A temporal perspective on stress hormones and memory
Pu, Z.

Citation for published version (APA):
CHAPTER IV

Interactive effects of corticosterone and isoproterenol on synaptic plasticity in the rat basolateral amygdala in vitro

Zhenwei Pu, Harm J. Krugers, Marian Joëls

SILS-CNS, University of Amsterdam, The Netherlands

An abbreviated version of this chapter was published in Learning & Memory, 2009; 16:155-160; under the title – “β-adrenergic facilitation of synaptic plasticity in the rat basolateral amygdala in vitro is gradually reversed by corticosterone”.

The basolateral amygdala plays a significant role in emotional learning. Earlier behavioral studies have indicated noradrenaline and corticosterone as important modulators of this process. In the present study, we examined the effectiveness of corticosterone and the $\beta$-adrenergic agonist isoproterenol in modulating, separately or by interaction, synaptic plasticity occurring in the basolateral amygdala induced by high-frequency stimulation of lateral amygdala fibers. Isoproterenol (1 $\mu$M) markedly enhanced synaptic plasticity induced by a relatively weak (theta-burst) stimulation paradigm but was ineffective when applied in conjunction with stronger stimulation protocols. Brief application of corticosterone just before and during high-frequency stimulation – thus focusing on nongenomic actions of the hormone – did not markedly affect synaptic plasticity, nor did it rapidly alter the modulatory action of isoproterenol. However, two observations support a delayed suppressive effect of corticosterone on the facilitatory action by isoproterenol. First, when corticosterone was given at the same time as isoproterenol (i.e. just before and during theta-burst stimulation), the facilitatory effect of isoproterenol was gradually reversed. Second, when corticosterone was applied for 20 minutes several hours in advance of isoproterenol, the facilitatory effect of the $\beta$-adrenergic agonist was entirely suppressed. These data suggest that for the lateral to basolateral amygdala pathway, $\beta$-adrenergic rather than glucocorticoid influences promote synaptic plasticity within the short term, especially when synaptic strengthening is relatively weak. The $\beta$-adrenergic facilitatory action may be gradually normalized through a presumably gene-mediated modulation by corticosterone, thus preventing the basolateral amygdala network from overshooting after stress.

Introduction

Information that strongly evokes emotional responses is generally much better retained than less significant events. The amygdala is crucially involved in the modulation of emotional memory (Cahill and McGaugh, 1998; LeDoux, 2000; McGaugh, 2004; Richter-Levin, 2004), as clearly demonstrated in the well-established animal model of fear-conditioning (LeDoux et al., 1990; Romanski et al., 1993; Rogan et al., 1997; Nader et al., 2001; Blair et al., 2003). In addition, it was proposed that the amygdala – more precisely, the basolateral nucleus (BLA) – can modulate memory-related processes in other brain regions, e.g. the hippocampus (McGaugh et al., 1996; Kim and Diamond, 2002; Pare, 2003; Richter-Levin and Akirav, 2003; Roozendaal, 2003; Richter-Levin, 2004; Roozendaal et al., 2006b); thus, memory traces constructed in the hippocampus that are “emotionally tagged” are rendered the competitive advantage for retention (Richter-Levin and Akirav, 2003; Diamond et al., 2005).

Within the BLA, multiple neuromodulatory systems exert their influences on memory, including, notably, the noradrenergic system and the glucocorticoid system (Roozendaal, 2003; Roozendaal et al., 2006b). Interestingly, these systems are necessary for the amygdala-mediated modulation of activity-dependent synaptic plasticity in the hippocampal dentate gyrus (Vouimba et al., 2007). The adrenal hormones are rapidly secreted into the circulation when an organism confronts an acute stress response. This rapid release can activate the glucocorticoid system, resulting in the rapid release of corticosterone, which can then interact with the amygdala to modulate synaptic plasticity.
stressful situation to which negatively emotional arousal is collateral. While glucocorticoids can readily cross the brain-blood barrier, adrenaline stimulates the noradrenaline release within the central nervous system (Roozendaal, 2003). Glucocorticoids and noradrenaline bind to their respective receptors in the brain, including the BLA. Animal behavioral studies suggested that noradrenergic activity within the BLA plays a central role in mediating a memory-enhancing effect, while glucocorticoid receptor (GR) activation may exert a “permissive” function (Roozendaal et al., 2002; Roozendaal et al., 2006a). However, experiments in which the two hormones were not given concurrently showed that glucocorticoids may otherwise have a suppressive impact on the noradrenergic effect (Borrell et al., 1984). This suggests that the interactive hormonal functions affecting the memory systems are not always uniform.

Support for this non-uniformity was recently obtained at the neuronal network level, in the hippocampal dentate gyrus (Pu et al., 2007). Corticosterone time-dependently modulated noradrenergic action on long-term potentiation (LTP) – which is the best-described neurobiological substrate of learning and memory to date (Goosens and Maren, 2002; Martin and Morris, 2002; Morris, 2003). Thus, in the dentate gyrus, β-adrenergic activation could facilitate the induction of LTP by high-frequency stimulation (HFS). If corticosterone was co-applied with a β-adrenergic agonist and temporally linked to the occurrence of HFS, the β-adrenergic-mediated facilitation was further enhanced during the initial stage of LTP. However, if corticosterone was transiently applied several hours before HFS – allowing gene-mediated effects to develop, it prevented the β-adrenergic effect on LTP (Pu et al., 2007). Therefore, in the dentate gyrus, corticosterone can bidirectionally modify the capability of the noradrenergic system in regulating synaptic plasticity.

In view of the behavioral observations that β-adrenoceptor agonists and glucocorticoids both affect the memory processes involving the BLA (Roozendaal et al., 2002), we here investigated the time-dependent hormonal interactions in this region. LTP was induced in the lateral-to-basolateral amygdala (LA-BLA) pathway with three different levels of tetanization: strong, moderate and weak. We were particularly interested in identifying: 1) β-adrenergic effects on amygdala LTP induced by different stimulation paradigms; and 2) the effect of corticosterone on amygdala LTP or on β-adrenergic modulation of amygdala LTP, either when corticosterone was co-applied with the β-adrenergic agonist (isoproterenol) around tetanization, or was applied more than 2 hours in advance of isoproterenol.

Materials and Methods

**Animals**

The Animal Committee for Bioethics of University of Amsterdam approved all of the experiments. Male Wistar rats (Harlan CPB, the Netherlands) were housed in groups, with food and water ad libitum available. A 12 hr :12 hour light-dark cycle (light-on at 8.00 a.m.) was maintained; the temperature kept at 20 – 22 °C, and the humidity at 55 ± 15 %. After arrival, the rats were not disturbed for at least one week before experiments started. The body weights ranged between 200 g and 300 g at the time of experiment.
**In vitro slice preparation**

The animals were decapitated early in the morning, between 9:30 and 10:30 hours – when plasma corticosterone levels are low. The brain was rapidly removed from the skull and immersed in chilled (4 °C) dissection buffer which consisted of 120 mM NaCl, 3.5 mM KCl, 5.0 mM MgSO\(_4\cdot7\)H\(_2\)O, 1.25 mM NaH\(_2\)PO\(_4\), 0.2 mM CaCl\(_2\cdot2\)H\(_2\)O, 10 mM glucose and 25 mM NaHCO\(_3\); oxygenated with 95 % O\(_2\) and 5 % CO\(_2\). 500 μm-thick coronal slices containing the basolateral complex of the amygdala were made with a vibroslicer (Leica VT1000S, Germany). Slices were then kept in artificial cerebrospinal fluid (aCSF) consisting of 120 mM NaCl, 3.5 mM KCl, 1.3 mM MgSO\(_4\cdot7\)H\(_2\)O, 1.25 mM NaH\(_2\)PO\(_4\), 2.5 mM CaCl\(_2\cdot2\)H\(_2\)O, 10 mM glucose and 25 mM NaHCO\(_3\); oxygenated with 95 % O\(_2\) and 5 % CO\(_2\). Slices remained in aCSF for at least 1 hour before being transferred to the recording chamber.

**Electrophysiology and protocols**

One slice at a time was transferred to the recording chamber, where the temperature was maintained at 30 – 32 °C. For field potential recordings in the BLA, a bipolar stimulation electrode (60 μm in diameter, stainless steel, insulated except for the tip) was placed in the lateral amygdala to stimulate the LA-BLA pathway (Rammes et al., 2000; DeBock et al., 2003; Schimanski and Nguyen, 2005; Huang and Kandel, 2007). Field potential signals were recorded with a glass microelectrode (impedance 2 – 5 MΩ, filled with aCSF). The stimulus intensity was adjusted to evoke a synaptic response at around the half value of the maximal amplitude, and this intensity was applied throughout the experiment. The amplitudes were measured by projecting the negative peak of the field potential onto a plotted line between the onset of the signal and the most positive point after signal decay (Figure 1A). The LA-BLA pathway was stimulated four times each minute, and four consecutive responses were averaged to represent the mean value for each minute.

Baseline synaptic transmission was monitored for 10 minutes, followed by the perfusion of either: 1) corticosterone (Sigma-Aldrich, 100 nM); 2) the β-adrenergic agonist, (-)-isoproterenol (+)-bitartrate (Sigma-Aldrich, 1.0 μM); 3) a combination of 1.0 μM (-)-isoproterenol (+)-bitartrate and 100 nM corticosterone; or 4) vehicle solution, as a control, into aCSF for 15 minutes. All perfusions co-terminated with a tetanic stimulation (see below), after which synaptic responses were monitored for another 60 minutes.

In order to reveal the delayed effect of corticosterone, part of the slices were preincubated with 100 nM corticosterone at 32 °C for 20 minutes, starting one hour after decapitation. After a resting period of no less than two hours (thus allowing the full development of the late effect), in one half of these slices, baseline transmission was monitored for 10 minutes, followed by a 15-minute period of perfusion with 1.0 μM (-)-isoproterenol (+)-bitartrate; this co-terminated with high-frequency stimulation, and synaptic responses were further observed for 60 minutes. In another half of the slices, baseline transmission was monitored for 25 minutes under the control condition, followed by high-frequency stimulation; afterwards, synaptic responses were monitored for 60 minutes.
To induce LTP, the following high-frequency stimulation protocols were used: 1) 5 trains of 100 Hz pulses, each train lasting 1 sec, with an inter-train interval of 10s (Rammes et al., 2000); 2) 1 train of 100 Hz pulses, lasting for 1 sec (Rammes et al., 2000; DeBock et al., 2003); 3) theta-burst stimulation (TBS): a burst of 4 pulses at 100 Hz, repeated 200 msec later by another 4 pulses at 100 Hz; this sequence was repeated 5 times, with an inter-train interval of 30 sec (Alfarez et al., 2003; Wiegert et al., 2005).

Data analysis
Synaptic potentiation after tetanus was expressed as percentage change from the baseline; the average of the measurements during the 25 minute pre-tetanus period served as the baseline value. A two-tailed, paired Student’s t-test was used to compare synaptic responses before versus after high-frequency stimulation within each group. The general linear model for repeated measures (GLM) was performed for between-group comparisons of overall difference in LTP, followed by post hoc least significant difference (LSD) multiple comparison tests. Between-group comparisons were performed for 1) the entire 60 minutes post-tetanus period; 2) the final part of the post-tetanus period (50’ – 60’); and 3) in certain cases, the initial post-tetanus phase (0’ – 10’). All data are expressed as average ± S.E.M.; n indicates the number of animals. P-value < 0.05 was accepted as significantly different.

Results
Identification of field potential signals in the BLA
The field potential signal that was recorded in the LA-BLA pathway was characterized by a negative-going waveform with a constant latency of 2 – 3 msec (Figure 1A). It could follow 100 Hz stimulation without failure (Figure 1B). The signals disappeared in the presence of the AMPA receptor antagonist CNQX (10 μM) and were restored after the antagonist was washed away (Figure 1C). Therefore, the field potentials could be identified as AMPA-receptor mediated, monosynaptic responses, comparable to signals described in literature (Rammes et al., 2000; Huang and Kandel, 2007).

In the presence of vehicle medium, three protocols were tested for their abilities to induce LTP in the LA-BLA pathway: 1) 5 x (100 Hz x 1s), the strongest one; 2) 1 x (100 Hz x 1s), the intermediate one; and 3) theta-burst stimulation (TBS), the weakest protocol (see Materials and Methods for details). LTP was effectively induced by the strongest and intermediate protocols (Figure 2A and B); over 60 minutes after tetanus, the degree of potentiation amounted to 152 ± 9 % (mean amplitude ± S.E.M., normalized to the pre-tetanus baseline, n = 5) and 153 ± 10 % (n = 5) respectively, both of which indicated a significant increase from their baseline values (both P < 0.01). Accordingly, during the final 10 minutes of the recording period (50’ – 60’, post-tetanus), strong and significant potentiation was observed in both cases (156 ± 13 %, P < 0.05 and 157 ± 14 %, P < 0.05, respectively). The weakest protocol that we applied, i.e. TBS, did not result in stable LTP (Figure 2C). The potentiation after tetanus only amounted to 113 ± 9 % (n = 5) over 60 minutes and 108 ± 10 % over the last 10 minutes (both P > 0.05).
A temporal perspective on stress hormones and memory

Figure 1 Field potential signals as induced in the LA-BLA pathway that represent a monosynaptic, AMPA-mediated response. (A) Positioning of the stimulating electrode and the recording electrode at their respective sites within the lateral and basolateral amygdala. The magnitude of the recorded field potential signal was measured by projecting the most negative point of the negative-going signal onto a line that connected the onset of the signal and the most positive point during its decay phase. The depicted signal represents an average of the responses to four consecutive stimuli. (B) This signal could follow multiple pulses of stimulation at 100 Hz without failure. (This trace specifically displays a not-averaged signal). (C) The signal was abolished in the presence of the AMPA-receptor antagonist CNQX (10 μM), but restored when the drug was cleared away from the medium. Signals represent averaged responses to four consecutive stimuli. The asterisk indicates where the stimulus artifact appeared.
For these 3 protocols, over the entire 60 minute post-tetanus period, there was an overall difference ($F_{(2, 12)} = 5.79, P < 0.05$). Post hoc tests did not indicate any difference between the strongest and intermediate protocols ($P > 0.05$); by contrast, the effect of the weakest protocol: TBS was significantly different from the other two (both $P < 0.05$) (Figure 2D). This suggests that a single train of 100 Hz pulses (for 1 sec) was adequate to produce amygdala LTP in our current setting, which may well achieve a maximal level of potentiation. On the other hand, theta bursts were a subthreshold protocol for LTP induction.

Figure 2 The effect of isoproterenol on synaptic plasticity in the basolateral amygdala with 3 stimulation protocols of different strengths. (A) Strong tetanic stimulation, using 5 trains of 100 Hz for 1 sec, induced stable LTP in the LA-BLA pathway. Administration of isoproterenol (ISO, 1 μM, n = 4) instead of vehicle (VEH, n = 5) did not affect the degree of potentiation. Perfusion with ISO or VEH is indicated by the horizontal grey bar; the tetanus was given at $t = 25$ min (arrow). (B) Similarly, tetanic stimulation with a single train of 100 Hz for 1 sec yielded stable LTP in the LA-BLA pathway under VEH condition (n = 5). Perfusion with ISO (n = 5) resulted in a comparable level of potentiation. (C) With a weak stimulation paradigm (theta-burst stimulation, TBS), only brief post-tetanic potentiation was observed under VEH condition (n = 5). If ISO was perfused just before and during TBS (n = 4), a markedly stable form of synaptic potentiation was achieved. (D) Mean values (± S.E.M.) representing the averaged responses during the final 10 minutes of the post-tetanus recording period. Under VEH conditions (white column), both the strong and intermediate stimulation paradigms resulted in stable and significant potentiation in comparison to the pre-tetanus baselines; and comparable results were found for ISO perfusion conditions (black column). TBS did not induce significant potentiation under the VEH condition, whereas strong potentiation was observed if ISO was rapidly perfused. #, ##: $P < 0.05$, $P < 0.01$, respectively, compared with the individual pre-tetanus baseline. **: $P < 0.01$, compared between the groups.
**Effect of isoproterenol on LTP**

Subsequently, we investigated the effect of the β-adrenergic agonist isoproterenol (1.0 μM) on synaptic plasticity with these 3 protocols. Importantly, the concentration of the agonist was based on the positive effects seen with this concentration in the dentate gyrus (Pu et al., 2007); moreover, this was the lowest dose that yielded reproducible effects on single neuron synaptic responses in the BLA (Liebmann et al., unpublished data). When isoproterenol was applied for 15 minutes before and during the strongest stimulation protocol (5 x (100 Hz x 1s)), long-lasting potentiation was apparent (155 ± 18 % of baseline over 60 minutes, n = 4; 151 ± 15 % over the last 10 minutes, \( P < 0.05 \); Figure 2A). However, there was no difference between the vehicle- and isoproterenol-treated slices (neither over 60 minutes nor the last 10 minutes, both \( P > 0.05 \); Figure 2D).

When the intermediate protocol (1 x (100 Hz x 1s)) was tested in the presence of isoproterenol, a similar pattern was found. After perfusion of isoproterenol and tetanization, a significant level of potentiation was observed (n = 5; 143 ± 8 % of baseline, over 60 minutes and 143 ± 9 % over the last 10 minutes, both \( P < 0.01 \); Figure 2B). Yet, again there was no difference between isoproterenol-treated and vehicle-treated groups (both \( P > 0.05 \), over 60 minutes and the last 10 minutes; Figure 2D).

By contrast, a clear effect of isoproterenol became apparent when the β-adrenergic agonist was administered in conjunction with the weakest protocol, TBS. Thus, when isoproterenol was applied before and during TBS, a significant level of potentiation was observed (n = 4; 161 ± 10 % of baseline over 60 minutes, \( P < 0.01 \), and 166 ± 17 % over the last 10 minutes, \( P < 0.05 \); Figure 2C), which was significantly different from the vehicle condition (over 60 minutes as well as the last 10 minutes, both \( P < 0.01 \); Figure 2D). We conclude that modulation of synaptic plasticity in the LA-BLA pathway by a β-adrenergic agonist is only seen along with a mild degree of synaptic strengthening, and not revealed in a context where synaptic strength is already boosted to the maximum.

**Interactions between isoproterenol and corticosterone**

We next examined whether corticosterone could alter the efficacy of isoproterenol to modulate LTP in the LA-BLA pathway, either in a rapid (thus, nongenomic) or in a delayed (presumably, gene-mediated) way.

Corticosterone perfusion just before and during TBS (i.e. without isoproterenol) resulted in a marginal level of potentiation over 60 minutes after high-frequency stimulation (114 ± 5 %, n = 5, \( P < 0.05 \); Figure 3A). Over time the potentiation became less obvious so that it was no longer significant during the last 10 minutes (111 ± 8 %, \( P = 0.27 \); Figure 3A and C). Interestingly, application of corticosterone in conjunction with a stronger protocol (1 x (100 Hz x 1s)) produced an analogous pattern that indicates a less efficient potentiation later on (data not shown). Hence, while over the entire 60 minutes post-tetanus period the average synaptic response amounted to 126 ± 4 % of the pretetanus value (n = 6, \( P < 0.01 \)), this reached a level of 121 ± 7 % during the last 10 minutes (\( P < 0.05 \)), which resulted in a significant reduction from the level of potentiation as could be induced by this protocol in the control condition (\( P < 0.05 \)).
Figure 3 Modulation by isoproterenol and/or corticosterone of synaptic plasticity in the LA-BLA pathway with theta-burst stimulation. (A) Brief perfusion with ISO lead to stable LTP after TBS (n = 4). When 100 nM corticosterone was perfused together with isoproterenol (ISO + CORT, n = 7), a significant level of LTP was seen over the entire 60 minute post-TBS period; however, it declined during the late stage of potentiation. Perfusion of corticosterone alone (CORT, n = 5) before and during TBS did not result in synaptic potentiation, similar to
VEH (depicted in Figure 2, not shown in this graph). The horizontal grey bar indicates the perfusion period; the arrow points to the moment of tetanus. (B) If corticosterone was briefly (20 min) administered more than 2 hours in advance of isoproterenol and TBS (CORT before ISO, n = 6), any form of LTP attributable to isoproterenol-mediated facilitation was completely lost. Pretreatment with corticosterone alone (CORT preincubation, n = 5) did not result in significant synaptic potentiation, similar to VEH treatment (not shown in this graph). (C) Between-group comparisons (mean + S.E.M.) show that isoproterenol alone enhanced synaptic responses after TBS (the final 10 minutes value of the post-tetanus period). This facilitatory effect of β-adrenergic activation was not further enhanced by concomitant application of corticosterone (through its rapid effect); by contrast, a decrease was noticed during the last 10 minutes of the post-tetanus period. The facilitative effect of β-adrenergic activation was completely prevented if corticosterone was applied more than 2 hours in advance (through its delayed effect). (Veh: white column; ISO: black column; CORT: gradiently grey column; ISO + CORT: full grey column; CORT preincubation: striped white column; CORT before ISO, striped grey column.) #: P < 0.05, compared with the individual pre-tetanus baseline. *, **: P < 0.05, P < 0.01, respectively, compared between the groups.

Next, the ability of corticosterone to alter the facilitatory effects of isoproterenol on TBS was studied. If corticosterone was given in addition to isoproterenol before and during tetanus (Figure 3A), a significant level of LTP was observed during the entire period of 60 minutes (135 ± 10 %, n = 7, P = 0.01); however, for the last 10 minutes, it only reached a trend of significance (126 ± 12 %, P = 0.07; Figure 3A and C). In the between-group comparison we were particularly interested in the initial stage of potentiation, since an earlier experiment in the dentate gyrus revealed rapid interactive actions of corticosterone and isoproterenol in this time window (Pu et al., 2007). For the combined treatment, a significant level of potentiation (142 ± 11 %, P = 0.01) was observed during the first 10 minutes after tetanus. Comparing the co-application, isoproterenol only, corticosterone only and the vehicle-treated group, there was an overall difference during the first 10 minutes after tetanus (F(3,17) = 3.53, P < 0.05); this could be attributed to a difference between corticosterone and isoproterenol or between corticosterone and co-application of the two hormones. Importantly, signals recorded after co-application of corticosterone and isoproterenol did not differ from those seen after isoproterenol application only (P > 0.05).

For the last 10 minutes (50' – 60', post-TBS), there was an overall difference (F(3,17) = 4.09, P < 0.05) between the four groups. Post hoc comparisons between the groups demonstrated significant differences between the isoproterenol-treated and vehicle- (P < 0.01) or corticosterone-treated groups (P < 0.01), and between isoproterenol treatment and co-application of both hormones (P < 0.05) (Figure 3C). Collectively, these results indicate that under the current experimental conditions, corticosterone did not rapidly facilitate noradrenergic action in the BLA. Rather, brief application of corticosterone around the time of TBS gradually diminished the facilitation of synaptic strengthening caused by β-adrenergic activation, implying a delayed suppressive corticosteroid action.

To further examine a putative delayed suppressive effect of corticosterone, slices were incubated for 20 min with corticosterone more than 2 hours in advance of TBS induction of LTP. With such brief preincubation of corticosterone, only a transient post-tetanus potentiation was seen; over 60 minutes the synaptic responses amounted to 109 ± 7 % (n = 5, P > 0.05; Figure 3B), with a declination to 89 ± 9 % (P > 0.05) during the last 10 minutes. If isoproterenol was rapidly perfused to
slices that were previously incubated with corticosterone, the isoproterenol-mediated facilitation of post-TBS signals was not seen (Figure 3B). Under this condition, synaptic responses amounted to $118 \pm 15\%$ ($n = 6$, $P > 0.05$) and $105 \pm 12\%$ ($P > 0.05$) during the first and the last 10 minutes after tetanization respectively, very similar to the baseline values. Comparison during the first 10 minutes after tetanus among the four experimental groups, i.e. slices preincubated with corticosterone, preincubated prior to isoproterenol perfusion, with isoproterenol perfusion only or with vehicle treatment, revealed no overall difference ($F_{(3,16)} = 1.43$, $P > 0.05$) and no between-group differences. However, for the last 10 minutes (50’ – 60’, post-TBS), an overall difference was identified ($F_{(3,16)} = 6.96$, $P < 0.01$). As shown in Figure 3C (50’ – 60’, post-TBS), post hoc analysis among all groups revealed a significant difference between corticosterone preincubation prior to isoproterenol perfusion and isoproterenol perfusion only ($P < 0.01$). Clearly, a delayed, suppressive effect of corticosterone on β-adrenergic facilitation of synaptic plasticity was evident in our study.

**Baseline transmission with the hormones**

All of the above-mentioned differences in LTP between the groups were not driven by any drug-mediated changes in baseline transmission, such as arising from modulation of basal transmission by hormone application alone, irrespective of LTP induction. This was evident from the fact that there was no overall difference with regard to the baseline transmission during perfusion of different hormone(s) (pooled data, including all 3 stimulation protocols: $F_{(4,69)} = 0.16$, $P > 0.05$) and no between-group differences for any individual comparison against the vehicle treatment ($all P > 0.05$) (see Figure 4).

**Figure 4** The baseline neurotransmission was not affected by rapid perfusion of the hormone(s) in any condition. The values of synaptic responses during perfusion were not different from their individual pre-perfusion baselines, nor different from those in another treatment condition or from those during the vehicle perfusion. (Pooled data; Veh: white column, $n = 18$; ISO: black column, $n = 13$; CORT: gradiently grey column, $n = 18$; ISO + CORT: full grey column, $n = 19$; CORT before ISO, striped grey column, $n = 6$.)
Discussion

Aversive situations are generally well remembered (Cahill and McGaugh, 1998; Richter-Levin and Akirav, 2003; Olsson and Phelps, 2007). Behavioral studies have shown that this phenomenon critically depends on noradrenaline- and corticosterone-mediated actions exerted in the BLA (Quirarte et al., 1997). It has been postulated that, in particular, β-adrenergic activation is of paramount importance to the memory of stressful, aversive events (Roozendaal et al., 2006b). Some studies have described the role of corticosteroids as permissive (Roozendaal, 2003; Roozendaal et al., 2006b), although at least one study found that corticosterone may counteract the effect of adrenaline (Borrell et al., 1984). Attempting to unravel this paradox, we earlier performed experiments in the hippocampal dentate gyrus (Pu et al., 2007) in which we tested the hypothesis that the character of the corticosteroid modulation is associated with its mode of action: genomic versus nongenomic. This was based on the finding that corticosterone nongenomically enhances LTP in the CA1 area whereas suppresses LTP through a delayed gene-mediated pathway (Wiegert et al., 2005; Wiegert et al., 2006). Also, time-dependency was found to be crucial for the influence of amygdala activity on dentate gyrus function – activation of the amygdala enhances LTP induction in the dentate gyrus when the two processes are closely temporally linked, but mediates a suppression with longer delays (Akirav and Richter-Levin, 2002; Vouimba and Richter-Levin, 2005); such studies have implied the engagement of the noradrenergic and glucocorticoid systems (Akirav and Richter-Levin, 2002; Vouimba et al., 2007).

In the dentate gyrus, we have observed that corticosterone rapidly, nongenomically accelerates the facilitatory action of isoproterenol on (a weak form of) synaptic plasticity (Pu et al., 2007). Conversely, via a delayed and presumably gene-mediated mechanism, corticosterone suppresses the efficacy of isoproterenol in facilitating this plasticity. Based on these observations, we proposed that the effects of the two hormones synergize when they are present in the dentate gyrus at around the same time (as occurs during and immediately after a stressful event), but in the meanwhile, due to the gene-mediated mechanism, corticosterone suppresses any further activation of noradrenergic pathways for hours to come (Pu et al., 2007).

In view of the significant role of the BLA in memory retention of aversive situations, we here tested if a comparable, time-dependent interaction between corticosterone and isoproterenol also exists in the major afferent pathway to the BLA. We found that β-adrenergic activation facilitates synaptic plasticity, though only in a condition that synaptic strengthening was not yet saturated. There was yet no evidence of a permissive action of corticosterone at the network level within the BLA. However, corticosterone consistently and slowly diminished the facilitatory action of isoproterenol on synaptic strength.

The network function and isoproterenol
The lateral amygdala supplies one of the major afferent pathways to the BLA (Pitkanen et al., 1995; Wang et al., 2002). The AMPA-receptor mediated signals described in the current study in the LA to
BLA pathway are highly consistent with those observed by literature (Rammes et al., 2000; DeBock et al., 2003; Schimanski and Nguyen, 2005). *In vitro*, relatively strong stimulation protocols are necessary to evoke lasting potentiation of the LA-BLA pathway. Thus, LTP was consistently found with the LA being stimulated with five trains of 100 Hz (for 1 sec) (Rammes et al., 2000; DeBock et al., 2003; Schimanski and Nguyen, 2005), but fewer trains only resulted in transient forms of potentiation (Rammes et al., 2000). In our hands, one train of 100 Hz (1 sec) stimulation was sufficient to evoke stable potentiation, which was indistinguishable from the LTP seen with five trains. Contrarily, weaker stimulation by a theta-burst protocol was ineffective to produce stable LTP. It should be realised that the *in vitro* slice preparation lacks tonic excitatory input, which could render this preparation relatively insensitive to synaptic potentiation. This was not unprecedented; for instance, *in vivo* theta-burst stimulation in the entorhinal cortex produces a robust increase in BLA field potentials (Yaniv et al., 2003; Kavushansky and Richter-Levin, 2006), whereas *in vitro* this pathway demands multiple tetanic stimuli to achieve lasting potentiation (Rodriguez Manzanares et al., 2005).

Noradrenaline is known to exert bidirectional actions on the BLA. Generally, inhibitory actions appear to act via α2-adrenergic receptors (Ferry et al., 1997; DeBock et al., 2003; Buffalari and Grace, 2007), whereas β-adrenergic receptors facilitate excitatory transmission and synaptic potentiation in the BLA (Huang et al., 1996; Ferry et al., 1997; Wang et al., 1999) or LA (Huang et al., 2000). Isoproterenol (15 μM) was found to increase AMPA-receptor-mediated responses via presynaptic enhancement of P/Q calcium currents (Huang et al., 1996), while postsynaptically isoproterenol is able to enhance NMDA-receptor-mediated currents (Huang et al., 1998a). At the field potential level, we did not observe significant changes in response to baseline stimulation during the 15-minute perfusion with 1 μM isoproterenol. The lack of effect is probably due to the moderately low concentration of the β-adrenergic agonist used in the present study. It seems likely that the effect of isoproterenol on synaptic potentiation is caused by postsynaptic actions on the NMDA receptors, although we cannot fully exclude the role of putative subthreshold effects of isoproterenol on AMPA-receptor-mediated transmission.

**Modulatory effects of corticosterone**

We did not observe any rapid effects of (100 nM) corticosterone on synaptic responses evoked in the BLA, neither during baseline stimulation nor posterior to high-frequency stimulation. This is in line with a recent study reporting no rapid effects of (100 nM) corticosterone on excitatory postsynaptic potentials evoked in identified BLA principal neurons (Duvarcı and Pare, 2007). It should be taken into account that the period during which corticosterone was applied was relatively short (15 minutes totally). However, it is considered a sufficient length of time to alter the frequency of miniature excitatory synaptic currents in the CA1 area (Karst et al., 2005), but slower kinetic properties of putative “rapid” effects of corticosterone in the BLA cannot be ruled out.

The lack of rapid corticosteroid effects on the facilitatory action by isoproterenol on TBS in the BLA differs from what was observed in the dentate gyrus (Pu et al., 2007). Notably, in the dentate gyrus, isoproterenol mediated a gradually-developing enhancement of synaptic strength, a process that
was accelerated by corticosterone. In the BLA, however, facilitation by isoproterenol was visible immediately after TBS; this left little room for further acceleration by corticosterone to occur. Possibly, the enhancing effects of corticosterone could be revealed if even lower concentrations of isoproterenol were applied; we presently refrained from doing so, because lower concentrations of isoproterenol do not give consistent effects in single cell recordings (Liebmann et al., unpublished data).

With corticosterone applied just before and during a moderate high-frequency stimulation paradigm (1 train of 100 Hz x 1 sec), a gradual declination of potentiation took place, resulting in significantly decreased signals (compared to vehicle treatment) during 50’ – 60’ after the tetanus. With the weakest paradigm, potentiation during the post-TBS 50’ – 60’ period was not significant, as opposed to that of earlier time points; but in view of the marginal potentiation seen with the latter paradigm, these data should be interpreted with extreme care. Notwithstanding, the overall data suggest that corticosterone can modulate synaptic potentiation in the BLA, but only in a particular (i.e. intermediate) range of activity-dependent synaptic plasticity.

The gradually-appearing effect of corticosterone on synaptic potentiation could signify that the hormonal actions were mediated by a slow gene-mediated pathway and/or that a specific late phase of synaptic potentiation was targeted. In the LA and the dentate gyrus, this late phase of LTP was found to depend on protein kinase A and mitogen-activated protein kinase (Huang et al., 2000; Wu et al., 2006), the second messengers that are also influenced by β-adrenergic agonists (Huang et al., 1998a; Huang et al., 1998b; Price et al., 2004). By sharing a common endpoint, acting in an opposite direction, corticosterone could gradually reduce noradrenergic efficacy via β-adrenergic receptors, as exactly shown in this study. However, other gene-mediated pathways by which corticosterone could interfere with the development and maintenance of LTP and the efficacy of β-adrenergic agonists also need to be explored. Presently, the information about corticosteroid actions on identified BLA neurons still remains relatively sparse. At the single cell level, 100 nM corticosterone was reported to slowly attenuate GABAergic neurotransmission and spike frequency accommodation in the BLA (Duvarci and Pare, 2007; Liebmann et al., 2008). As this is expected to promote rather than impair synaptic potentiation, contribution of these phenomena to the currently observed delayed actions by corticosterone seems limited.

Functional implications
The present electrophysiological studies indicate a facilitatory role of noradrenaline in synaptic plasticity via β-adrenergic receptors in the BLA, which is in line with behavioral observations that pinpoint a quintessential function of BLA β-adrenergic receptors in the consolidation of emotionally arousing learning events (Ferry et al., 1999; Roozendaal et al., 2006b). Our findings would suggest that activation of β-adrenergic receptors is particularly effective in relatively weak learning paradigms, but less so with stronger forms of aversive learning.

Under the current experimental conditions we did not find evidence for a permissive role of corticosterone in the BLA, which differs from the effect observed earlier in the dentate gyrus (Pu et al.,
The lack of effect may contrast with behavioral observations (Roozendaal et al., 2002; Roozendaal et al., 2006b). The latter studies, however, used the selective glucocorticoid receptor agonist RU 28362, whereas we administered corticosterone, which has, in addition, a very high affinity for mineralocorticoid receptors. Moreover, we could not, at this stage, exclude the possibility that corticosterone may be effective with a lower degree of \( \beta \)-adrenergic receptor activation.

While corticosterone did not promote the action of isoproterenol, a consistently suppressive effect was observed, developing in a delayed (and presumably gene-mediated) manner. This fits well with the result at a behavioral level, which showed that post-learning administration of adrenaline to adrenalectomized rats facilitated memory retention in a passive avoidance paradigm – a facilitation that was largely impaired by pretreatment with corticosterone (Borrell et al., 1984). Interestingly, we did not only see a gradual suppressive effect when corticosterone was administered several hours in advance of isoproterenol, but also when the two compounds were given simultaneously. As intra-BLA elevations in the levels of noradrenaline and corticosterone are indeed expected to occur within a restricted time window after stress exposure, this strongly argues that noradrenaline, via \( \beta \)-adrenergic receptor system, rapidly promotes activity-dependent synaptic plasticity in the BLA, which is subsequently and gradually normalized by corticosterone. If so, corticosteroids would serve to contain the initial stress response and prevent it from dysfunctional overshooting.