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

completion of behaviour testing, as well as from experimentally naive and unpaired control rats, to investigate the synaptic alterations induced by whole-animal emotional learning.

Excitatory postsynaptic currents (EPSCs) in lateral amygdala neurons were elicited by stimulating fibres emerging from the internal capsule, shown to carry efferents from the medial geniculate nucleus[12]. The EPSC was completely blocked by co-application of 50 μM D-2-amino-5-phosphovalerate (D-AP5; an NMDA-receptor antagonist) and 100 μM GYKI 52466 (a non-competitive AMPA-receptor antagonist[13]; $n = 3$), suggesting mediation by both NMDA and AMPA receptors. The EPSC threshold was significantly lower in fear-conditioned (FC) animals (4.6± 0.2 V, $n = 23$) than in naive controls (NC: 5.5 ± 0.2 V, $P < 0.01$, $n = 28$) or in unpaired controls (UC: 5.4 ± 0.2 V, $P < 0.01$, $n = 24$) animals. Action potential threshold (FC: 9.0 ± 0.4 V, NC: 9.5 ± 0.4 V, UC: 10.2 ± 0.5 V) and input resistance (FC: 138.9 ± 7.3 MΩ, NC: 136.5 ± 8.5 MΩ, UC: 133.1 ± 10.7 MΩ) did not differ between the three groups.

EPSCs are potentiated in neurons from fear-conditioned rats relative to EPSCs recorded in neurons from naive and unpaired controls (Fig. 1b). This potentiation is reflected both in the composite response, a measure of the native synaptic response, and in the AMPA-receptor-mediated component of the EPSC (Fig. 1c). Input–output relationships, measuring EPSC amplitude (pA, output) as a function of afferent fibre stimulus intensity (V, input) for each neuron, were compared in the three groups (naive control, unpaired control and fear conditioned) using a one-way ANOVA. The effect of treatment (fear-conditioned, unpaired control, or naive control) was significant ($F(2, 51) = 26.27$, $P < 0.0001$). Whereas the slopes of the curves from naive controls (15.1 ± 1.2 pA/V, $n = 14$) and unpaired controls (11.2 ± 1.0 pA/V,

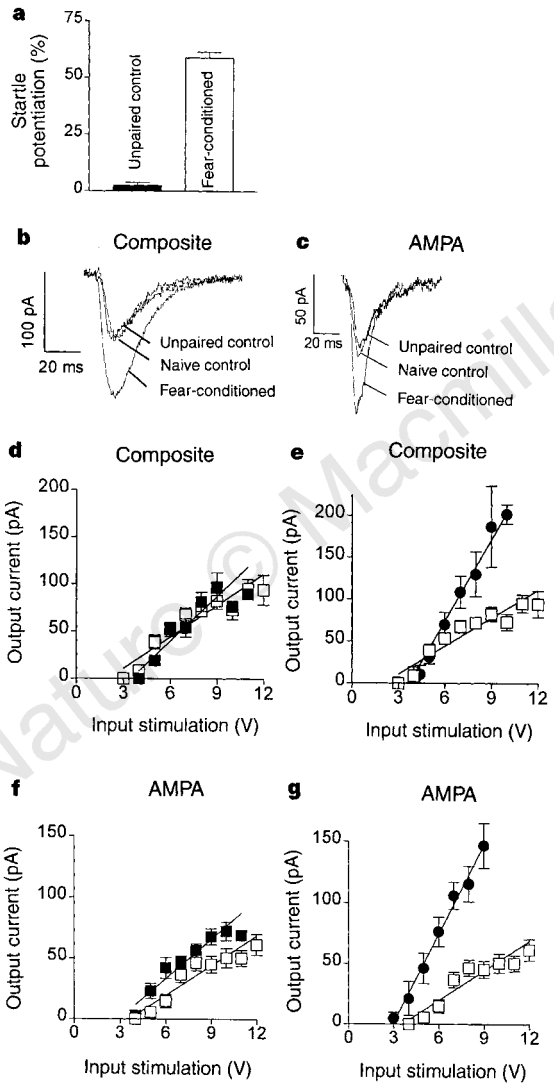

Figure 1 Fear conditioning results in potentiation of evoked EPSCs. **a**, Plot of per cent startle potentiation in fear-conditioned and unpaired control animals. **b**, Composite EPSCs evoked with input stimulations of 9 V. **c**, AMPA EPSCs evoked with input stimulations of 9 V. **d**, Input-output curves for composite EPSCs in naive control (■, $n = 14$) and unpaired control (□, $n = 23$) animals. **e**, Input-output curves for composite EPSCs in unpaired control (□, $n = 23$) and fear conditioned (●, $n = 22$) animals. **f**, Input-output curves for AMPA EPSCs in unpaired control (□, $n = 16$) and naive control (■, $n = 12$) animals. **g**, Input-output curves for AMPA EPSCs in unpaired control (□, $n = 16$) and fear conditioned (■, $n = 17$) animals.

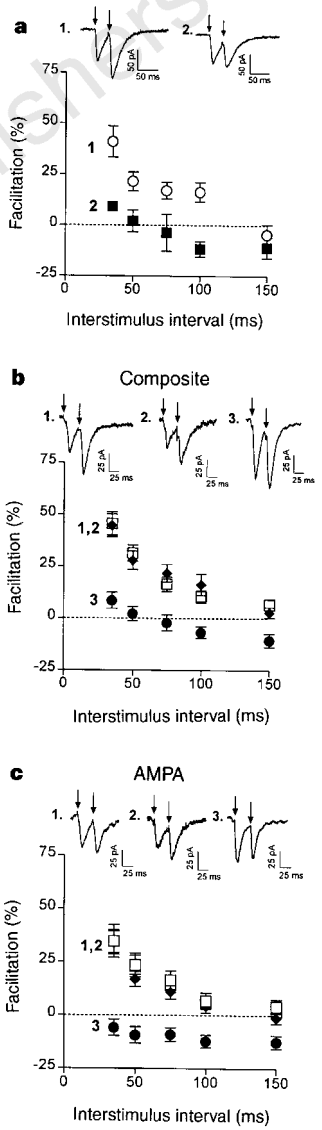

Figure 2 PPF is decreased in fear-conditioned animals. Numbered traces in **a**, **b** and **c** correspond to numbers in the plot; traces shown are averages of five recorded traces. $V_{HOLD} = -60$ mV. **a**, Decreasing the external $[Mg^{2+}]/[Ca^{2+}]$ ratio from 0.48 (□) to 0.16 (■) decreases the amount of PPF in naive control animals ($n = 4$). **b**, PPF is significantly decreased in fear-conditioned animals (●, $n = 18$) compared to naive (♦, $n = 16$) and unpaired (□, $n = 22$) controls. **c**, PPF of the AMPA component is significantly decreased in fear-conditioned animals (●, $n = 17$) compared to naive (♦, $n = 11$) and unpaired (□, $n = 17$) controls.

$n = 23$) were not significantly different ($P > 0.05$, Fig. 1d), the slope of the input–output curve in neurons from fear-conditioned animals (32.3 ± 3.7 pA/V, $n = 22$) was significantly greater than that in neurons from unpaired control animals ($P < 0.001$; Newman–Keuls post test). These data suggest an increase in synaptic efficacy in lateral amygdala neurons from fear-conditioned animals (Fig. 1e).

As many reports on LTP, a model of learning, suggest a predominant facilitation of AMPA-receptor-mediated transmission[14–16], and as AMPA receptors have been proposed to mediate the expression of fear conditioning *in vivo*[10], we focused on the AMPA component of the EPSC by recording input–output curves in the presence of D-AP5 (50 μM). Comparing the slopes of the input–output curves in the three groups with a one-way ANOVA showed that the effect of treatment was significant ($F(2, 35) = 18.5$, $P < 0.0001$). Whereas the slope of the AMPA component curve in unpaired control animals (8.2 ± 0.9 pA/V, $n = 16$) did not differ ($P > 0.05$) from that in the naive control animals (10.9 ± 0.9 pA/V, $n = 12$) (Fig. 1f), the slope of the input–output curve in fear-conditioned animals (24.3 ± 2.7 pA/v, $n = 17$) was significantly ($P < 0.001$; Newman–Keuls post test) greater than that of the curve from unpaired control animals, suggesting that AMPA-mediated transmission in the amygdala is potentiated in emotional learning (Fig. 1g).

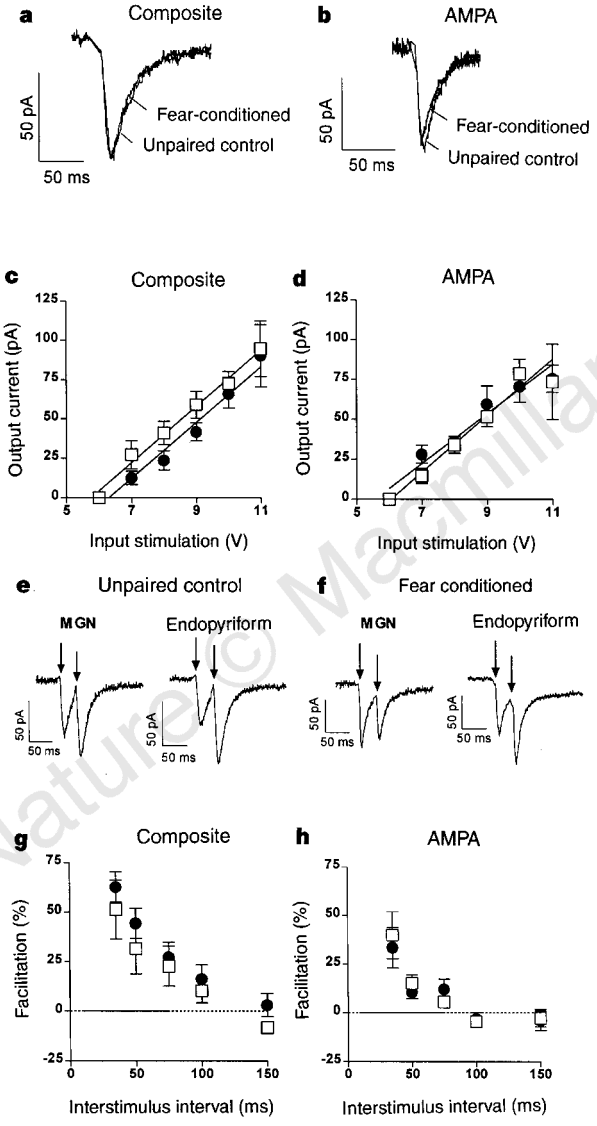

Figure 3 The endopyriform–LA pathway is not potentiated in fear-conditioned animals. **a**, Example composite EPSCs evoked with an input stimulation of 10 V. **b**, Example AMPA EPSCs evoked with an input stimulation of 10 V. **c**, Input–output curves for composite EPSCs in unpaired control (□, $n = 6$) and fear-conditioned (●, $n = 6$) animals. **d**, Input–output curves for the AMPA EPSC in unpaired control (□, $n = 5$) and fear-conditioned (● $n = 6$) animals. **e**, The same neuron from an unpaired control animal displays PPF in both the MGN–LA pathway and the endopyriform–LA pathway. **f**, Fear-conditioned animals display a selective loss of PPF in the MGN–LA pathway while maintaining PPF in the endopyriform–LA pathway. **g**, PPF does not differ in fear-conditioned animals (●, $n = 5$) and unpaired controls (□, $n = 6$). **h**, PPF of the AMPA component does not differ in fear-conditioned animals (●, $n = 5$) and unpaired controls (□, $n = 5$).

Fear conditioning results in a potentiation of the evoked MGN–LA response. To examine whether increases in presynaptic neurotransmitter release could be a contributing factor in this potentiation, we analysed paired-pulse facilitation (PPF) in neurons from the three groups. PPF is a phenomenon by which a second synaptic stimulation of equal magnitude evokes a larger synaptic response than the first, if the interval between the two pulses is sufficiently brief. PPF has traditionally been attributed to short-term increases in presynaptic intracellular calcium levels, and has been used as a tool to implicate presynaptic involvement; manipulations that increase the probability (p) of transmitter release, such as decreasing the external $[Mg^{2+}]/[Ca^{2+}]$ ratio, have been shown to decrease PPF[17–21]. Decreasing the external $[Mg^{2+}]/[Ca^{2+}]$ ratio from 0.48 to 0.16 produced the expected decrease in the degree of PPF at this MGN–LA synapse ($n = 4$) (Fig. 2a). Comparison of the time course and magnitude of PPF between the three experimental groups showed that the effect of treatment group was significant ($F(2, 225) = 37.26$, $P < 0.0001$, two-way ANOVA). A significant treatment effect ($F(2, 55) = 13.89$, $P < 0.01$, one-way ANOVA) was observed at the interstimulus interval showing maximal PPF, 35 ms, with neurons from fear-conditioned animals ($n = 18$) showing significantly less PPF than neurons from naive ($P < 0.001$, $n = 16$; Newman–Keuls) and unpaired ($P < 0.001$, $n = 22$; Newman–Keuls) control animals. PPF in neurons from naive and unpaired control animals did not differ significantly ($P > 0.05$) (Fig. 2b).

Some studies report a postsynaptic contribution to PPF mediated through NMDA receptors, although PPF mediated through AMPA receptors is thought to be primarily presynaptic[22–24]. Presynaptic regulation of AMPA PPF at this synapse was confirmed by the lack of correlation between EPSC1 and EPSC2 amplitudes and a lack of postsynaptic voltage dependence[25]. To assess AMPA-mediated PPF, we analysed PPF in neurons from the three groups of animals in the presence of D-AP5 (50 μM). Comparison of the time course and magnitude of PPF between groups indicated that the effect of treatment group was significant ($F(2, 210) = 76.50$, $P < 0.0001$, two-way ANOVA). The effect of treatment was significant ($F(2, 41) = 22.48$, $P < 0.0001$, one-way ANOVA) at the 35-ms interstimulus interval, with neurons from fear-conditioned animals ($n = 17$) exhibiting significantly less PPF of the AMPA EPSC than those from naive ($P < 0.001$, $n = 11$; Newman–Keuls) and unpaired ($P < 0.001$, $n = 17$; Newman–Keuls) control animals. PPF in neurons from naive and unpaired controls did not differ significantly ($P > 0.05$) (Fig. 2c). These data, coupled with the quantitative and qualitative similarity of PPF in fear-conditioned animals and PPF in decreased external $[Mg^{2+}]/[Ca^{2+}]$, suggest that increased presynaptic release contributes to synaptic potentiation in lateral amygdala neurons from fear conditioned animals.

The lack of synaptic potentiation in the unpaired control animals suggests that potentiation observed in fear-conditioned animals is not the result of nonspecific exposure to stress. However, it does not rule out the possibility that potentiation is a nonspecific result of the learning paradigm, not limited to fear-conditioning circuitry. To test this, we recorded EPSCs evoked in lateral amygdala neurons by

stimulating the endopyriform nucleus, a structure not believed to be involved in fear-conditioning. In this pathway, neither the composite nor the AMPA EPSC exhibited potentiation in fear-conditioned animals relative to unpaired controls (Fig. 3a, b). The slope of the input–output curves did not differ between fear-conditioned and unpaired control animals for the composite EPSC ($F(1, 61) = 0.006$, $P = 0.940$, one-way ANOVA) or the AMPA EPSC ($F(1, 50) = 0.616$, $P = 0.563$, one-way ANOVA) (Fig. 3c, d).

The endopyriform–lateral amygdala pathway also displayed PPF. In the same neuron, PPF was evident in both the MGN–LA pathway and the endopyriform–LA pathway in unpaired control animals; a paired *t*-test revealed that the degree of facilitation at the 35-ms interstimulus interval did not differ between the two inputs (MGN: $+55.3\% \pm 20.6\%$, endopyriform: $+56.5\% \pm 30.0\%$, $n = 3$, $P = 0.98$) (Fig. 3e). However, neurons from fear-conditioned animals show a selective loss of PPF in the MGN–LA pathway (MGN: $-0.3 \pm 4.8\%$, endopyriform: $+62.7 \pm 7.6\%$, $n = 5$, $P < 0.001$, paired *t*-test) (Fig. 3f). The overall time course and magnitude of PPF in the endopyriform–LA pathway did not differ between neurons from unpaired control animals and fear-conditioned animals either for the composite EPSC ($F(1, 45) = 2.49$, $P = 0.121$) or the AMPA EPSC ($F(1, 35) = 0.82$, $P = 0.817$, two-way ANOVA) (Fig. 3g, h). The finding that potentiation in fear-conditioned animals is specific to synapses involved in fear-conditioning circuitry provides additional support for this potentiation serving as a substrate for emotional learning.

The findings presented here provide direct evidence that the learning associated with fear-conditioning produces changes in the MGN–LA synapse that can be measured *in vitro*. The synaptic potentiation associated with fear-conditioning is the result, at least in part, of a presynaptic increase in transmitter release; potential postsynaptic changes occurring with fear conditioning remain to be investigated. Furthermore, the lack of potentiation in the endopyriform–LA pathway in fear-conditioned animals provides strong evidence for the specificity of learning-induced synaptic potentiation to fear-conditioning circuitry. The ability to record and analyse the expression of whole-animal emotional learning in an *in vitro* slice, using many of the same parameters traditionally used in the study of learning models such as LTP, represents an important means of revealing the neural basis of behaviour. □

Methods

Slice preparation. *In vitro* brain slices were obtained from male Sprague–Dawley rats (weight range, 100–150 g) and prepared as described[26]. Rats were decapitated and the brains were removed and cooled rapidly in a modified ACSF solution (0–6 °C) bubbled continuously with 95% O_2 and 5% CO_2. Three to four serial coronal slices of 500 μm thickness per hemisphere were cut with a Vibroslice. In the recording chamber, the slice was fully submerged and continuously perfused with oxygenated ACSF maintained at 32 ± 2 °C. Modified ACSF is (millimolar): NaCl, 117; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2, $NaHCO_3$, 25; NaH_2PO_4, 1.2; and glucose, 11.5.

Whole-cell recording. Voltage-clamp recordings in the whole-cell configuration were obtained as described[27]. Neurons were recorded in discontinuous single electrode whole-cell voltage clamp. The composition of the internal electrode solution was: Cs-gluconate, 115 mM; NaCl, 5 mM; EGTA, 1 mM; $CaCl_2$, 0.3 mM; $MgCl_2$, 2 mM; Na-ATP, 5 mM; Na-GTP, 0.4 mM; HEPES 10 mM; the internal solution was adjusted to a pH of 7.2 and osmolarity of 280 mOsm; tip resistance was 4–5 MΩ.

Stimulation and recording parameters. Bipolar concentric stimulating electrodes were placed (1) on fibres emerging from the internal capsule which originate in the medial geniculate nucleus of the thalamus and project monosynaptically to the lateral nucleus of the amygdala, and (2) within the endopyriform nucleus. Neurons used for recording were located in the dorsal portion of the lateral amygdala, where fibres from the medial geniculate terminate. Input–output curves were constructed by varying stimulus intensity and measuring EPSC amplitude from EPSC threshold to spike threshold. Peak EPSC amplitude was measured as the peak inward current within a time window defined as current onset to return to baseline. Peak amplitude for individual responses in the paired-pulse paradigm was measured as the difference between the current level before the stimulus artefact and the peak of the EPSC[28]. Paired-pulse facilitation (PPF) was calculated as [(EPSC2 − EPSC1)/EPSC1] × 100. Paired-pulse EPSCs were elicited at a frequency of 0.1 Hz.

Fear conditioning. Fear conditioning was measured using the potentiated startle paradigm[29]. Male Sprague–Dawley rats (100–150 g) were trained and tested in a stabilimeter device, in which cage movement results in the displacement of an accelerometer located beneath the stabilimeter (San Diego Instruments). Startle amplitude was defined as peak accelerometer voltage within 200 ms after startle stimulus onset. 50-ms bursts of white noise at 95 dB were used as the acoustic startle stimulus. The conditioned stimulus (CS) was a 3.7 s 70-dB white-noise bandpass filtered with low and high passes at 2,000 Hz (24 dB per octave attenuation). This stimulus was paired 10 times a day for two days with a 0.5-mA footshock of 0.5 s in experimental animals to provide the aversive component necessary for fear conditioning. Unpaired controls received the same number of CS and US presentations, but in an unpaired, pseudorandom fashion. Amygdala slices were prepared from animals 24 h after the final testing session; 1–2 neurons were recorded per animal, typically with a single neuron recorded per slice. To investigate possible experimenter bias, experiments on 5 fear-conditioned and 5 unpaired animals were blinded to animal group during data acquisition and analysis. The data from these experiments were not significantly different from those obtained during non-blinded experiments, so all data were pooled.

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How opioids inhibit GABA-mediated neurotransmission

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The midbrain region periaqueductal grey (PAG) is rich in opioid receptors and endogenous opioids and is a major target of analgesic action in the central nervous system[1]. It has been proposed that the analgesic effect of opioids on the PAG works by suppressing the inhibitory influence of the neurotransmitter GABA (γ-aminobutyric acid) on neurons that form part of a descending antinociceptive pathway[2]. Opioids inhibit GABA-mediated (GABAergic) synaptic transmission in the PAG and other brain regions by reducing the probability of presynaptic neurotransmitter release[3,4], but the mechanisms involved remain uncertain. Here we report that opioid inhibition of GABAergic synaptic currents in the PAG is controlled by a presynaptic voltage-dependent potassium conductance. Opioid receptors of the μ type in GABAergic presynaptic terminals are specifically coupled to this potassium conductance by a pathway involving phospholipase A_2, arachidonic acid and 12-lipoxygenase. Furthermore, opioid inhibition of GABAergic synaptic transmission is potentiated by inhibitors of the enzymes cyclooxygenase and 5-lipoxygenase, presumably because more arachidonic acid is available for conversion to 12-lipoxygenase products. These mechanisms account for the analgesic action of cyclooxygenase inhibitors in the PAG[5] and their synergism with opioids[6].

To determine the presynaptic mechanism of opioid inhibition of $GABA_A$ergic synaptic transmission, we examined the action of opioids on spontaneous action-potential-independent miniature GABAergic postsynaptic currents (mISPCs) in the rat PAG. Superfusion of methionine-enkephalin (ME; 10 μM) reduced the frequency of mIPSCs by 64 ± 3%, without any significant reduction in their mean amplitude (8 ± 6%) in all PAG neurons tested (Fig. 1a–d; $n = 15$)[4]. This effect was mimicked by the μ agonist DAMGO ([D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]-enkephalin; 0.1–1 μM; Fig. 3b) and was abolished by the μ antagonist CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Pen-Thr-NH_2; 1 μM; $n = 5$). The decrease in mIPSC frequency without any change in mIPSC amplitude is indicative of a reduction in the probability of transmitter release from presynaptic GABAergic terminals mediated by μ-receptors. Although opioid modulation of postsynaptic Ca^{2+} conductances and Ba^{2+}-sensitive, inwardly rectifying K^+ conductances is well established[7], opioid inhibition of GABAergic synaptic transmission in PAG is not mediated by similar conductances in presynaptic terminals[4]. ME produced a significant reduction in mIPSC frequency in Ca^{2+}-free, high-Mg^{2+} (10 mM) solutions containing Cd^{2+} (100 μM; $n = 4$), or Ba^{2+} (10 mM; $n = 4$) (Fig. 1e). Similar observations in cultured hippocampal neurons have led to the proposition that opioids directly inhibit the spontaneous GABA exocytotic process, without modulation of presynaptic Ca^{2+} or K^+ conductances[3]. However, opioids can potentially modulate several K^+ conductances[8]. We therefore examined the effects of other K^+-channel blockers on presynaptic inhibition by opioids and found that the ME-induced reduction in mIPSC frequency was blocked by the voltage-dependent K^+ channel blockers 4-aminopyridine (4-AP, 0.1–1 mM; $n = 9$) and dendrotoxin (100 nM; $n = 5$), but was unaffected by tetraethylammonium chloride (TEA; 10 mM; $n = 4$) (Fig. 1a–e)[9].

We tested the specificity of the effect of these K^+-channel blockers on inhibition of synaptic transmission in PAG. The $GABA_B$-receptor agonist baclofen (10 μM) reduced the frequency of mIPSCs by 74 ± 3% ($n = 10$), without any significant reduction in their amplitude (9 ± 5%; Fig. 1a–e)[10]; baclofen also reduced mIPSC frequency in the presence of 4-AP (1 mM; $n = 5$) and dendrotoxin (100 nM; $n = 4$; Fig. 1a–e). Furthermore, ME (10 μM) reduced the frequency of glutamatergic miniature excitatory postsynaptic currents (mEPSC) in the absence (53 ± 3%; $n = 6$)[4] or presence of 4-AP (1 mM; 59 ± 7%; $n = 6$). Thus, 4-AP and dendrotoxin selectively abolished presynaptic μ-receptor-mediated inhibition without affecting either $GABA_B$-receptor-mediated inhibition of GABAergic mIPSC frequency, or μ-receptor inhibition of glutamatergic mEPSC frequency.

Electrically evoked synaptic transmission is dependent on Ca^{2+} entry, so the mechanism of opioid inhibition of mIPSCs and electrically evoked GABAergic postsynaptic currents (eIPSCs) might differ. ME inhibited the amplitude of eIPSCs by 69 ± 7% ($n = 6$), and this was reduced by 4-AP (100 μM; 16 ± 5% inhibition; $n = 6$) and dendrotoxin (100 nM; 26 ± 12% inhibition; $n = 5$; Fig. 1f–h). Baclofen reduced the amplitude of eIPSCs in the absence (87 ± 3% inhibition; $n = 4$) or presence of 4-AP (63 ± 3% inhibition; $n = 5$) and dendrotoxin (85 ± 3% inhibition; $n = 4$; Fig. 1f–h). Thus, it is likely that the μ-opioid inhibition of electrically evoked GABA release and of spontaneous action potential independent GABA release in PAG results from a common mechanism.

These data define the site of the inhibitory action of opioids on GABAergic neurotransmission in PAG as a dendrotoxin- and 4-AP-sensitive, presumably *Shaker*-like[9], voltage-dependent K^+ conductance in presynaptic terminals. The mechanism by which a presynaptic K^+ conductance regulates mIPSC frequency (in the absence of Ca^{2+} entry) remains to be determined, but probably involves hyperpolarization resulting from enhanced K^+-channel activity. A similar mechanism of presynaptic inhibition might occur in other brain regions. Inhibition of electrically evoked excitatory postsynaptic currents in hippocampal CA3 neurons that is mediated by opioid receptor of the κ type is also abolished by voltage-dependent K^+-channel blockers[11], although this could have been due to actions in somata or terminals. Inhibition of this presynaptic K^+ conductance does not generalize to all forms of μ-opioid or other G-protein-receptor-mediated forms of presynaptic inhibition in PAG. μ-Opioid-mediated inhibition of glutamatergic transmission and $GABA_B$-receptor-mediated inhibition of GABAergic neurotransmission in PAG are mediated by as yet unidentified mechanisms because neither was abolished by 4-AP.

A variety of G-protein-modulated second-messenger pathways could be involved in the presynaptic opioid action. The sulphydryl alkylating agent *N*-ethylmaleimide (NEM) blocked the reduction in mIPSC frequency produced by ME ($n = 4$) and baclofen ($n = 3$) (Fig. 2f). However, NEM disrupts a number of cellular proteins in addition to pertussis-toxin-sensitive G-protein-mediated actions[12]. Both the cyclic-AMP-dependent protein kinase A and protein kinase C systems regulate synaptic transmission[13]. We therefore examined whether maximal concentrations of activators of protein