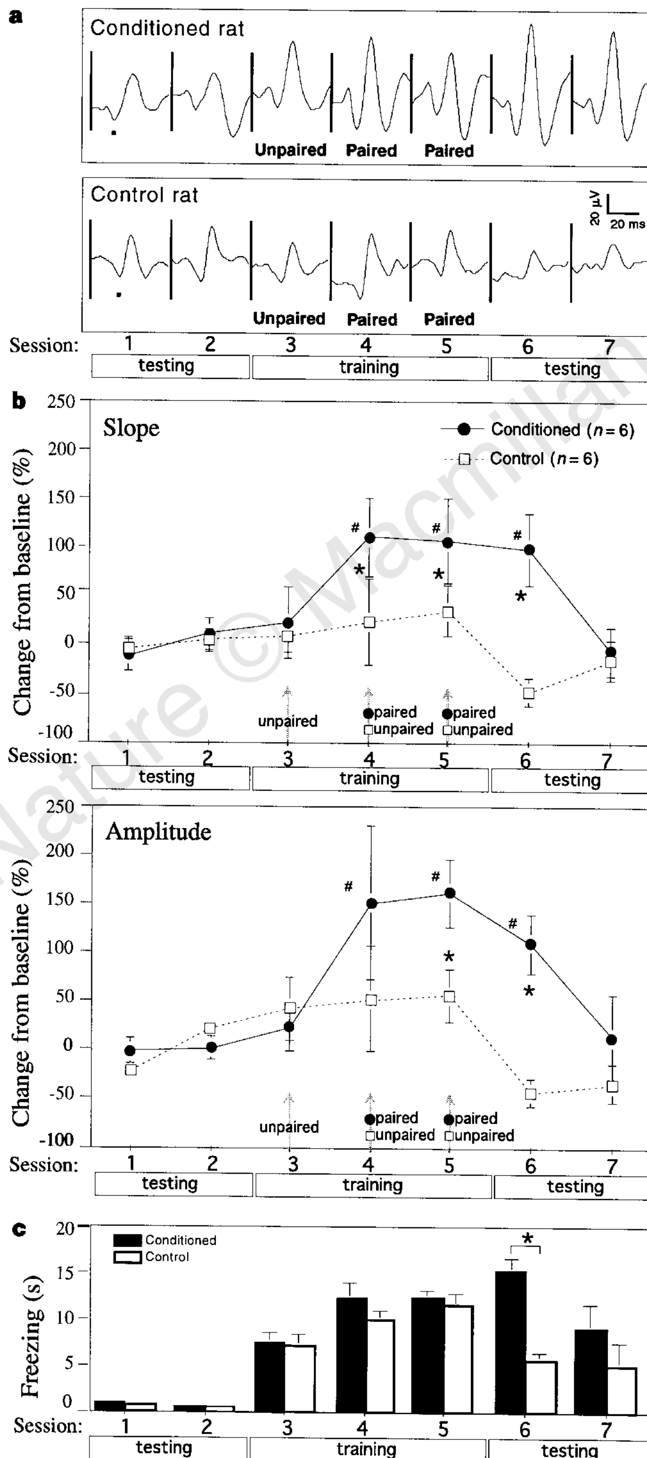

Figure 1 The effect of paired and unpaired training on CS-evoked field potentials and behaviour. Sessions are numbered 1–7; one session occurred per day, except that sessions 3 and 4 occurred on the same day. **a**, CS-evoked field potentials from a conditioned rat (top) and a control rat (bottom), covering the full-time course of the experiment. Quantitative analysis was performed on the first negative (downward)-going deflection (dot). Our previous studies of these waveforms have concentrated on this feature as it has the shortest latency, is reliably present, coincides with local evoked unit activity, shows experience-dependent plasticity, and reflects transmission from the auditory thalamus to the amygdala[7,30]. The other components of the waveform visible in these examples are not reliably present across trials and subjects, and little is known about their origin and mechanisms[7]. **b**, Fear conditioning increases the slope and amplitude of CS-EPs, but unpaired training does not. Slope and amplitude of the negative-going potential are normalized as a percentage of the mean values before training (sessions 1 and 2). The normalized slope and amplitude of the evoked potentials were evaluated statistically with two-factor ANOVAs with group (conditioned, control) as the between-subjects factor and experimental session as within-subject factor. A significant group–session interaction was observed for both measures (slope, $F(6, 60) = 2.59$, $P < 0.05$; amplitude, $F(6, 60) = 2.70$; $P < 0.05$). Significant differences of post hoc analyses are indicated (* Duncan, $P < 0.05$, between groups; # Duncan, $P < 0.05$, within group with respect to pretraining sessions 1 and 2). Error bars, ± s.e.m. **c**, Fear conditioning leads to associative conditioning of fear behaviour. Freezing responses during the CS and pre-CS period were evaluated statistically with two-factor ANOVAs with group (conditioned, control) as the between-subjects factor and experimental session as within-subject factor. A significant group–session interaction was observed for CS freezing ($F(6, 60) = 5.23$, $P < 0.001$) but not for pre-CS freezing ($F(6, 60) = 0.42$, $P > 0.1$) (not shown). Significant difference of post hoc analysis are indicated (* Duncan, $P < 0.05$, between groups). Freezing in sessions in which US presentations occur (sessions 3–5) is not useful as a measure of fear conditioning. Associative conditioning of freezing is best shown in session 6, in which only the CS was presented. The reduction in freezing in session 7 relative to session 6 reflects extinction of the CS–US association. The small amount of freezing exhibited by the control group after training (~5 s) reflects normal acquisition of freezing to the experimental context that extends into, but which is not elicited by, the CS[8,10]. Error bars, ± s.e.m.

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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[11,16]. Also, hippocampal field potentials are generally susceptible to modulation by behavioural state at the time of evoked potential sampling[11,17–19]. 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 (15–30 ms) that invariably corresponded to histologically confirmed electrode placement within the LA[7]. The mean latency of CS-EPs across all rats (18.52 ± 3.58 ms) matched that measured in the LTP study (18.50 ± 2.65 ms), which used similar auditory stimulation parameters[7], 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[7]. LTP induction in anaesthetized animals produced effects of similar magnitude on both auditory evoked field potentials (change in slope over baseline, +129.59 ± 6.88%) and on electrical single-pulse stimulation (the typical test stimulus for LTP studies; change in slope over baseline, +108.2 ± 10.93%)[7]. Fear conditioning produced effects of similar magnitude on CS-EPs (change in slope over baseline, +98.5 ± 36.94%). Fear conditioning also alters single unit responses in the LA[20], 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[21,22]. Other studies have used behavioural tasks that are dependent on the hippocampus[23], 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[5,6]. 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[7] 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

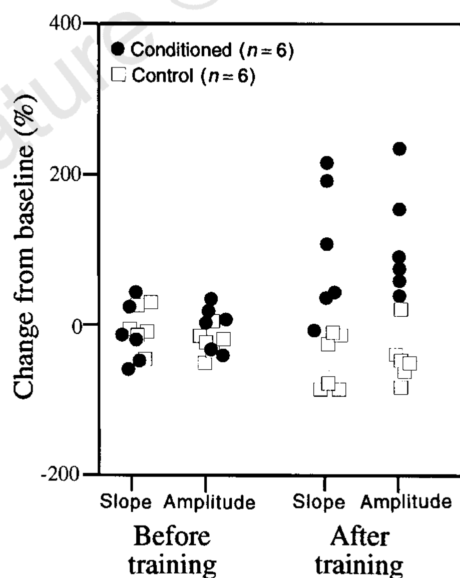

Figure 2 Scattergram of slope and amplitude values for each of the control and conditioned animals, before and after training. Pictured are normalized slope and amplitude values for each animal at the beginning of the experiment (testing, session 1), and in the first testing session after training (session 6). Points have been offset horizontally as needed to allow every point to be seen. Training had a reliable effect on the slope and amplitude of the CS-EP in the conditioned group, but not the control group.

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mechanisms, particularly processes mediated by the NMDA (N-methyl-D-aspartate) receptor[1,2]. CS processing in LA involves glutamatergic transmission[24–26], and the blockade of NMDA receptors in LA and adjacent regions interferes with fear conditioning[27–29]. 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[9]. 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 mΩ) 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 mA, 500 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 s; range, 60–120 s). The sequence of testing and training sessions over 6 days is shown in Fig. 1.

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Fear conditioning induces a lasting potentiation of synaptic currents *in vitro*

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The amygdala plays a critical role in the mediation of emotional responses, particularly fear, in both humans and animals[1–4]. Fear conditioning, a conditioned learning paradigm, has served as a model for emotional learning in animals, and the neuroanatomical circuitry underlying the auditory fear-conditioning paradigm is well characterized[5]. Synaptic transmission in the medial geniculate nucleus (MGN) to lateral nucleus of the amygdala (LA) pathway, a key segment of the auditory fear conditioning circuit, is mediated largely through *N*-methyl-D-aspartate (NMDA) and non-NMDA (such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)) glutamate receptors[6]; the potential for neural plasticity in this pathway is suggested by its capacity to support long-term potentiation (LTP)[7,8]. Here we report a long-lasting increase in the synaptic efficacy of the MGN–LA pathway attributable to fear-conditioning itself, rather than an electrically induced model of learning. Fear-conditioned animals show a presynaptic facilitation of AMPA-receptor-mediated transmission, directly measured *in vitro* with whole-cell recordings in lateral amygdala neurons. These findings represent one of the first *in vitro* measures of synaptic plasticity resulting from emotional learning by whole animals.

Fear-conditioned rats, when exposed to a tone (conditioned stimulus, CS) repeatedly paired with an aversive footshock (unconditioned stimulus, US), respond with a potentiated acoustic startle reflex (+58.9% ± 11.6%, $n = 27$; see Methods) immediately following CS presentation, whereas unpaired control rats, exposed to the CS and US in an unpaired, pseudorandom fashion, do not (+2.6% ± 5.6%, $n = 23$; unpaired t-test: $P < 0.0001$) (Fig. 1a). *In vivo* experiments suggest that the amygdala is involved in both the acquisition and expression of fear-potentiated startle[9–11]. We prepared coronal slices from fear-conditioned rats 24 hours after

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