

α_2 -Adrenoreceptor activation inhibits LTP and LTD in the basolateral amygdala: involvement of $G_{i/o}$ -protein-mediated modulation of Ca^{2+} -channels and inwardly rectifying K^+ -channels in LTD

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Abstract

Activation of adrenoreceptors modulates synaptic transmission in the basolateral amygdala. Here, we investigated the effects of α_2 -adrenoreceptor activation on long-term depression and long-term potentiation in an *in vitro* slice preparation of the mouse basolateral amygdala. Field potentials and excitatory postsynaptic currents were evoked in the basolateral amygdala by stimulating the lateral amygdala. Norepinephrine (20 μ M) reduced synaptic transmission and completely blocked the induction of long-term potentiation and long-term depression. The α_2 -adrenoreceptor antagonist yohimbine (2 μ M) reversed this effect. The α_2 -adrenoreceptor agonist clonidine (10 μ M) mimicked the effects of norepinephrine. The $G_{i/o}$ -protein inhibitor pertussis toxin (5 μ g/mL) reversed the effect of clonidine. Long-term depression was blocked in the presence of ω -conotoxin GVIA, but not ω -agatoxin IVA. Clonidine inhibited voltage-activated Ca^{2+} currents mediated via N- or P/Q-type Ca^{2+} -channels. The inhibitory action of clonidine on long-term depression was reversed when inwardly rectifying K^+ -channels were blocked by Ba^{2+} (300 μ M). The present data suggest that α_2 -adrenoreceptor activation impairs the induction of long-term depression in the basolateral amygdala by a $G_{i/o}$ -protein-mediated inhibition of presynaptic N-type Ca^{2+} -channels and activation of inwardly-rectifying K^+ -channels.

Introduction

The amygdala is an integral component of the limbic circuitry, and appears to be critically involved in the control of emotional and autonomic behaviour (Rogan & LeDoux, 1996; McKernan & Shinnick-Gallagher, 1997; Swanson & Petrovich, 1998) such as conditioned fear and anxiety (Davis *et al.*, 1994; Maren & Fanselow, 1996; Pitkanen *et al.*, 1997). Extensive evidence indicates that stress hormones affect memory storage (McGaugh *et al.*, 1996; Salinas *et al.*, 1997) and memory consolidation (Galvez *et al.*, 1996) via noradrenergic mechanisms in the amygdala. The amygdala receives its noradrenergic input from the locus coeruleus (Fallon & Loughlin, 1987). Consistent with the evidence for a high density of β -adrenoreceptor subtypes within the amygdala (Bylund & Snyder, 1976), infusion of β -adrenergic receptor antagonists into the amygdala block norepinephrine (NE)-mediated effects on memory. The regional infusion of β -adrenergic receptor agonists into the basolateral amygdala (BLA) improves memory (Roozendaal *et al.*, 1997), probably by an increase in glutamatergic transmission. The amygdala also contains α -adrenoreceptors (Zilles *et al.*, 1993). Activation of α -adrenoreceptors

decreases glutamatergic transmission (Ferry *et al.*, 1997). The α_1 -adrenoreceptor subtype is located postsynaptically (Hardmann *et al.*, 1996). A recent report suggests that, in the BLA, α_1 -adrenoreceptors mediate their effects on memory storage only during concurrent β -adrenoreceptor activation (Ferry *et al.*, 1999). Presynaptically located α_2 -adrenoreceptors inhibit the release of neurotransmitter (Starke, 2001) and decrease amygdala kindling (Shouse *et al.*, 1994). In line with this antiepileptic effect, α_2 -adrenoreceptor activation decreases the firing rate of amygdala neurons *in vivo* (Freedman & Aghajanian, 1985). The role of α_2 -adrenoreceptors in synaptic plasticity in the BLA has not yet been established. The present study is the first attempt to elucidate the involvement of α_2 -adrenoreceptor-coupled effector systems in the modulation of the induction of long-term potentiation (LTP) and long-term depression (LTD) in the BLA.

Materials and methods

Slice preparation

Coronal slices of the amygdala (400 μ m thick) were obtained from 6–8-week-old mice (FVB/N; Charles River, Sulzfeld, Germany), anaesthetized with isoflurane before decapitation. After rapidly removing the brain, slices were prepared in ice-cold artificial cerebrospinal fluid (aCSF) using a vibroslicer (FTB, Villingen, Germany). The experimental protocols were approved by the Ethical Committee on Animal

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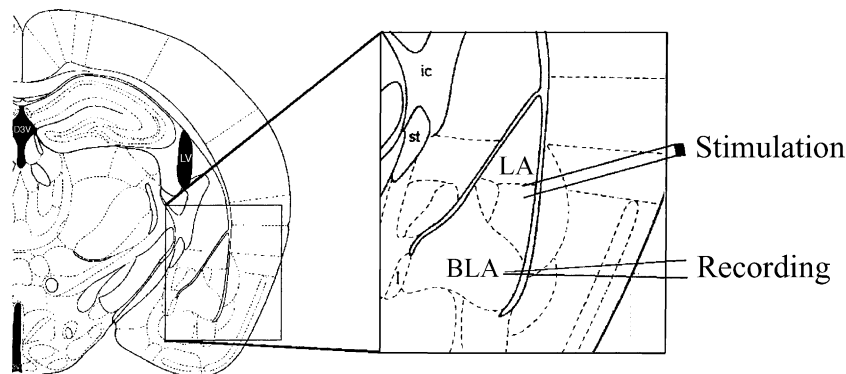


FIG. 1. Schematic illustration of a coronal brain slice containing the amygdala with the position of the stimulus and recording electrodes.

Care and Use of the Government of Bavaria, Germany. All slices were placed in a storage chamber (22 °C) for at least 60 min and then transferred to the superfusion chamber (Luigs & Neumann, Ratingen, Germany) for recording. The flow rate of the aCSF through the chamber was 1.5 mL/min. The aCSF contained (in mM): NaCl, 125; KCl, 2.5; NaHCO₃, 25; CaCl₂, 2; MgCl₂, 1; D-glucose, 25; NaH₂PO₄, 1.25; bubbled with a 95% O₂/5% CO₂ mixture, and had a final pH of 7.4.

In the present study, we used a total of 45 mice and all experiments were performed on 163 slices. Usually one neuron was recorded from one slice. For each mouse, three–four slices were used.

Synaptic transmission and synaptic plasticity

Excitatory postsynaptic currents (EPSCs) and field potentials (FPs) were evoked by square pulse stimuli (0.066 Hz, 5–12 mA, 200 μs) delivered via bipolar tungsten electrodes insulated to the tip (5 μm tip diameter) and positioned in the lateral amygdaloid nucleus close to the external capsule (EC) (Fig. 1). The FPs and EPSCs reflect the activity of a monosynaptic connection because their latency (2–3 ms) remained constant. FP recordings were made using glass microelectrodes (2–3 MΩ) filled with aCSF. The stimulus intensity was adjusted to produce a FP of approximately 50% of the maximum amplitude. High-frequency stimulation (HFS) trains of stimuli [5 trains at 100 Hz for 1 s, and 10 s interstimulus intervals (ISI)] to induce LTP were applied at the same stimulus intensity. For HFS and low-frequency stimulation (LFS; 1 Hz for 15 min) of FPs, the same stimulus intensities were used. Before HFS or LFS was applied, the responses to single stimuli had to remain stable for at least 20 min. Control LTP/LTD experiments were intercolated with pharmacological testing and results were only considered for analysis when control LTP slices showed at least a 20% change from baseline values 30 min after HFS. The voltage difference between the onset and the negative peak (*a*) and the difference between the negative peak and the succeeding positive peak (*b*) were measured. The amplitude of the FP was calculated as (*a* + *b*)/2. All experiments were performed at room temperature (22–25 °C). The FPs were averaged before analysis. We stimulated every 15 s and averaged four consecutive FPs into one response, of which the amplitudes were then analysed, and which are shown as representative traces. Each dot in the graph represents the normalized amplitude of one averaged trace and is equal to 1 min.

Somata of principal neurons in the basolateral amygdala were identified by infrared-phase-contrast-enhanced videomicroscopy (Zeiss, Oberkochen, Germany; for details see Dodt *et al.*, 2002), and with these cells, whole-cell recordings were performed with glass pipettes (4–6 MΩ) containing (in mM): K-D-gluconate, 130; KCl, 5; EGTA, 0.5; Mg-ATP, 2; HEPES, 10; D-glucose, 5. Currents were recorded with a switched voltage-clamp amplifier (SEC 10L, NPI

electronic, Tamm, Germany) with switching frequencies of 60–80 kHz (25% duty cycle). Series resistance was monitored continuously and compensated in bridge mode (for details see Swandulla & Misgeld, 1990). Neuronal input resistance was monitored by injecting a hyperpolarizing pulse (300 ms, –10 mV) periodically through the patch electrode (no current was injected during LTD induction). All patch-clamp experiments were performed at room temperature (22–25 °C) at a holding potential of –70 mV. For LTD induction, LFS (1 Hz/900 pulses) was applied in bridge mode. Before LFS, the EPSC amplitudes to single stimuli had to remain stable for at least 15 min. Data are expressed as means ± standard error of the mean (SEM) of EPSC amplitude. The significance was tested (*P* < 0.05) using the Student's *t*-test at 30 min following termination of HFS or LFS. The recordings were amplified, filtered (3 kHz) and digitized (9 kHz) (ITC-16 Computer Interface, Instrutech, New York). The digitized responses were stored to disk on a Power Macintosh G3 computer with a data acquisition program (Pulse vs. 8.31; Heka electronic GmbH, Lambrecht, Germany).

Recording of Ca²⁺ currents

The solution for preparation of slices contained (in mM): NaCl, 130; KCl, 3; NaHCO₃, 26; CaCl₂, 1; MgCl₂, 5; NaH₂PO₄, 1.25; D-glucose, 10; bubbled with a 95% O₂/5% CO₂ mixture to obtain a final pH of 7.38. Slices were maintained in a similar solution, but with CaCl₂ and MgCl₂ (both at 2 mM). For voltage-clamp recordings of Ba²⁺ currents through N- and P/Q-type voltage-gated calcium channels (VGCCs), we used an external HEPES-based Ringer solution that contained (in mM): NaCl, 145; KCl, 3.5; HEPES, 10; BaCl₂, 2; MgCl₂, 2; D-glucose, 25; tetrodotoxin (TTX), 0.001; Ni²⁺, 0.05; nifedipine, 0.02 (pH 7.3 using NaOH); the internal solution contained (in mM): TEACl, 30; CsOH₃O₃S, 100; NaCl, 4; MgCl₂, 1; CaCl₂, 0.5; HEPES, 10; EGTA, 10; Mg-ATP, 5; GTP-Tris, 0.3; creatine-PO₄-Tris salt, 10 (pH 7.3 using TEOH). Transmembrane currents were evoked by stepping the holding potential from –70 mV to 0 mV for 300 ms.

Recording of K⁺ currents

After whole-cell access was established, inwardly-rectifying K⁺-channels (K_{ir}-channels) currents were investigated in standard aCSF; patch electrodes (3.5–4.5 MΩ) were filled with (in mM): KCH₃SO₃, 120; CsCH₃SO₃, 120; CdCl₂, 0.01; NaCl, 4; MgCl₂, 0.5; CaCl₂, 0.5; HEPES, 10; EGTA, 10; GTP-Tris, 0.3; Na₂-creatine phosphate, 20 (pH 7.3 using NaOH). TTX (1 μM) has been added to block Na⁺-channels. Cells were held at –70 mV and K_{ir} currents were elicited by ramp voltage commands from –130 to 0 mV. To enhance K_{ir} currents, controls and drug exposure were carried out in solutions with elevated extracellular K⁺ (30 mM) by substitution for Na⁺.

For investigating Ca^{2+} currents, a standard P/4 protocol was used for the subtraction of leakage currents prior to analysis. (This protocol provides the option to generate leak pulses while averaging to eliminate slow capacitive currents arising from the jump from holding to leak holding).

Chemicals

Drugs were applied via the superfusion system. Compounds used: NE, clonidine, yohimbine, TEA (tetraethylammonium), Na_2 -creatine phosphate, SPcAMPs (sp-adenosine 3',5'-cyclic monophosphothionate), MDL12,330A (*cis*-N-(2-phenylcyclopentyl)-azacyclotridec-1-en-2-amine monohydrochloride), forskolin (7 β -acetoxy-6 β -hydroxy-8,13-epoxy-labd-14-en-11-one) and nifedipine from RBI/Sigma, WAY100635 ((S)-N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropan-amide dihydrochloride) from Tocris. ω -Conotoxin GVIA (ω -CnTx) and ω -agatoxin IVA (ω -AgTx) were purchased from the Peptide Institute, Japan.

Results

The effect of α_2 -adrenoreceptor activation on basal synaptic transmission and synaptic plasticity

Bath application of NE (20 μM) reduced evoked EPSCs to $48.9 \pm 7.4\%$ and increased the paired pulse facilitation (PPF; 50 ms pulse interval) ratio from 1.16 ± 0.14 to 1.51 ± 0.21 (both $n = 7$, $P < 0.05$; Fig. 2A). In addition, NE slightly depolarized BLA neurons (-62.2 ± 0.7 mV to -57.9 ± 0.7 mV) and reduced input resistance (267.4 ± 30.9 M Ω to 229.6 ± 30.2 M Ω). Previous studies from our laboratory demonstrated that a strong tetanic stimulation (5×100 Hz for 1 s, and 10 s ISI) produced a robust potentiation of excitatory synaptic currents outlasting 60 min (Rammes *et al.*, 2000). In the present study, HFS potentiated synaptic responses to $135.9 \pm 5.7\%$ (Fig. 2B). After application of NE (20 μM), HFS only induced short-term potentiation (STP), and the amplitude of synaptic responses returned to control levels after 20 min (Fig. 2B). LTD was induced by LFS (1 Hz, 15 min; see Rammes *et al.*, 2001), which reduced the FP amplitude to $78.4 \pm 6.0\%$ (Fig. 2C). NE (20 μM) completely blocked LTD (Fig. 2C). The blocking effect of NE on LTP and LTD was significant ($P < 0.001$, both $n = 7$). These effects on synaptic plasticity were mimicked by the α_2 -adrenoreceptor agonist clonidine (10 μM). Clonidine reduced EPSC amplitude to $76.6 \pm 7.4\%$ ($n = 7$; $P < 0.01$), increased PPF ratio (control: 1.15 ± 0.05 ; in clonidine: 1.37 ± 0.08 , $P < 0.05$; Fig. 3A), hyperpolarized neurons from a resting potential of -62.2 ± 2.1 mV to -70.7 ± 2.4 mV ($n = 10$, $P < 0.01$) and increased input resistance from 219.1 ± 28.5 M Ω to 270.9 ± 33.8 M Ω ($n = 10$, $P < 0.05$). In the presence of clonidine, HFS resulted only in STP, and LFS failed to induce LTD (Fig. 3B and C).

To provide further evidence that the inhibition of synaptic plasticity in the BLA is mediated by α_2 -adrenoreceptor activation, we applied NE (20 μM) and the α_2 -adrenoreceptor antagonist yohimbine (2 μM) simultaneously. Because yohimbine also affects the serotonin type 1A (5-HT_{1A}) receptor and mere activation of 5-HT_{1A} receptors blocks LTP (G. Rammes, unpublished results) and LTD (Rammes *et al.*, 2001), we additionally added the specific 5-HT_{1A} receptor antagonist WAY100635 (300 nM). Under these conditions, HFS of the lateral nucleus of the amygdala (LA) resulted in LTP in the BLA, increasing the FP amplitudes to $133.6 \pm 14.4\%$ ($n = 8$; $P < 0.05$; Fig. 4B). LFS produced a robust LTD, reducing the amplitude of the synaptic responses to $78.3 \pm 4.6\%$ (Fig. 4C). Importantly, the 5-HT_{1A} receptor antagonist WAY100635 alone had no effect on basal synaptic transmission or synaptic plasticity (Fig. 4A). These experiments provide strong evidence

that the activation of presynaptically located α_2 -adrenoreceptors might be responsible for the inhibition of LTP and LTD in the BLA. There is further evidence in favour of a presynaptic locus of expression for LTD in the mouse BLA from a previous study (Rammes *et al.*, 2001). In order to investigate the α_2 -adrenoreceptor-stimulated signal cascade responsible for the inhibition of synaptic plasticity, we now focused on the induction of LTD.

G_{i/o}-proteins mediate α_2 -adrenoreceptor effects on LTD

Adrenergic α_2 -receptors are coupled to inhibitory G-proteins (Saunders & Limbird, 1999). To determine whether the inhibitory effect of α_2 -adrenoreceptor activation on the induction of LTD involves pertussis toxin-sensitive (PTX-sensitive) G-proteins, we incubated slices with PTX (5 $\mu\text{g}/\text{mL}$, 4–6 h at 37 °C). Treatment with PTX alone neither altered evoked FPs nor prevented LFS from inducing stable LTD (FPs were reduced to $81.0 \pm 7.6\%$; Fig. 5A, left; $n = 8$, $P < 0.05$). In slices pretreated with PTX, clonidine did not inhibit LTD (Fig. 5A, right). In these experiments, LFS induced a pronounced LTD (FP reduction to $69.7 \pm 8.7\%$; $n = 8$, $P < 0.05$), indicating that blocking PTX-sensitive G-proteins reverses the inhibitory influence of clonidine on LTD. Thus, activation of G_{i/o}-proteins seems to be a prerequisite for the α_2 -adrenoreceptor-mediated effect on LTD induction.

Inhibition of protein kinase A, but not of adenylate cyclase, is involved in the α_2 -adrenoreceptor-mediated effect of clonidine on LTD

α_2 -Adrenoreceptors display various functions via G_{i/o}-proteins. Their activation decreases adenylate cyclase (AC) activity (Saunders & Limbird, 1999), thereby reducing protein kinase A (PKA) activity. First, we tested whether activation of AC affects the induction of LTD. After application of the specific AC inhibitor MDL 12,330A (Lippe & Ardizzone, 1991; 10 μM) for 40 min, LFS still induced robust LTD (MDL 12,330A itself slightly increased basal synaptic transmission to $108.7 \pm 6.3\%$, data not shown). This finding indicates that activation of AC is not required to induce LTD (the FP amplitude was reduced to $78.4 \pm 6.0\%$ and $80.4 \pm 6.1\%$ for control and in MDL 12,330A, respectively; $n = 7$ both $P < 0.05$; Fig. 5B). Furthermore, if the inhibition of LTD by α_2 -adrenoreceptor activation was mediated by a decrease in AC activity, the effect of clonidine should be occluded by MDL 12,330A. However, in the presence of MDL 12,330A, clonidine still blocked the induction of LTD, and the FP amplitude returned to control levels (FP amplitude: $102.9 \pm 2.6\%$; $n = 7$; Fig. 5B, open circles). Consistent with a previous report (Wang *et al.*, 1999), application of the direct AC activator forskolin (25 μM) caused a LTP-like increase in FP amplitude ($131.8 \pm 6.9\%$, $P < 0.05$; data not shown) without occluding LTD. LFS reduced FP amplitudes to $76.9 \pm 4\%$ ($n = 7$, $P < 0.01$; Fig. 5C, left) under these conditions. In the presence of forskolin, clonidine (10 μM) was still able to inhibit the induction of LTD. The FP amplitude returned to control levels after 30 min (FP amplitude: $100.2 \pm 10.3\%$; $n = 7$; Fig. 5C, right). Together, these results demonstrate that the α_2 -adrenoreceptor-mediated effect on LTD does not depend on changes in AC activity.

In order to determine whether activation of PKA is crucial for LFS-induced LTD, we tested the influence of the PKA inhibitor H89 on LTD. When the slices were perfused with H89 (10 μM), the expression of LTD was significantly reduced (Fig. 6A). Control LTD showed a decrease in FP amplitude to $75.4 \pm 1.2\%$. In slices pretreated with H89, the FP amplitudes were decreased to $88.9 \pm 5.9\%$ ($n = 7$, $P < 0.05$; right). These results indicate that PKA activation plays a role in LFS-induced LTD. Next, we investigated the role of cAMP and PKA in the α_2 -adrenoreceptor-mediated inhibitory effect of clonidine on LTD. We used the membrane-permeable cAMP analogue and PKA activator

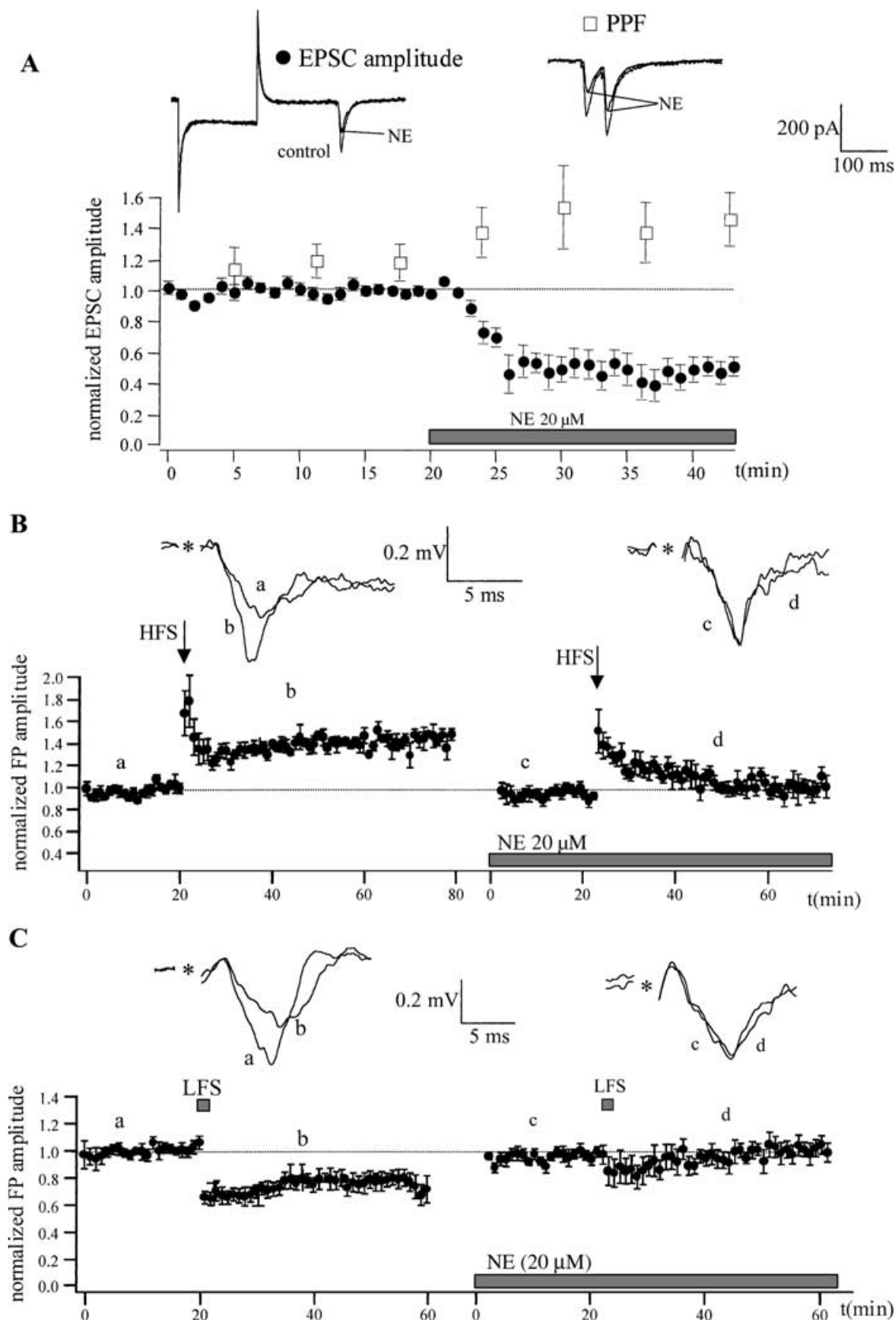


FIG. 2. NE reduces synaptic transmission and blocks synaptic plasticity in the BLA. (A) NE (20 μ M) reduces EPSC amplitude to $48.9 \pm 7.4\%$ and increases PPF ratio significantly from 1.16 ± 0.14 to 1.51 ± 0.21 ($n = 7$, $P < 0.05$). (B, left) HFS induces LTP ($135.9 \pm 5.7\%$ increase in FP amplitude). (right) After application of NE, HFS only induces STP (after 30 min FP amplitude returned to $99.1 \pm 8.6\%$). (C, left) LTD under control conditions (FPs were reduced to $78.4 \pm 6.0\%$). (right) NE completely blocks LTD (FP amplitude returned to $105.5 \pm 6.8\%$). The effect of NE on LTP and LTD is significant (for LTP and LTD: $P < 0.001$, $n = 7$). Insets show representative traces.

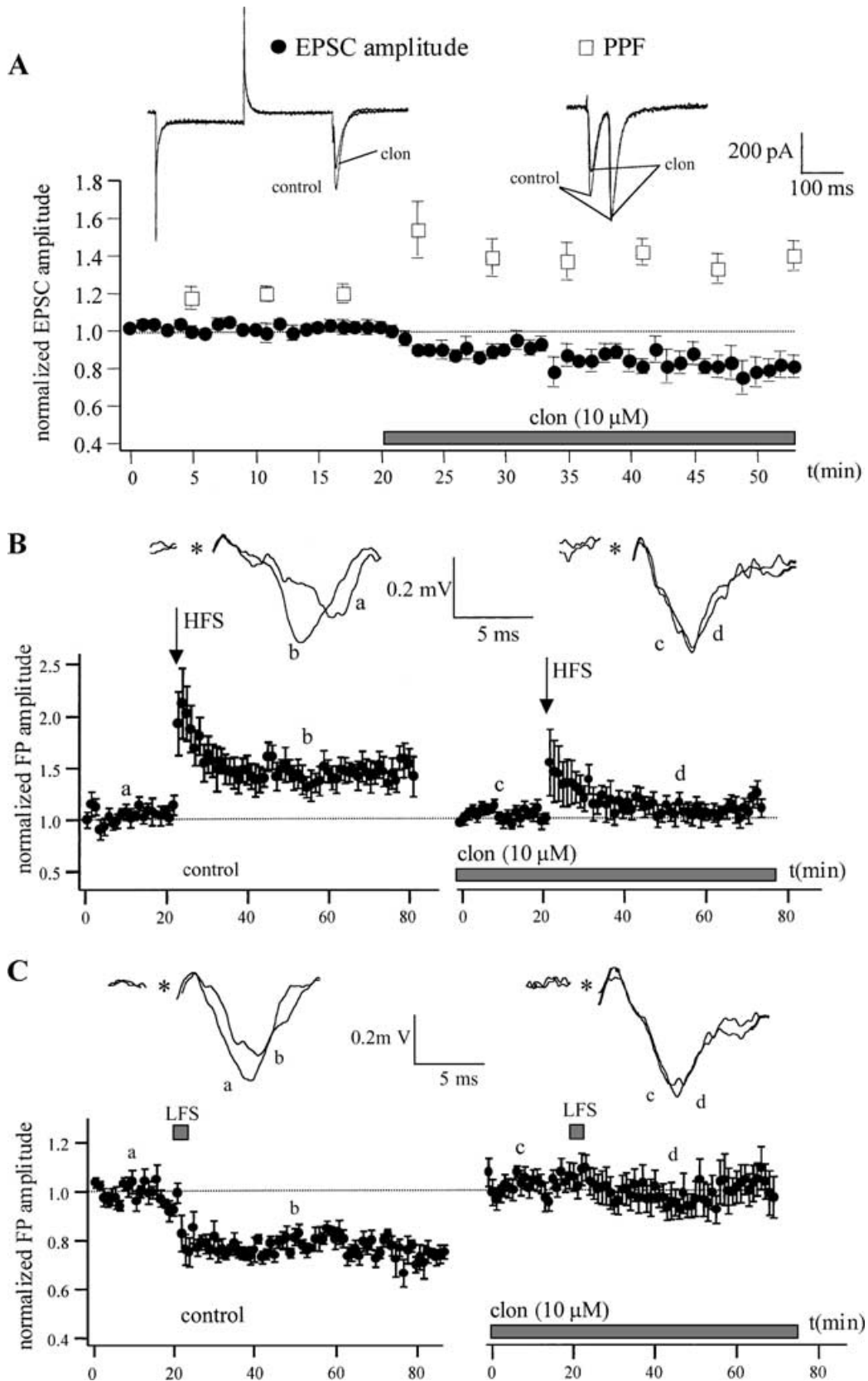


FIG. 3. The α_2 -adrenoreceptor agonist clonidine (10 μ M) mimics the inhibitory effect of NE on EPSC amplitude and synaptic plasticity. (A) Clonidine reduces EPSC amplitude to $76.6 \pm 7.4\%$ ($n = 7$; $P < 0.01$) and increases PPF ratio (control: 1.15 ± 0.05 ; in clonidine: 1.37 ± 0.08). (B) In the presence of clonidine, HFS induces STP (after 30 min FP amplitude returned to $108.4 \pm 9.3\%$; control is $144.9 \pm 10.6\%$). (C) In the presence of clonidine, LFS fails to induce LTD (after 30 min FP amplitude returned to $100.7 \pm 7.6\%$; control is $74.8 \pm 3.9\%$). The effect of clonidine on LTP and LTD is significant (for LTP and LTD: $P < 0.01$, $n = 8$). Insets show representative traces.

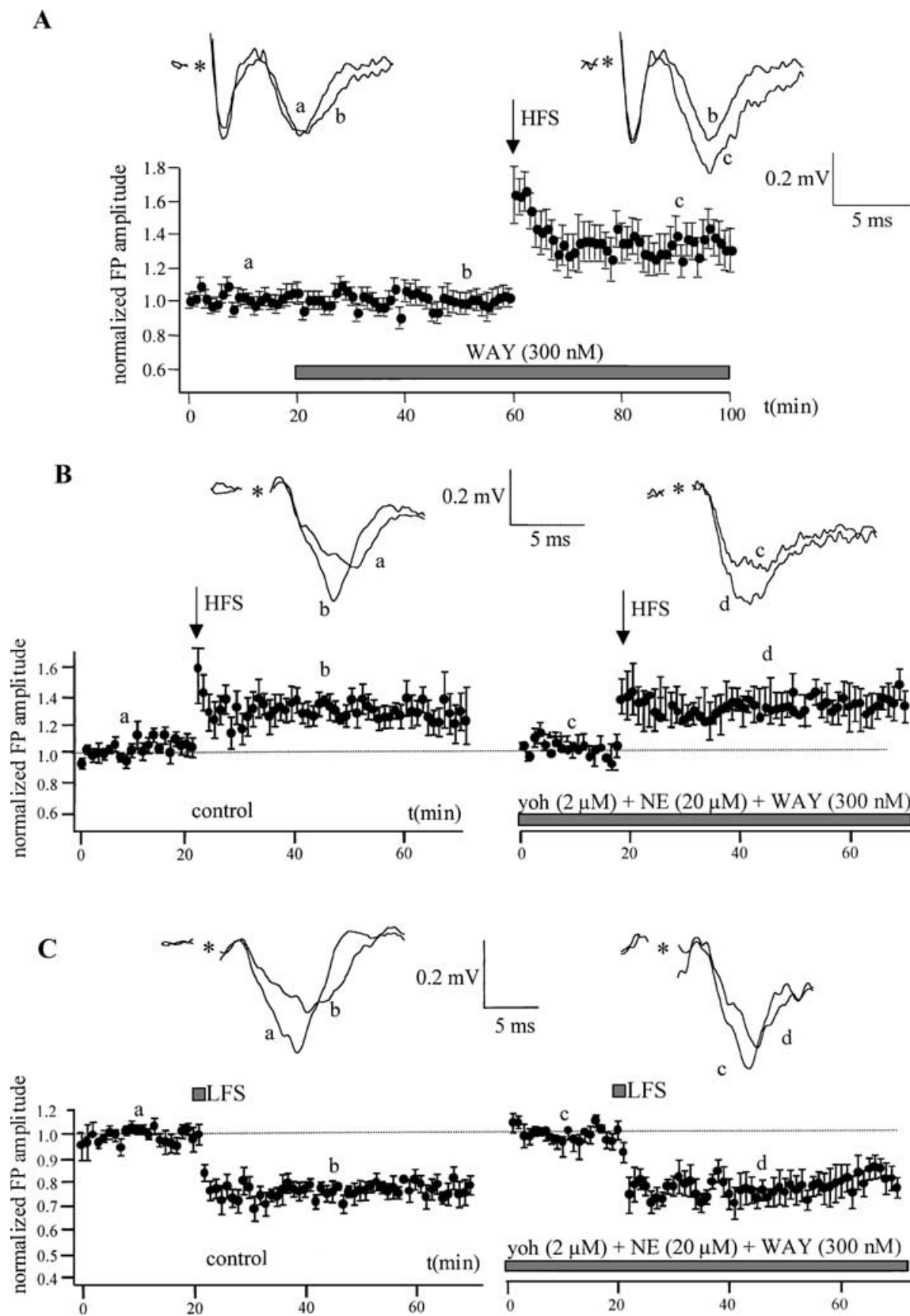


FIG. 4. The effect of NE and clonidine are mediated by α_2 -adrenoreceptor. (A) The 5-HT_{1A} receptor antagonist WAY100635 (300 nM) neither affects basal synaptic transmission nor LTP induction. (B) The α_2 -adrenoreceptor antagonist yohimbine (Yoh; 2 μ M) reverses the effect of NE. (left) Control LTP and (right) LTP in the presence of NE, yohimbine and the specific 5-HT_{1A} receptor antagonist WAY100635 (300 nM). Under these conditions, HFS induces LTP and potentiates FP amplitude to $133.6 \pm 14.4\%$ ($n = 7$). (C) In yohimbine, NE and WAY100635 (300 nM), LFS produces reliable LTD and reduces the amplitude of the synaptic responses to $78.3 \pm 4.6\%$ ($n = 7$, $P < 0.05$; right). Insets show representative traces.

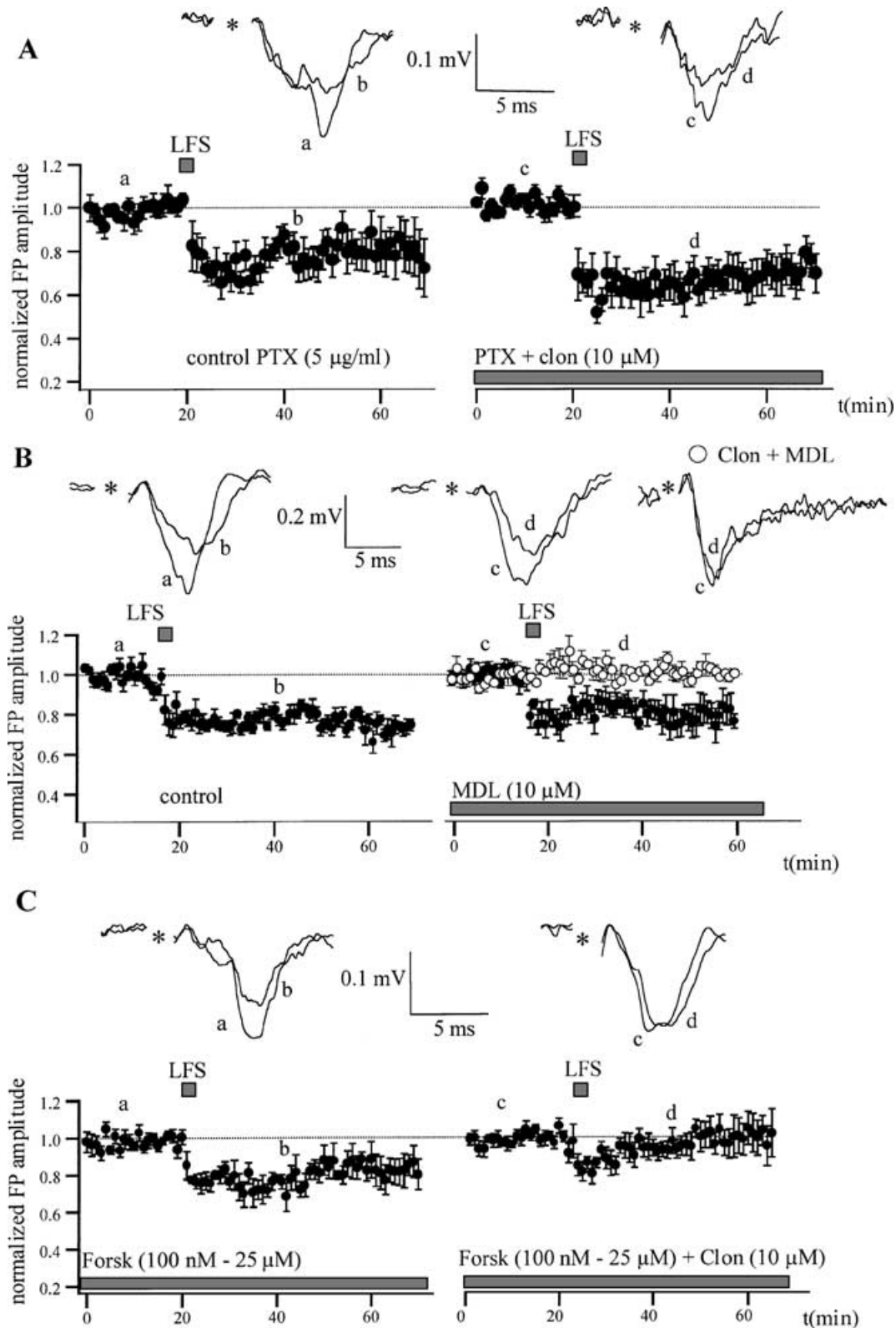


FIG. 5. (A) Activation of $G_{i/o}$ -proteins is a prerequisite for the α_2 -adrenoreceptor-mediated effect on inhibition of LTD. (left) In PTX-treated slices (5 µg/mL, 4–8 h at 37 °C), neither FPs nor LFS-induced LTD are altered as compared with untreated slices (FPs were reduced to $81.0 \pm 7.6\%$; $n = 8$). (right) Pre-incubation with PTX reverses the effect of clonidine on LTD induction (FP reduction to $69.7 \pm 8.7\%$; $n = 8$). (B) Activation of AC is not required for the induction of LTD *per se*. (left) In control, LFS reduces FP amplitude to $78.4 \pm 6.0\%$ ($n = 7$). (right) After the application of the specific AC inhibitor MDL 12,330A (10 µM), LFS causes LTD and reduces FP amplitude to $80.4 \pm 6.1\%$ ($n = 7$). Adenylate cyclase is not involved in the α_2 -adrenoreceptor-mediated inhibitory effect on LTD. In the presence of MDL 12,330A (10 µM), clonidine blocks the induction of LTD (FP amplitude remains stable at $102.9 \pm 2.6\%$, $n = 7$; right, open circles). (C, left) The AC activator forskolin (25 µM) does not occlude LTD (LFS reduced FP amplitude to $76.9 \pm 4\%$; $n = 7$; left). In the presence of clonidine (10 µM), forskolin does not reverse the inhibition of LTD, and after 30 min FP amplitude returns to control levels ($100.2 \pm 10.3\%$; $n = 7$; right). Insets show representative traces.

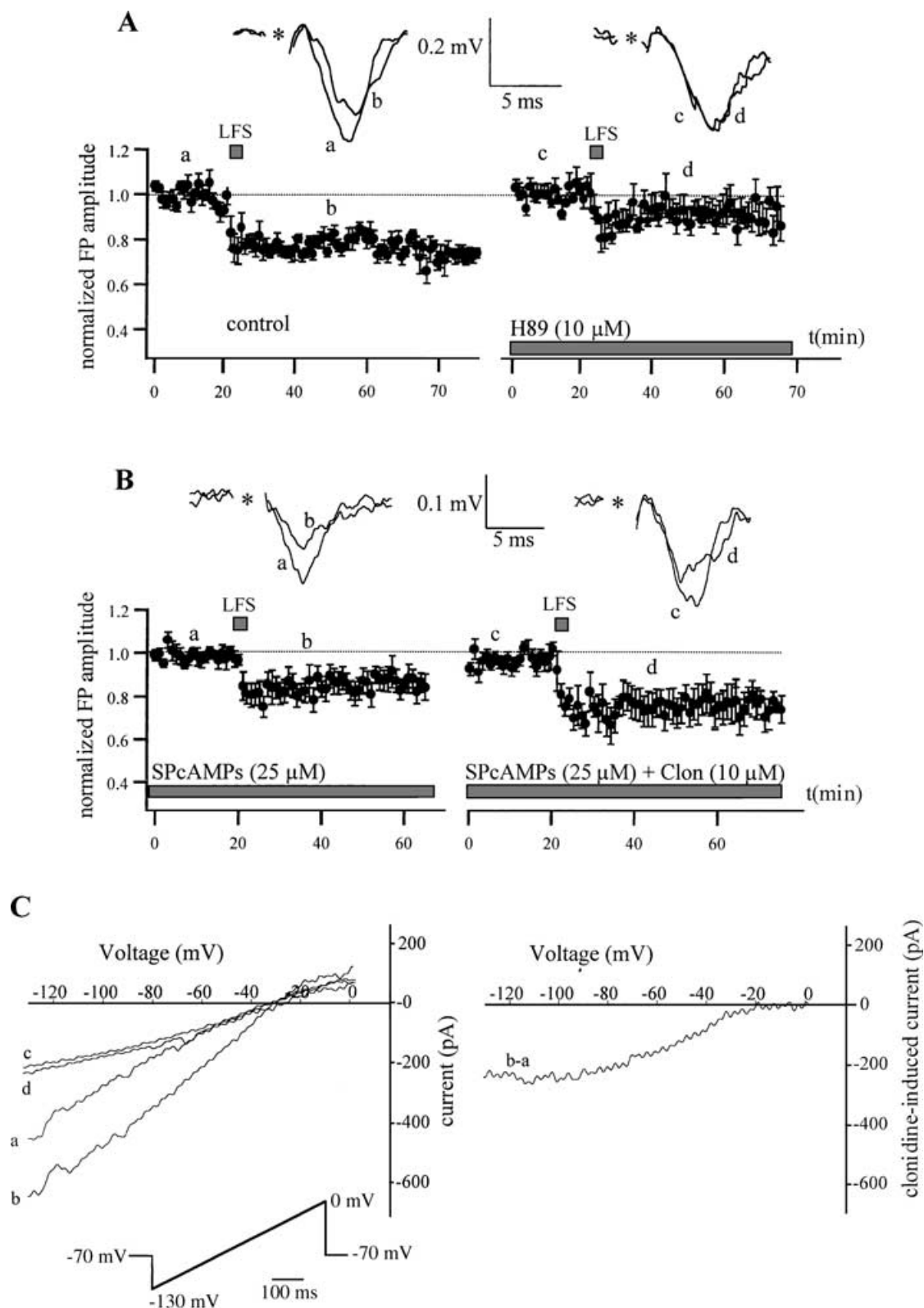


FIG. 6. PKA inhibition is involved in the clonidine-mediated effect on LTD. (A) Activation of PKA is necessary for LFS-induced LTD. (right) When slices were perfused with H89 (10 μ M), LTD expression is significantly reduced ($88.9 \pm 5.9\%$; $n = 7$). (left) In control, LFS decreases FP amplitude to $75.4 \pm 1.2\%$ ($P < 0.05$, $n = 7$). (B, left) Superfusion of the PKA activator SPcAMPs (25 μ M) does not affect the induction of LTD (FP reduction to $86.3 \pm 4.4\%$, $n = 8$, $P < 0.05$). (right) In the presence of clonidine, SPcAMPs reverses the effect of α_2 -adrenoreceptor activation, and LFS induces pronounced LTD (FP amplitude is reduced to $77.4 \pm 6.3\%$, $n = 8$, $P < 0.05$; right). Insets show representative traces. (C, left) Current–voltage (I–V) curves of membrane currents elicited by hyperpolarization ramps at time points of control (a), clonidine (b), Ba^{2+} 300 μM (c) and Ba^{2+} 300 μM + clonidine (d). (right) The I–V curve of clonidine-induced current was obtained by subtracting the current in the control from that during exposure to clonidine (b–a). Please note the shift of the reversal potential to more negative values due to the elevated extracellular K^+ concentration.

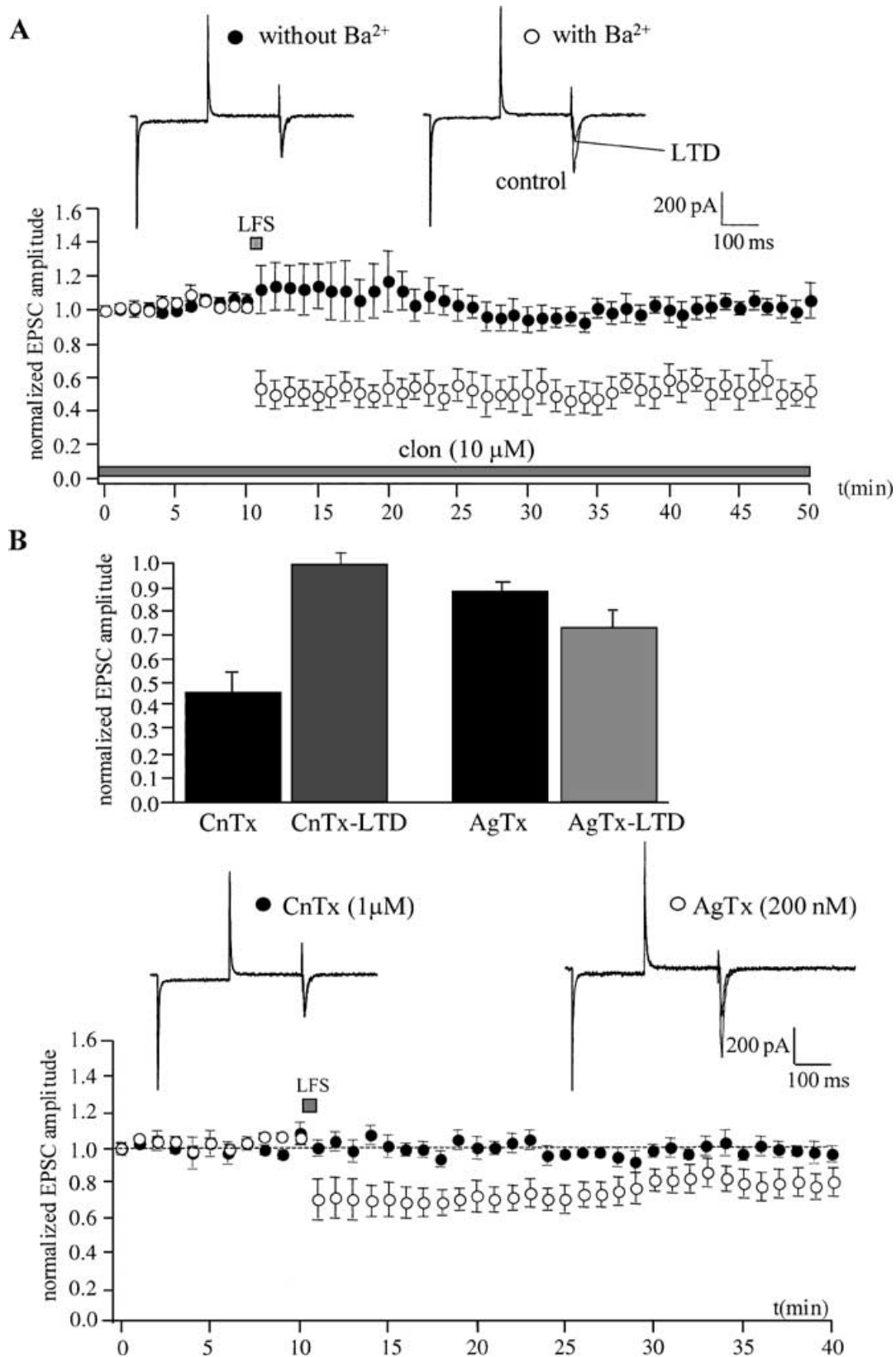


FIG. 7. (A) Activation of α_2 -adrenoreceptors blocks LTD via increasing K_{ir} -channel conductance. α_2 -Adrenoreceptor activation by clonidine blocks LTD (closed circles). This effect is abolished while antagonizing K_{ir} -channels with Ba^{2+} ($300 \mu M$). (B) Antagonizing presynaptic N-type Ca^{2+} -channels blocks the induction of LTD. (top) Application of ω -CnTx ($1 \mu M$) and ω -AgTx ($200 nM$) reduces EPSC amplitude to $45.5 \pm 8.6\%$ and $87.6 \pm 3.4\%$, respectively ($n = 5$; black bars). After the selective blockade of N-type Ca^{2+} -channels, LFS does not induce LTD (grey bar, left; ω -CnTx-LTD). Antagonizing P/Q-type Ca^{2+} -channels with ω -AgTx does not block LTD induction (EPSC amplitude is depressed to $77.7 \pm 7.8\%$; $n = 7$, $P < 0.05$; grey bar, right; ω -AgTx-LTD). (bottom) The same data shown as an amplitude plot. Insets show representative traces.

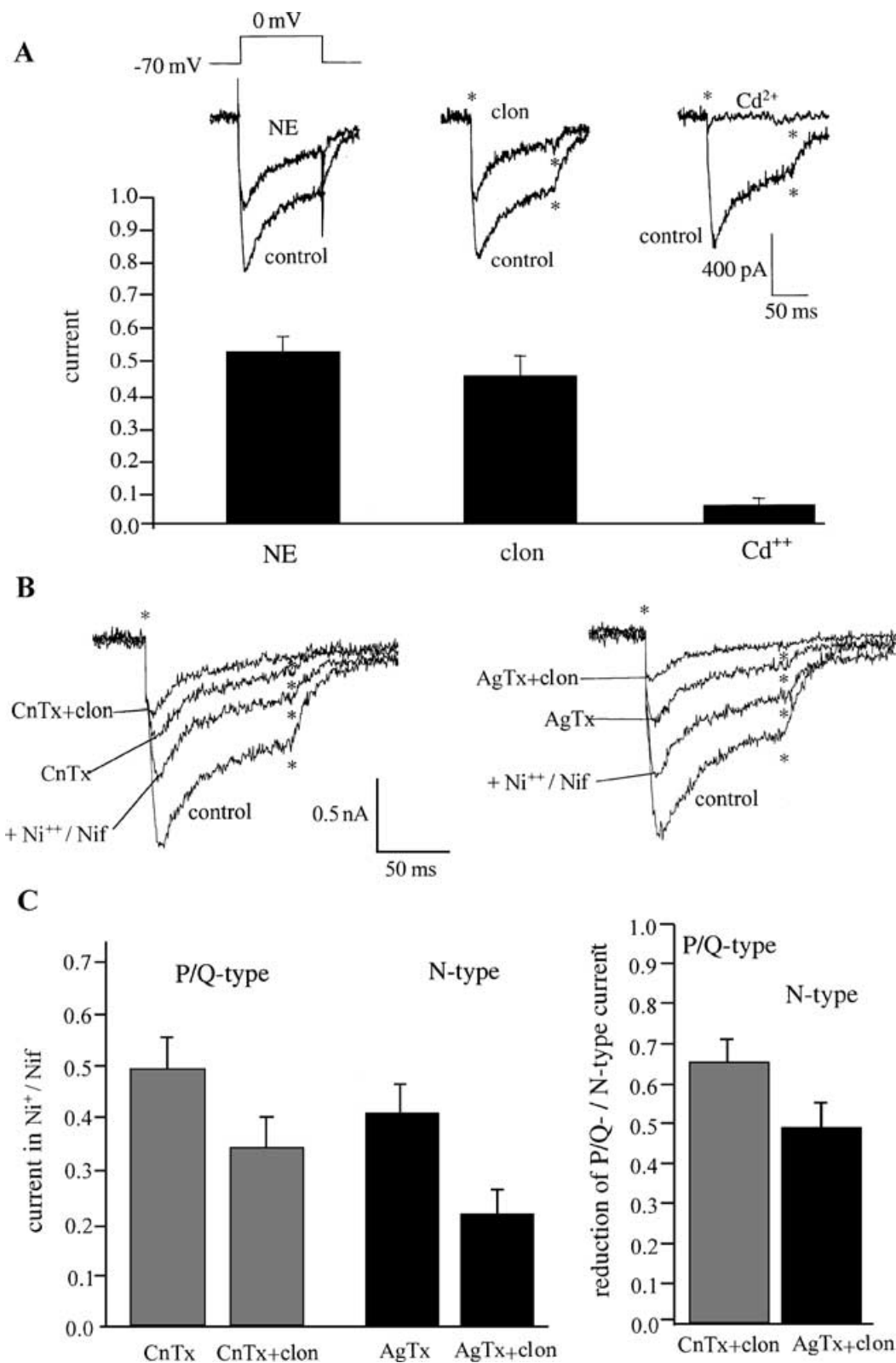


Fig. 8. Activation of α_2 -adrenoreceptors inhibits N- and P/Q-type Ca^{2+} -channels. (A) Ca^{2+} currents are evoked by voltage steps (300 ms) from a holding potential of -70 mV to 0 mV. Both NE ($20 \mu\text{M}$) and clonidine ($10 \mu\text{M}$) reduce evoked Ca^{2+} currents (insensitive to Ni^{2+} and nifedipine) to $52.3 \pm 5.1\%$ ($n = 8$, $P < 0.05$) and $44.3 \pm 6.7\%$ ($n = 8$, $P < 0.05$) of control, respectively. Representative traces of the effects of NE, clonidine and Cd^{2+} are shown. (B) Superimposed traces of evoked VGCC currents obtained in control, in the presence of Ni^{2+} /nifedipine followed by either ω -CnTx or ω -AgTx in the absence or presence of clonidine. (C) P/Q- and N-type currents are isolated by applying ω -CnTx ($1 \mu\text{M}$) and ω -AgTx (200 nM), respectively. (left) ω -CnTx or ω -AgTx reduce the whole-cell currents to $50.1 \pm 5.6\%$ ($n = 8$) and $40.2 \pm 6.2\%$ ($n = 9$), respectively. In the presence of either ω -CnTx or ω -AgTx, clonidine produces a further reduction. (right) To emphasize the effect of clonidine on Ca^{2+} currents, the residual current in either ω -CnTx or ω -AgTx is set to 1.0. Clonidine reduces the P/Q-type Ca^{2+} current to $65.2 \pm 6.2\%$ ($n = 8$, $P < 0.05$) and the N-type Ca^{2+} current to $48.7 \pm 5.5\%$ ($n = 9$, $P < 0.05$).

SPcAMPs (25 μ M). Superfusion with SPcAMPs for 40 min neither affected the expression of LTD (FP reduction to $86.3 \pm 4.4\%$, $n = 8$, $P < 0.05$; Fig. 6B, left), nor FP amplitudes (data not shown). However, SPcAMPs reversed the effect of clonidine-induced α_2 -adrenoreceptor activation. Under these conditions, LFS permitted the induction of a pronounced LTD (FP amplitude was reduced to $77.4 \pm 6.3\%$, $n = 8$, $P < 0.05$; Fig. 6B, right). These results are unexpected, as they suggest that the blocking effect of clonidine on LTD depends on α_2 -adrenoreceptor-regulated PKA activity, but is not mediated via the AC signal cascade.

Activation of α_2 -adrenoreceptors blocks LTD via modulation of K_{ir} channels

Activation of the α_2 -adrenoreceptor can open K_{ir} channels (Evans & Surprenant, 1993). Clonidine increased the membrane current elicited by a hyperpolarization ramp in the BLA in a voltage-dependent manner, i.e. greater at more negative potentials (Fig. 6C, left). At a membrane potential of -100 mV, clonidine increased the membrane current to $158.2 \pm 18.2\%$ ($n = 5$, $P < 0.05$). Resting K_{ir} current was subtracted from that additionally seen in the presence of clonidine (Fig. 6C, right). In the presence of 300μ M Ba^{2+} , clonidine failed to affect the membrane current (Fig. 6C, left). Due to the elevated extracellular K^+ concentration, the current activated has a reversal potential near -35 mV. To test whether the effect of clonidine on LTD is mediated by activating K_{ir} -channels, we used Ba^{2+} (300μ M), which blocks K_{ir} -channels. In the neurons tested, clonidine hyperpolarized the membrane potential from -65.8 ± 2.7 mV to -73.7 ± 2.6 mV, an effect which was reversed by adding Ba^{2+} ions to the superfusion medium ($n = 8$), indicating the involvement of K_{ir} -channels. Ba^{2+} also reversed the clonidine-induced blockade of LTD, and LFS then reduced the EPSC amplitude to $52.1 \pm 9.2\%$ ($n = 7$; Fig. 7A). Ba^{2+} did not reverse the clonidine-induced effect on EPSC amplitude, and Ba^{2+} alone had no effect on LTD induction (data not shown).

Antagonizing N-type VGCCs suppresses LTD

There is evidence that activation of α_2 -adrenoreceptors inhibits presynaptic N- and P/Q-type VGCCs in caudal Raphé neurons (Li & Bayliss, 1998). We tested whether the blockade of these channels with nonoverlapping concentrations of ω -CnTx (1μ M; blocks N-type) and ω -AgTx (200 nM; blocks P/Q-type) could affect the induction of LTD. Application of either ω -CnTx or ω -AgTx had no effect on input resistance or on membrane potential. ω -CnTx or ω -AgTx reduced the amplitude of EPSCs to $45.5 \pm 8.6\%$ and $87.6 \pm 3.4\%$, respectively ($n = 5$, $P < 0.05$; Fig. 7B, top). After a selective blockade of P/Q-type Ca^{2+} -channels by AgTx, LTD could still be induced (EPSC amplitude was depressed to $77.7 \pm 7.8\%$, $n = 7$, $P < 0.05$; Fig. 7B). In contrast, antagonizing N-type Ca^{2+} -channels by ω -CnTx blocked the induction of LTD by LFS (Fig. 7B). These results indicate that the induction of LTD requires a rise of Ca^{2+} through N-type but not through P/Q-type Ca^{2+} -channels.

Activation of α_2 -adrenoreceptors modulates VGCCs

It is still unknown whether α_2 -adrenoreceptor activation inhibits presynaptic N- or P/Q-type Ca^{2+} -channels in the BLA. We examined the effects of NE and clonidine on VGCCs using Ba^{2+} as the charge carrier. We applied Ni^{2+} (50μ M) and nifedipine (20μ M) to block T- and L-type Ca^{2+} -channels, respectively, as activation of T- and L-type Ca^{2+} -channels does not play a role in modulation of LTD in the BLA (Rammes *et al.*, 2000). The Ca^{2+} current, remaining after the T- and L-type Ca^{2+} -channels were blocked, was $46.8 \pm 2.2\%$ of the total Ca^{2+} -current (Fig. 8B). For the following analysis, this value was set to 100%. Step depolarization of 300 ms duration from a holding potential

of -70 mV evoked a whole-cell VGCC current which was reduced by NE (20μ M) or clonidine (10μ M) within 1–2 min after drug application to $52.3 \pm 5.1\%$ ($n = 8$) and $44.3 \pm 6.7\%$ ($n = 8$) of control (both $P < 0.05$), respectively (Fig. 8A). To assess the contribution of N- and P/Q-type Ca^{2+} -channels to the effect of clonidine, we used saturating, but nonoverlapping, concentrations of ω -CnTx (1μ M) and ω -AgTx (200 nM; Wheeler *et al.*, 1994; Li & Bayliss, 1998; Sidach & Mintz, 2000). Figure 8B shows the superimposed traces of evoked VGCC currents obtained in control, in the presence of Ni^{2+} /nifedipine followed by either ω -CnTx or ω -AgTx in the absence or presence of clonidine. ω -CnTx and ω -AgTx reduced whole-cell currents to $50.1 \pm 5.6\%$ ($n = 8$) and $40.2 \pm 6.2\%$ ($n = 9$), respectively (Fig. 8C, left). Applying ω -CnTx and ω -AgTx together reduced the current to $14.3 \pm 2.9\%$ (data not shown). Clonidine reduced the current in the presence of either ω -CnTx or ω -AgTx and produced a further reduction to $65.2 \pm 6.2\%$ ($n = 8$, $P < 0.05$) and $48.7 \pm 5.5\%$ ($n = 9$), respectively (Fig. 8C, right; residual current either in ω -CnTx or ω -AgTx was set to 100%). When the N-type Ca^{2+} current was blocked by ω -CnTx, clonidine inhibited approximately 35% of the remaining current, and after blocking P/Q-type Ca^{2+} current with ω -AgTx, clonidine reduced approximately 51% of the residual current. These results indicate that α_2 -adrenoreceptor activation can reduce Ca^{2+} entry through N-type and P/Q-type Ca^{2+} -channels.

Discussion

The enhancement of learning and memory triggered by the release of stress hormones and NE after emotional arousal involves neuronal circuits in the BLA (McGaugh *et al.*, 1996). The aim of the present study was to investigate the influence of NE on synaptic plasticity in this limbic structure. We used the combined application of extra- and intracellular recordings. There is no difference in the pharmacology of LTP/LTD in the BLA, when recording either FPs or EPSCs (Rammes *et al.*, 2001; see also Huang & Kandel, 1998). Interestingly, NE blocked both LTP and LTD in the BLA, suggesting that they share common mechanisms despite their functionally opposite effects on measured excitability. It remains to be shown whether intracellular Ca^{2+} ions, which activate kinases as well as phosphatases (Lisman, 1994; Otani & Connor, 1998) and evoke bi-directional effects on calmodulin (DeMaria *et al.*, 2001), trigger these opposite effects. A recently suggested biophysical model of synaptic plasticity supports the notion of such a bi-directional effect (Castellani *et al.*, 2001).

The effects of NE on LTP and LTD were mimicked by the α_2 -adrenoreceptor agonist clonidine. Furthermore, the α_2 -adrenoreceptor antagonist yohimbine blocked the effects of NE on synaptic plasticity. Although clonidine is not a selective α_2 -adrenoreceptor agonist, these data speak in favour of an α_2 -adrenergic mechanism. α_1 -Adrenoreceptor- or imidazole receptor-dependent pathways, which might be additionally activated by clonidine, are obviously not involved in LTD suppression. Imidazole receptor activation even stimulates hippocampal LTD (Bolshakov & Siegelbaum, 1995; Normandin *et al.*, 1996), an effect which could be due to a phospholipase A2-dependent rise of intracellular arachidonic acid.

Presynaptic neurotransmitter release is controlled by N- and P/Q-type Ca^{2+} -channels (Wheeler *et al.*, 1994). In the present study, both NE and clonidine inhibited N- and P/Q-type Ca^{2+} -channels, however, with clonidine being slightly less effective on the amplitudes of EPSCs than NE. This may be explained by the fact that the opening of K_{ir} -channels by α_2 -adrenoreceptor activation alone triggers a robust hyperpolarization, which augments the driving force for Na^+ ions and might counteract the N- and P/Q-type Ca^{2+} -channel-mediated reduction of synaptic transmission under clonidine. NE activates α_1 ,

α_2 - and β -adrenoreceptors. The fact that noradrenaline reduced EPSCs by 60% suggests a rather minor contribution of β -adrenoreceptors. Because α_1 -activation slightly reduces EPSP amplitude (Ferry *et al.*, 1997), it may explain why the simultaneous activation of α_1 - and α_2 -adrenoreceptors by NE reduces EPSC amplitude more effectively than the selective activation of α_2 -adrenoreceptors by clonidine. However, both noradrenaline and clonidine activate presynaptic α_2 -adrenoreceptors and thereby induce a similar increase of PPF.

LTD could be induced in the presence of clonidine, when α_2 -adrenoreceptor signal transduction was disrupted by PTX pretreatment. $G_{i/o}$ -proteins are coupled to the opening of K_{ir} -channels as well as the blockade of voltage-gated Ca^{2+} -channels (Clapham & Neer, 1997; Garcia *et al.*, 1998). Clonidine produces a robust hyperpolarization of BLA neurons. Previous studies have attributed this hyperpolarization to the opening of K_{ir} -channels (Kavanaugh *et al.*, 1991; Wickham *et al.*, 1994). In fact, clonidine opens K_{ir} -channels in BLA neurons. Furthermore, in the presence of Ba^{2+} , which blocks K_{ir} -channels, LTD could be induced while α_2 -adrenoreceptors are activated. These findings suggest that $G_{i/o}$ -protein-coupled K_{ir} -channel opening is a possible mechanism by which α_2 -adrenoreceptor activation inhibits LTD.

In a previous study (Rammes *et al.*, 2001), we found that bath-applied BAPTA-AM, but not intracellularly applied BAPTA (1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid), blocked LFS-induced LTD in the BLA. These results suggest that elevation of presynaptic, but not postsynaptic, calcium is a prerequisite for LTD induction, which is compatible with the view that LTD in the BLA is modulated primarily at presynaptic sites via α_2 -adrenoreceptor activation (Starke, 2001). Such a mechanism enables a neuron to modulate the firing patterns of its afferents (Abbott *et al.*, 1997). In caudal Raphe neurons, activation of α_2 -adrenoreceptors causes a reduction of Ca^{2+} currents (Li & Bayliss, 1998), especially of Ca^{2+} influx through N- and P/Q-type Ca^{2+} -channels. Their role seems to be crucial for the induction of LTD, as presynaptic Ca^{2+} influx links transmitter release to depolarization of the membrane (Cummings *et al.*, 1996; Lee *et al.*, 2000), thereby modulating synaptic strength (DeMaria *et al.*, 2001). In the present study, we tested the importance of presynaptic N- and P/Q-type Ca^{2+} -channels for the induction of LTD. Acute modulation of P/Q-type Ca^{2+} -channels may contribute to synaptic plasticity (Borst & Sakmann, 1998; Forsythe *et al.*, 1998) by enriching the neural computational capabilities of the synapse (Abbott *et al.*, 1997; Tsodyks & Markram, 1997). In our study, antagonizing N-type Ca^{2+} -channels suppressed LTD, whereas antagonizing P/Q-type Ca^{2+} -channels had no effect. In previous studies, it has been shown that neither antagonizing L-type Ca^{2+} -channels nor T-type Ca^{2+} -channels affected LTD (Norris *et al.*, 1998; Wang *et al.*, 1999; Rammes *et al.*, 2000). The EPSC amplitude proved clearly less dependent on P/Q-type than N-type Ca^{2+} -channel activation, a finding which reflects a smaller fraction of P/Q-type Ca^{2+} -channels on presynaptic terminals. The reduction of Ca^{2+} currents by NE via a $G_{i/o}$ -protein link can thus be seen as another pathway by which α_2 -adrenoreceptors can inhibit LTD.

$G_{i/o}$ -proteins are also coupled to the inhibition of AC, which in turn regulates PKA activity. Unexpectedly, neither activation nor inactivation of AC affected the clonidine-induced suppression of LTD, whereas the activation of cAMP-dependent PKA reversed the inhibitory effect of clonidine on LTD. These findings suggest that the α_2 -adrenoreceptor-dependent suppression of LTD does not rely on AC but on PKA. Similar evidence for a cAMP-independent PKA signalling via activation of G-protein-coupled receptors has also been provided by investigating other signal transduction pathways. It has been found that corticotropin-releasing hormone, vasoactive peptides and the α -subunit of G13 protein can interact with PKA in a cAMP-independent

manner (Kuryshv *et al.*, 1995; Dulin *et al.*, 2001; Niu *et al.*, 2001). Concerning the α_2 -adrenoreceptor-dependent signalling, further investigations are necessary to elucidate these contradictory results.

Various reports stress the importance of PKA and a Ca^{2+} -dependent equilibrium of kinase and phosphatase activity in memory consolidation and synaptic plasticity (Lisman, 1994; Abel *et al.*, 1997; Bevilacqua *et al.*, 1997). In the present study, the activation of cAMP-dependent PKA reversed the inhibitory effect of clonidine on LTD. Blocking PKA impeded the expression of LTD. It has been reported that PKA inhibits K^+ -channels and activates Ca^{2+} -channels by direct phosphorylation (Riedel *et al.*, 1999), counteracting $G_{i/o}$ -protein activity. Thus, a reduced PKA phosphorylation of K^+ -channels and Ca^{2+} -channels after α_2 -adrenoreceptor activation might block LTD. There is evidence that the phosphorylation of GABA_A receptor subunits by PKA (Moss *et al.*, 1995; Poisbeau *et al.*, 1999) decreases GABAergic activity and, thus, could impair LTD induction (Rammes *et al.*, 2001). Furthermore, it has been shown that the G_S -protein-coupled β -adrenoreceptor couples to $G_{i/o}$ -proteins following phosphorylation (Daaka *et al.*, 1997). Because the compound used in the present study to block PKA also inhibits cGMP-dependent protein kinase (cGK; Burkhardt *et al.*, 2000), it is still possible that induction of LTD depends at least partly also on cGK activation.

Both NE and clonidine reduced synaptic transmission and blocked synaptic plasticity in the BLA. Ba^{2+} did not reverse the effect on synaptic transmission, but reversed the clonidine-induced inhibition of LTD. These results suggest that there is no direct correlation between a K_{ir} -channel-induced reduction in amplitude and inhibition of synaptic plasticity. However, after antagonizing presynaptic N-type Ca^{2+} -channels, there seems to be such a correlation, as blocking these channels reduces EPSC amplitude and inhibits LTD. These results are supported by previous data (Rammes *et al.*, 2001), showing that only buffering of presynaptic Ca^{2+} with BAPTA-AM blocked LTD. On the other hand, antagonizing GABA_A receptors, which led to an increase in EPSC, also inhibited LTD (Rammes *et al.*, 2001). Thus, both mechanisms which cause a decrease and an increase in baseline synaptic transmission can impair the induction of LTD, indicating that this effect is not merely secondary to changes in basal synaptic strength.

In summary, our findings demonstrate that the induction of LTD in the BLA requires Ca^{2+} influx through N-type Ca^{2+} -channels, which are presumably located on the presynaptic site. Furthermore, the present data suggest that activation of α_2 -adrenoreceptors blocks LTD via $G_{i/o}$ -protein-mediated inhibition of N-type Ca^{2+} -channels as well as activation of K_{ir} s.

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Abbreviations

5-HT_{1A} receptor, serotonin type 1A receptor; AC, adenylate cyclase; aCSF, artificial cerebrospinal fluid; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetra-acetic acid; BLA, basolateral amygdala; cGK, cGMP-dependent protein kinase; EC, external capsule; EPSC, excitatory postsynaptic current; FP, field potential; HFS, high-frequency stimulation; ISI, interstimulus interval; K_{ir} , inwardly-rectifying K^+ -channels; LA, lateral nucleus of the amygdala; LFS, low-frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; NE, norepinephrine; PKA, phosphokinase A; PPF, paired-pulse facilitation; PTX, pertussis toxin; SEM, standard error of the mean; STP, short-term potentiation; TEA, tetraethylammonium; TTX, tetrodotoxin; VGCCs, voltage-gated calcium channels; ω -AgTx, ω -agatoxin; ω -CnTx, ω -conotoxin.

References

- Abbott, L.F., Varela, J.A., Kamal, S. & Nelson, S.B. (1997) Synaptic depression and cortical gain control. *Science*, **275**, 220–224.
- Abel, T., Nguyen, P.V., Barad, M., Deuel, T.A., Kandel, E.R. & Bourchouladze, R. (1997) Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. *Cell*, **88**, 615–626.
- Bevilaqua, L., Ardenghi, P., Schroder, N., Bromberg, E., Schmitz, P.K., Schaeffer, E., Quevedo, J., Bianchin, M., Walz, R., Medina, J.H. & Izquierdo, I. (1997) Drugs acting upon the cyclic adenosine monophosphate/protein kinase A signalling pathway modulate memory consolidation when given late after training into rat hippocampus but not amygdala. *Behav. Pharmacol.*, **8**, 331–338.
- Bolshakov, V.Y. & Siegelbaum, S.A. (1995) Postsynaptic induction and presynaptic expression of hippocampal long-term depression. *Science*, **264**, 1148–1152.
- Borst, J.G. & Sakmann, B. (1998) Facilitation of presynaptic calcium currents in the rat brainstem. *J. Physiol. (Lond.)*, **513**, 149–155.
- Burkhardt, M., Glazova, M., Gambaryan, S., Vollkommer, T., Butt, E., Bader, B., Heermeier, K., Lincoln, T.M., Walter, U. & Palmethofer, A. (2000) KT5823 inhibits cGMP-dependent protein kinase activity in vitro but not in intact human platelets and rat mesangial cells. *J. Biol. Chem.*, **275**, 33536–33541.
- Bylund, D.B. & Snyder, S.H. (1976) Beta-adrenergic receptor binding in membrane preparations from mammalian brain. *Mol. Pharmacol.*, **12**, 568–580.
- Castellani, G.C., Quinlan, E.M., Cooper, L.N. & Shouval, H.Z. (2001) A biophysical model of bidirectional synaptic plasticity: dependence on AMPA and NMDA receptors. *Proc. Natl Acad. Sci. USA*, **98**, 12772–12777.
- Clapham, D.E. & Neer, E.J. (1997) G-protein beta gamma subunits. *Ann. Rev. Pharmacol. Toxicol.*, **37**, 167–203.
- Cummings, J.A., Mulkey, R.M., Nicoll, R.A. & Malenka, R.C. (1996) Ca²⁺ signalling requirements for long-term depression in the hippocampus. *Neuron*, **16**, 825–833.
- Daaka, Y., Luttrell, L.M. & Lefkowitz, R.J. (1997) Switching of the coupling of the beta2-adrenergic receptor to different G-proteins by protein kinase A. *Nature*, **390**, 88–91.
- Davis, M., Rainnie, D. & Cassel, M. (1994) Neurotransmission in the rat amygdala related to fear and anxiety. *Trends Neurosci.*, **17**, 208–214.
- DeMaria, C.D., Soong, T.W., Alseikhan, B.A., Alvania, R.S. & Yue, D.T. (2001) Calmodulin bifurcates the local Ca²⁺ signal that modulates P/Q-type Ca²⁺-channels. *Nature*, **411**, 484–489.
- Dodt, H.U., Eder, M., Schierloh, A. & Zieglgänsberger, W. (2002) Infrared-guided laser stimulation of neurons in brain slices. *Science online*.
- Dulin, N.O., Niu, J., Browning, D.D., Ye R.D. & Voyno-Yasenetskaya, T. (2001) Cyclic AMP-independent activation of protein kinase A by vasoactive peptides. *J. Biol. Chem.*, **276**, 20827–20830.
- Evans, R.J. & Surprenant, A. (1993) Effects of phospholipase A2 inhibitors on coupling of alpha 2-adrenoceptors to inwardly-rectifying potassium currents in guinea pig submucosal neurones. *Br. J. Pharmacol.*, **110**, 591–596.
- Fallon, J. & Loughlin, S. (1987) Monoamine innervation of cerebral cortex and a theory of the role of monoamines in cerebral cortex and basal ganglia. In Jones, E.G. & Peters, A. (Eds), *Cerebral Cortex*, Vol. 6. Further aspects of Cortical Function, including Hippocampus. Plenum Press, New York, pp. 41–109.
- Ferry, B., Magistretti, P.J. & Pralong, E. (1997) Noradrenaline modulates glutamate-mediated neurotransmission in the rat basolateral amygdala in vitro. *Eur. J. Neurosci.*, **9**, 1356–1364.
- Ferry, B., Roozendaal, B. & McGaugh, J.L. (1999) Basolateral amygdala noradrenergic influences on memory storage are mediated by an interaction between beta- and alpha1-adrenoceptors. *J. Neurosci.*, **19**, 5119–5123.
- Forsythe, I.D., Tsujimoto, T., Barnes-Davies, M., Cuttle, M.F. & Takahashi, T. (1998) Inactivation of presynaptic calcium current contributes to synaptic depression at a fast central synapse. *Neuron*, **20**, 797–807.
- Freedman, J.E. & Aghajanian, G.K. (1985) Opiate and alpha2-adrenoceptor responses of rat amygdaloid neurons: co-localization and interactions during withdrawal. *J. Neurosci.*, **5**, 3016–3024.
- Galvez, R., Mesches, M.H. & McGaugh, J.L. (1996) Norepinephrine release in the amygdala in response to footshock stimulation. *Neurobiol. Learn. Mem.*, **66**, 253–257.
- Garcia, E.G., Li, B., Garcia-Ferreiro, R.E., Hernandez-Ochoa, E.O., Yan, K., Gautam, N., Catterall, W.A., Mackie, K. & Hille, B. (1998) G-protein β -subunit specificity in the fast membrane-delimited inhibition of Ca²⁺ channels. *J. Neurosci.*, **18**, 9163–9170.
- Hardmann, J.G., Limbird, L.R., Molinoff, P.B., Ruddon, R.W. & Gilman, A.G. (1996) Catecholamines and sympathomimetic drugs: endogenous catecholamines. In Hardmann, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W. & Gilman, A.G. (eds), *The Pharmacological Basis of Therapeutics*. (9th edn). McGraw-Hill, New York, pp. 204–248.
- Huang, Y.Y. & Kandel, E.R. (1998) Postsynaptic induction and PKA-dependent expression of LTP in the lateral amygdala. *Neuron*, **21**, 169–178.
- Kavanaugh, M.P., Christie, M.J., Osborne, P.B., Busch, A.E., Shen, K.Z., Wu, Y.N., Seeburg, P.H., Adelman, J.P. & North, R.A. (1991) Transmitter regulation of voltage-dependent K⁺-channels expressed in *Xenopus* oocytes. *Biochem. J.*, **277**, 899–902.
- Kuryshv, Y.A., Childs, G.V. & Ritchie, A.K. (1995) Corticotropin-releasing hormone stimulation of Ca²⁺ entry in corticotropes is partially dependent on protein kinase A. *Endocrinology*, **136**, 3925–3935.
- Lee, A., Scheuer, T. & Catterall, W.A. (2000) Ca²⁺-calmodulin-dependent facilitation and inactivation of P/Q-type Ca²⁺-channels. *J. Neurosci.*, **20**, 6830–6838.
- Li, Y.W. & Bayliss, D.A. (1998) Activation of alpha2 receptors causes inhibition of calcium channels but does not modulate inwardly-rectifying K⁺-channels in caudal raphe neurons. *J. Neurosci.*, **82**, 753–765.
- Lippe, C. & Ardizzone, C. (1991) Actions of vasopressin and isoprenaline on the ionic transport across the isolated frog skin in the presence and the absence of adenylyl cyclase inhibitors MDL12330A and SQ22536. *Comp. Biochem. Physiol. C*, **99**, 209–211.
- Lisman, J. (1994) The CaM kinase II hypothesis for the storage of synaptic memory. *Trends Neurosci.*, **17**, 406–412.
- Maren, S. & Fanselow, M.S. (1996) The amygdala and fear conditioning: has the nut been cracked? *Neuron*, **16**, 237–240.
- McGaugh, J.L., Cahill, L. & Roozendaal, B. (1996) Involvement of the amygdala in memory storage: interaction with other brain systems. *Proc. Natl Acad. Sci. USA*, **93**, 13508–13514.
- McKernan, M.G. & Shinnick-Gallagher, P. (1997) Fear conditioning induces a lasting potentiation of synaptic currents in vitro. *Nature*, **390**, 607–611.
- Moss, S.J., Gorrie, G.H., Amato, A. & Smart, T.G. (1995) Modulation of GABA_A receptors by tyrosine phosphorylation. *Nature*, **377**, 344–348.
- Niu, J., Vaiskunaitė, R., Suzuki, N., Kozasa, T., Carr, D.W., Dulin, N. & Voyno-Yasenetskaya, T.A. (2001) Interaction of heterotrimeric G13 protein with an A-kinase-anchoring protein 110 (AKAP110) mediates cAMP-independent PKA activation. *Curr. Biol.*, **11**, 1686–1690.
- Normandin, M., Gagne, J., Bernard, J., Elie, R., Miceli, D., Baudry, M. & Massicotte, G. (1996) Involvement of the 1,2-lipoxygenase pathway of arachidonic acid metabolism in homosynaptic long-term depression of the rat hippocampus. *Brain Res.*, **730**, 40–46.
- Norris, C.M., Halpain, S. & Foster, T.C. (1998) Reversal of age-related alterations in synaptic plasticity by blockade of 1-type Ca²⁺-channels. *J. Neurosci.*, **18**, 3171–3179.
- Otani, S. & Connor, J.A. (1998) Requirement of rapid Ca²⁺ entry and synaptic activation of metabotropic glutamate receptors for the induction of long-term depression in adult rat hippocampus. *J. Physiol. (Lond.)*, **511**, 761–770.
- Pitkanen, A., Savander, V. & LeDoux, J.E. (1997) Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. *Trends Neurosci.*, **21**, 240–246.
- Poisbeau, P., Cheney, M.C., Browning, M.D., Mody, I. (1999) Modulation of synaptic GABA_A receptor function by PKA and PKC in adult hippocampal neurons. *J. Neurosci.*, **19**, 674–683.
- Rammes, G., Eder, M., Dodt, H.U., Kochs, E., Zieglgänsberger, W. (2001) Long-term depression in the basolateral amygdala of the mouse involves the activation of interneurons. *Neuroscience*, **107**, 85–97.
- Rammes, G., Steckler, T., Kresse, A., Schutz, G. & Zieglgänsberger, W. (2000) Synaptic plasticity in the basolateral amygdala in transgenic mice expressing dominant-negative cAMP response element-binding protein (CREB) in the forebrain. *Eur. J. Neurosci.*, **12**, 2534–2546.
- Riedel, G., Micheau, J., Lam, A.G., Roloff, E.V., Martin, S.J., Bridge, H., Hoz, L.D., Poeschel, B., McCulloch, J. & Morris, R.G. (1999) Reversible neural inactivation reveals hippocampal participation in several memory processes. *Nature Neurosci.*, **2**, 898–905.
- Rogan, M.T. & LeDoux, J.E. (1996) Emotion: systems, cells, synaptic plasticity. *Cell*, **85**, 469–475.
- Roozendaal, B., Quirarte, G.L. & McGaugh, J.L. (1997) Stress-activated hormonal systems and the regulation of memory storage. *Ann. NY Acad. Sci.*, **821**, 247–258.
- Salinas, J.A., Introini-Collison, I.B., Dalmaz, C. & McGaugh, J.L. (1997) Post-training intraamygdala infusions of oxotremorine and propranolol modulate storage of memory for reductions in reward magnitude. *Neurobiol. Learn. Mem.*, **68**, 51–59.

- Saunders, C. & Limbird, L.E. (1999) Localization and trafficking of alpha2-adrenergic receptor subtypes in cells and tissues. *Pharmacol. Therapeutics*, **84**, 193–205.
- Shouse, M.N., Bier, M., Langer, J., Alcalde, O., Richkind, M. & Szymusiak, R. (1994) The alpha2-agonist clonidine suppresses seizures, whereas the alpha2-antagonist idazoxan promotes seizures – a microinfusion study in amygdala-kindled kittens. *Brain Res.*, **648**, 352–356.
- Sidach, S.S. & Mintz, I.M. (2000) Low-affinity blockade of neuronal N-type Ca²⁺-channels by the spider toxin omega-agatoxin-IVA. *J. Neurosci.*, **20**, 7174–7182.
- Starke, K. (2001) Presynaptic autoreceptors in the third decade: focus on α_2 -adrenoceptors. *Neurochemistry*, **78**, 685–693.
- Swandulla, D. & Misgeld, U. (1990) Development and properties of synaptic mechanisms in a network of rat hypothalamic neurons grown in culture. *J. Neurophysiol.*, **64**, 715–726.
- Swanson, L.W. & Petrovich, G.D. (1998) What is the amygdala? *Trends Neurosci.*, **21**, 323–331.
- Tsodyks, M.V. & Markram, H. (1997) The neural code between neocortical pyramidal neurons depends on neurotransmitter release probability. *Proc. Natl Acad. Sci. USA*, **94**, 719–723.
- Wang, S.J., Cheng, L.L. & Gean, P.W. (1999) Cross-modulation of the synaptic plasticity by β -adrenergic and 5-HT_{1A} receptors in the rat basolateral amygdala. *J. Neurosci.*, **19**, 570–577.
- Wheeler, D.B., Randall, A. & Tsien, G. (1994) Roles of N-type and P/Q-type Ca²⁺-channels in supporting hippocampal synaptic transmission. *Science*, **264**, 107–111.
- Wickham, K.D., Iniguez-Lluhl, J.A., Davenport, P.A., Taussig, R., Krapivinsky, G.B., Linder, M.E., Gilman, A.G. & Clapham, D.E. (1994) Recombinant G-protein beta gamma-subunits activate the muscarinic-gated atrial potassium channel. *Nature*, **368**, 255–257.
- Zilles, K., Qu, M. & Schleicher, A. (1993) Regional distribution and heterogeneity of alpha-adrenoceptors in the rat and human central nervous system. *J. Hirnforschung*, **34**, 123–132.