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# Amygdala β-noradrenergic influence on hippocampal long-term potentiation *in vivo*

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**WE have previously shown that hippocampal long-term potentiation (LTP), one form of synaptic plasticity that may underlie learning and memory, is attenuated by blocking neuron activity of the basolateral amygdala (BLA). In the present study we investigated the amygdala noradrenergic or cholinergic contribution to hippocampal LTP formation. When propranolol, a β-adrenoceptor antagonist, was injected into the BLA 10 min before tetanus, the formation of LTP in the perforant path–dentate granule cell synapses was significantly impaired. Scopolamine, a muscarinic cholinergic receptor antagonist, did not affect the formation of LTP. These results suggest that amygdala β-noradrenergic activity plays a critical role in modulation of hippocampal LTP.**

**Key words**: β-Adrenoceptor; Basolateral amygdala; Dentate gyrus; Hippocampus; Long-term potentiation; Muscarinic cholinergic receptor

## Introduction

The amygdala is known to be involved in emotional and motivational aspects of behaviors,[1] and has recently been found to be associated with memory formation.[2,3] In particular, various lines of evidence suggest that the amygdala modulates functions of the hippocampus which are essentially required for some types of memory formation. c-*fos* is expressed in the hippocampus following intraamygdala injection of the excitatory amino acid.[4] Electrophysiological studies have shown that a single-pulse stimulation of the basolateral amygdala (BLA) facilitates synaptic transmission in the perforant path–dentate gyrus (DG) synapses[5] and that high-frequency stimulation of the basal amygdala affects the pattern of electroencephalogram in the hippocampus.[6] Furthermore, we have previously reported that hippocampal long-term potentiation (LTP), one form of synaptic plasticity that may underlie learning and memory,[7] is attenuated by injecting a local anesthetic tetracaine into the BLA[8] and that high-frequency stimulation of the BLA facilitates the induction of LTP.[9] These findings suggest that neuron activity in the BLA affects the formation of hippocampal LTP and support behavioral evidence that the amygdala modulates hippocampal-dependent memory.[10,11] However, it remains unclear which neurotransmitter system in the BLA contributes to the modulation of hippocampal synaptic plasticity. Previous behavioral data[12,13] indicate that the β-noradrenergic and muscarinic cholinergic systems in the amygdala are important for the modulation of memory. In the present study, therefore, we investigated possible contribution of the amygdala β-noradrenergic or cholinergic system to LTP induction in the DG.

## Materials and Methods

Evoked potential was recorded as described previously.[5,8] Briefly, male Wistar rats, 8–9 weeks old, were anesthetized with urethane (1 g/kg, i.p.) and α-chloralose (25 mg/kg, i.p.) and fixed in a stereotaxic frame. The medial perforant path was stimulated, and the evoked potential was extracellularly recorded from the granule cell layer of the DG. Test stimulation was applied at intervals of 30 s and stimulus intensity was set to a level which evoked a population spike of half of the maximum amplitude. All drugs were dissolved in saline and 1 μl of drug solution was injected over 90 s through a stainless steel cylindrical cannula aimed at the BLA (2.8 mm posterior to bregma, 5.2 mm lateral to midline, 7.6 mm ventral to dura)[8,14] or the central amygdala (CeA; 2.8 mm posterior to bregma, 4.2 mm lateral to midline, 6.9 mm ventral to dura). Ten minutes after the drug injection, tetanic stimulation (30 pulses at 60 Hz) was applied to induce LTP. LTP was evaluated by measuring changes in the amplitude of population spike because we have previously observed that lesion or stimulation of the BLA affected LTP of population spikes.[5,8] To check the placement of the injection cannula, the dye trypan blue was injected through the cannula at the end of each experiment.[14] The brain was then quickly

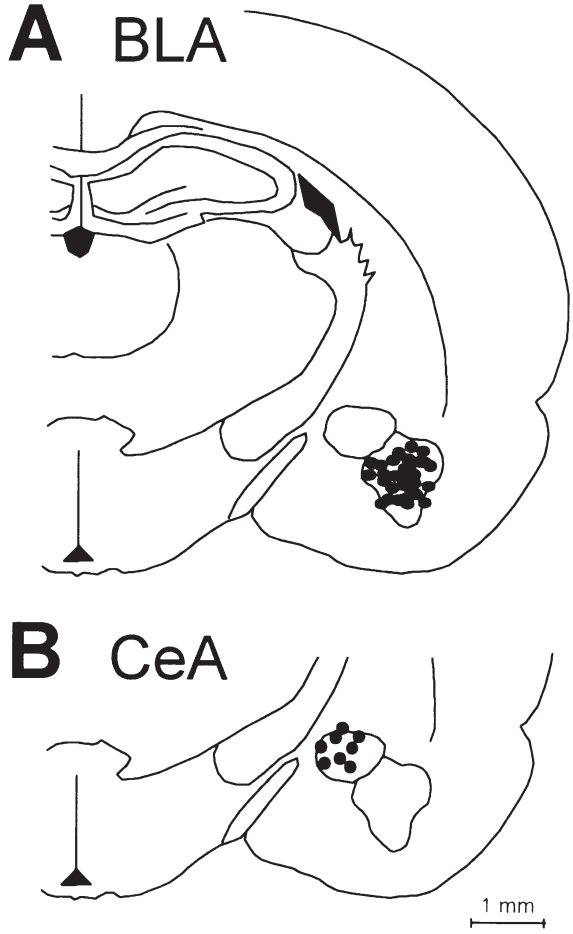


FIG. 1. Schematic drawings of the placement of the injection cannula. After the completion of the experiment, the rats with a cannula implanted into the BLA (**A**, $n = 27$) or the CeA (**B**, $n = 8$) were sacrificed, and each brain was coronally sectioned to verify the spot of trypan blue injected through the cannula. The diagrams show a coronal view of rat brain at a position 2.8 mm posterior to bregma. The centers of the area stained with trypan blue are indicated by black dots.

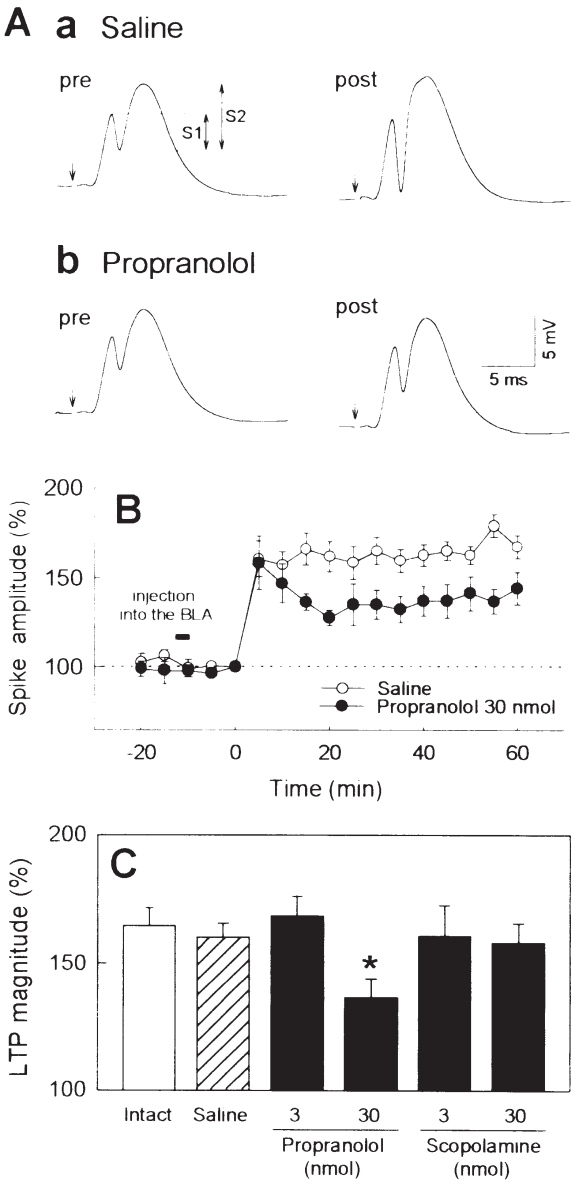


FIG. 2. Effect of propranolol or scopolamine injected into the BLA on the induction of DG LTP. (**A**) Typical evoked potentials recorded from the dentate granule cell layer in the saline-injected group (**a**) or in the propranolol-injected group (**b**). Saline or propranolol (30 nmol) was injected into the BLA 10 min before tetanic stimulation. Left and right traces in each panel show field potentials immediately before (pre) and 60 min after (post) tetanic stimulation, respectively. Test stimulation was delivered at time indicated by arrows. The amplitude of population spike was defined as the average of the amplitude from the first positive peak to the succeeding negative peak (S1) and the amplitude from the negative peak to the second positive peak (S2), i.c. (S1 + S2)/2. (**B**) Time-course of DG LTP induced by tetanic stimulation (30 pulses at 60 Hz) in the saline-injected group (○, $n = 6$) and in the propranolol-injected group (●, $n = 5$). Saline or propranolol (30 nmol) was injected into the ipsilateral BLA during the time indicated by black bars (12.5–10 min prior to tetanic stimulation) and tetanic stimulation was applied at time 0 min. Amplitude of population spike is expressed as a percentage of baseline value immediately before tetanic stimulation. (**C**) Dose-dependent effect of propranolol or scopolamine injected into the BLA on the magnitude of LTP. Intact group did not receive the cannula implantation. The average of percentage amplitude of population spikes 30–60 min after tetanic stimulation was calculated to compare the magnitude of LTP in each group. All data are represented as the means ± s.e.m. of 5–6 animals. Asterisks indicate significant difference from the saline-injected group: $*p < 0.05$; Duncan's multiple range test following analysis of variance.

removed, frozen and sectioned coronally at 14 μm with a freezing microtome. Histological examination confirmed that the drugs were injected precisely into the intended amygdaloid nuclei in all rats tested (Fig. 1). All efforts were made for the care and use of animals according to the Guideline for Animal Experiment of the Faculty of Pharmaceutical Sciences, the University of Tokyo.

## Results

In the intact or saline-injected group, the population spike in the DG was potentiated following tetanic stimulation, and robust LTP was induced in all cases tested. When 30 nmol propranolol was injected into the BLA, there was no change in baseline DG synaptic responses before tetanic stimulation, but the magnitude of DG LTP was significantly reduced

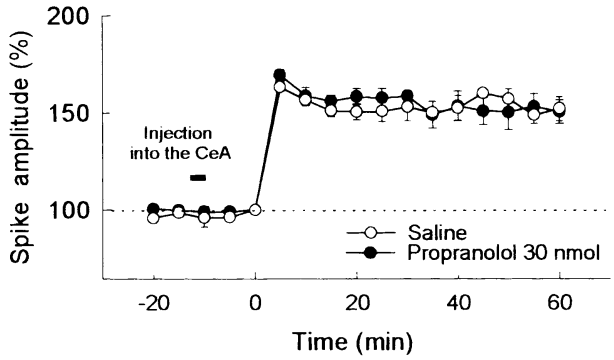


FIG. 3. Effect of propranolol injected into the CeA on the induction of DG LTP. Saline (○, $n = 4$) or 30 mnol propranolol (●, $n = 4$) was injected into the ipsilateral CeA during the time indicated by black bars. Data are the means ± s.e.m.

(Fig. 2A,B). The LTP-blocking effect of propranolol injected into the BLA was dose dependent (Fig. 2C). On the other hand, injection of 3 or 30 nmol scopolamine into the BLA did not affect the induction of DG LTP (Fig. 2C).

To determine whether propranolol injected into the BLA specifically affected the BLA neurons, we also examined the effect of propranolol injected into the CeA, the amygdaloid nucleus adjacent to the BLA. However, it did not significantly affect the induction of DG LTP (Fig. 3).

## Discussion

We have shown for the first time that injection of propranolol into the BLA significantly attenuates DG LTP without affecting basal synaptic responses. The effect of propranolol observed here is undoubtedly due to the blockade of β-noradrenergic activity in the BLA for the following reasons. First, we have previously confirmed that drugs injected by our procedure hardly diffuse and act within the BLA.[8,14] Second, when propranolol was injected into the CeA, which is adjacent to the BLA, DG LTP was not significantly affected. Finally, we used propranolol at the doses that have been reported to selectively block β-adrenoceptors *in vivo*.[15,16] This result clearly suggests, therefore, that β-noradrenergic activity in the BLA specifically modulates the mechanisms of LTP induction in the DG.

We observed previously that DG LTP was attenuated by lesion of the BLA but not by lesion of the CeA, suggesting that the amygdaloid subnuclei contribute differentially to hippocampal LTP.[17] Our present finding that DG LTP was attenuated by propranolol injection into the BLA but not into the CeA supports the major role of the BLA in modulating hippocampal LTP.

The critical role of amygdala β-noradrenergic system in modulation of memory formation has been demonstrated by numerous behavioral and pharmacological studies. It has been reported that behavioral effects of various memory effectors (e.g. glucocorticoid, opioid, GABA, etc.) are mediated by the activation of amygdala β-noradrenergic system.[3] Our physiological finding supports earlier behavioral data and provides a valuable model for studying the cellular basis of amygdala β-noradrenergic influences on memory processes.

The cellular mechanism by which amygdala β-noradrenergic activity contributes to the formation of DG LTP is not clear only from our present data, but several previous reports have provided useful information. First, Huang *et al.* reported that isoproterenol, a β-adrenoceptor agonist, potentiates NMDA receptor-mediated glutamatergic synaptic transmission in the BLA.[18] Second, the glutamatergic synapses of the amygdala display LTP[19–21] and the induction of LTP in the BLA requires the activation of NMDA receptors.[20] Third, we have previously observed that local injection of NMDA receptor antagonists into the BLA results in the attenuation of DG LTP.[14] Based on these data, we propose the mechanism underlying amygdala β-noradrenergic influences on DG LTP as follows: β-noradrenergic activity potentiates NMDA receptor-mediated glutamatergic synaptic transmission and thereby induces synaptic enhancement in the BLA; and the enhanced amygdala neuron activity facilitates the induction of hippocampal LTP. To prove this hypothesis, further investigations are underway in our laboratory.

Unlike propranolol, 3 or 30 nmol scopolamine injected into the BLA did not affect the induction of LTP in the DG. Because these doses of scopolamine are high enough to block muscarinic cholinergic receptors,[22,23] the result suggests that amygdala muscarinic system does not contribute to the formation of DG LTP. This finding seems to be contrary to several behavioral and pharmacological studies suggesting that the noradrenergic effect on memory involves subsequent muscarinic cholinergic activation in the amygdala.[3,13] However, the amygdala modulates not only hippocampal-dependent memory but also other types of memory. For example, the amygdala modulates memory by interacting with the caudate nucleus.[10,24] Therefore, our data rather suggest that amygdala muscarinic system contributes to memory processes in other brain regions than the hippocampus.

## Conclusion

Memory enhancement associated with aversive emotional arousal such as anxiety, fear and panic, is

required for animals to survive enemies or dangers. Our present study shows that the induction of hippocampal LTP is facilitated by amygdala noradrenergic activity, which is known to increase following the aversive emotional drive.[25] Our finding may, therefore, provide electrophysiological evidence for the hypothesis that emotional arousal-evoked activation of the amygdala modulates memory processing in the hippocampus.

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**General Summary**
The amygdala is considered to be involved in neuromodulatory influences on memory storage as well as emotional and motivational aspects of behaviors. Hippocampal long-term potentiation (LTP) is one form of synaptic plasticity that may underlie learning and memory. Here we report that hippocampal LTP was significantly attenuated by local injection of propranolol into the basolateral amygdala (BLA), suggesting that β-noradrenergic activity in the BLA plays a critical role in modulation of hippocampal function. Because the amygdala noradrenergic activity is known to increase following the emotional fear drive, our finding may provide electrophysiological evidence for the hypothesis that emotional arousal activation of the amygdala results in modulation of hippocampal-dependent memory processes.