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**RAPID PUBLICATION**

# Medial Amygdala Enhances Synaptic Transmission and Synaptic Plasticity in the Dentate Gyrus of Rats in Vivo

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## SUMMARY AND CONCLUSIONS

*1*. The present experiment was designed to test whether synaptic transmission and synaptic plasticity in the dentate gyrus were modulated by the medial amygdala (MeA). Field potentials in the dentate gyrus (DG) evoked by stimulations of the medial perforant path (PP) were extracellularly recorded in anesthetized rats.

*2*. Although single-pulse stimulation of the MeA augmented PP stimulation-evoked population spike amplitude in the DG transiently, high-frequency stimulation (100 Hz for 1 s) of the MeA induced long-lasting enhancement of synaptic transmission that was not occluded by PP tetanus-induced long-term potentiation (LTP).

*3*. When high-frequency stimulation of the MeA was applied concurrently with weak tetanus of the PP, which alone induced only marginal LTP, the magnitude of LTP increased considerably.

*4*. These results demonstrate that neuron activities in the MeA induce short- and long-lasting changes in the excitability of the PP-DG synapses and thereby enhance their synaptic plasticity.

## INTRODUCTION

The excitatory synapses in the hippocampus are known to display long-term potentiation (LTP) after a brief tetanus, which is a form of activity-dependent synaptic plasticity that may underlie learning and memory (Bliss and Collingridge 1993). LTP has been studied extensively as a part of investigations on synaptic mechanisms between intrinsic hippocampal neurons, but modulation by extrinsic inputs is also an important subject for elucidating the nature of LTP in vivo.

The present study focused on the presumable modulation of hippocampal synaptic transmission and LTP by afferents from the amygdala, which is involved in certain types of memory besides emotional and motivational aspects of behavior (Kesner 1992; LeDoux 1992). Because recent behavioral studies demonstrated that the amygdala modulated hippocampal-dependent memory processes (McGaugh et al. 1992; Packard et al. 1994), hippocampal LTP may also be modified by the amygdala. Indeed we already showed that high-frequency stimulation of the basolateral amygdala facilitated LTP of the population spike amplitude in the dentate gyrus (DG) (Ikegaya et al. 1995), indicating the critical role of the amygdala in the formation of hippocampal LTP.

The amygdaloid complex consists of several subnuclei, which play differential roles in various aspects (Amaral et al. 1992). Neural projections from the medial amygdala (MeA) to the hippocampus have been confirmed by several anatomic and physiological studies (Albeck et al. 1990; Caffe et al. 1987). In this experiment, therefore, we examined the effects of stimulations of the MeA on basal synaptic transmission and LTP in the DG of anesthetized rats.

## METHODS

Male Wistar rats 8–9 wk old were fixed in a stereotaxic frame under anesthesia with a combination of urethan (1 g/kg ip) and $\alpha$-chloralose (25 mg/kg ip). A bipolar electrode was placed in the left entorhinal cortex to stimulate the medial perforant path (PP), and evoked potentials were extracellularly recorded with a monopolar electrode positioned at the granule cell layer of the ipsilateral DG. Another stimulating electrode was inserted into the left (ipsilateral to the recording site) or right (contralateral) MeA (2.8 mm posterior to bregma, 3.2 mm lateral to midline, 8.2 mm ventral to dura), and a single-pulse stimulation (0.08 ms duration, 400 $\mu$A) or a conditioning stimulation (100 Hz for 1 s) was applied. Because we have previously observed that stimulation of the basolateral amygdala facilitated the LTP of population spikes, changes in evoked potentials were evaluated by measuring the population spike amplitude that was defined as shown in Fig. 1*A*.

At the end of each experiment, stimulated sites were marked by small lesions (400 $\mu$A of current passed across the tips, 10 s of each polarity). Each brain was coronally sliced at 20-$\mu$m thickness and stained with cresyl violet. Microscopic examination of the sections confirmed that we had stimulated the MeA accurately in all rats tested.

## RESULTS

### *Single-pulse stimulation of the MeA transiently augments synaptic efficacy in the DG*

Although single-pulse stimulation of the MeA alone did not change field potentials in the ipsilateral DG (Fig. 1*Aa*), MeA stimulation increased population spike amplitude evoked by PP stimulation when it was delivered simultaneously with or before PP stimulation (Fig. 1). The facilitatory effect of MeA stimulation was the maximum at intervals of 0–50 ms between MeA stimulation and PP stimulation. Stimulation of the contralateral MeA had no influence on the population spike amplitude (data not shown, $n = 3$).

### *High-frequency stimulation of the MeA induces long-lasting enhancement of neurotransmission in the DG*

Next, effects of repetitive stimulations of the MeA on baseline synaptic transmission in the DG was examined. When high-frequency stimulation (100 Hz for 1 s) of the ipsilateral MeA was applied, the population spike amplitude gradually increased in 20 min. This synaptic enhancement

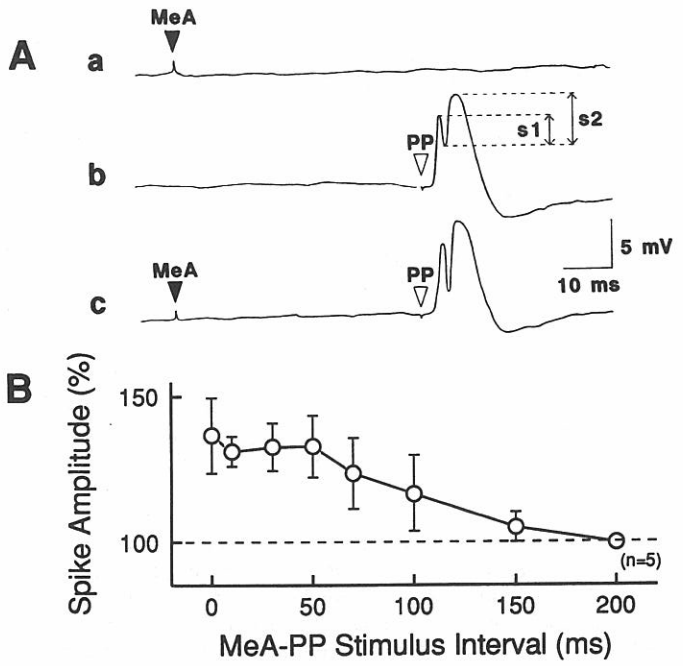


FIG. 1. *A*: field potentials evoked by medial amygdala (MeA) stimulation (*Aa*), perforant path (PP) stimulation (*Ab*), or PP stimulation 50 ms after MeA stimulation (*Ac*) were recorded from the dentate gyrus (DG) cell layer of anesthetized rats. Single-pulse test stimulations were delivered to the MeA and PP at the time indicated by closed arrowheads and open arrowheads, respectively. *B*: changes in population spike amplitude with the time between MeA stimulation and PP stimulation were shown as percent change from baseline (when PP stimulation delivered alone). Data are shown as means ± SE. The amplitude of a population spike was defined as the average of the amplitude from the 1st positive peak to the succeeding negative peak (s1) and the amplitude from the negative peak to the 2nd positive peak (s2), i.e., (s1 + s2)/2.

was maintained >60 min during our observation (Fig. 2, *A* and *B*). However, contralateral MeA stimulation did not affect basal synaptic transmission (Fig. 2*C*).

We also tested whether long-lasting enhancement induced by high-frequency stimulation of the MeA was occluded by preexisting LTP. PP tetanus (100 Hz for 2 s) generated LTP, which was considered to be saturated because no more LTP was produced by the second tetanus given 30 min later (data not shown, $n = 3$). When high-frequency stimulation of the MeA was applied 30 min after PP tetanus, the population spike amplitude was additionally enlarged (Fig. 2*D*). The magnitude of synaptic enhancement induced by MeA stimulation following PP tetanus was similar to that induced by MeA stimulation alone (Fig. 2*B*).

*High-frequency stimulation of the MeA facilitates DG LTP*

We attempted to investigate the effect of high-frequency stimulation of the MeA on the formation of LTP. Although PP week tetanus (20 Hz for 1 s) alone induced marginal LTP in the DG (Fig. 3*A*), robust LTP was generated by simultaneous application of high-frequency stimulation of the MeA (Fig. 3*B*). The difference between the magnitude of LTP induced by PP tetanus paired with MeA stimulation and the magnitude of long-lasting enhancement induced by MeA stimulation alone (indicated by dots in Fig. 3*B*) was larger than the magnitude of LTP induced by unpaired PP weak tetanus, indicating that substantial LTP was facilitated by MeA stimulation. On the other hand, a conditioning stimulation of the contralateral MeA did not influence the induction of LTP (Fig. 3*C*).

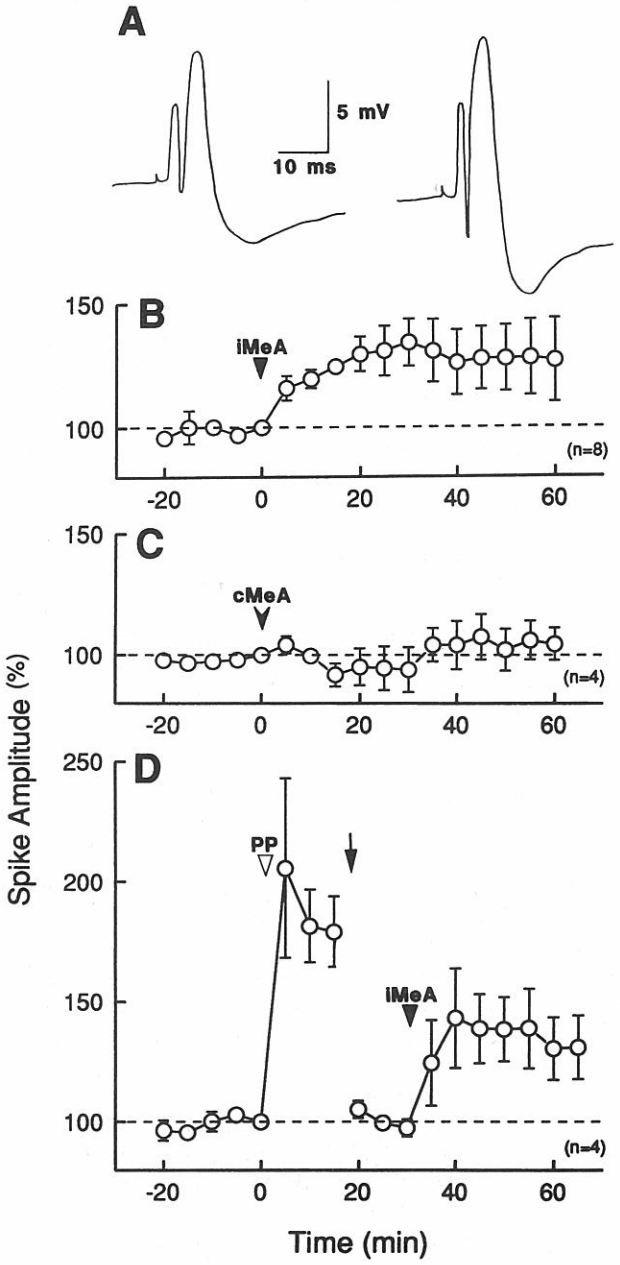


FIG. 2. *A*: typical evoked potentials recorded from the DG cell layer immediately before (*left*) and 60 min after (*right*) high-frequency stimulation of the MeA ipsilateral to the recording site (100 Hz for 1 s). *B*: changes in the population spike amplitude following ipsilateral MeA (iMeA) stimulation (closed arrowhead). MeA stimulation was applied at *time 0*, and population spike amplitude is expressed as a percentage of baseline value immediately before MeA stimulation. *C*: contralateral MeA (cMeA) stimulation was applied at *time 0*. *D*: Tetanus-induced long-term potentiation (LTP) did not occlude the MeA stimulation-induced synaptic enhancement. At an open arrowhead, the PP received a 100-Hz, 2-s tetanus. Test stimulation strength was reduced so that the amplitude of population spike elicited by PP stimulation was approximately the same as baseline amplitude before tetanus at the time indicated by an arrow. High-frequency stimulation of the ipsilateral MeA was applied 30 min after PP strong tetanus.

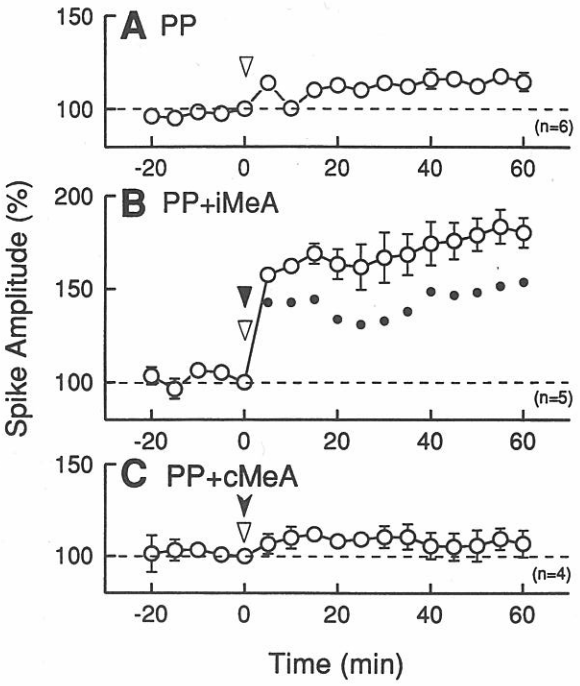


FIG. 3. Changes in the population spike amplitude following PP tetanus and MeA stimulation. All tetanic stimulation was applied at *time 0*. *A*: PP weak tetanus (20 Hz for 1 s) was applied. *B*: high-frequency stimulation of the ipsilateral MeA (100 Hz for 1 s) and PP weak tetanus were simultaneously applied. Dotted line indicates a deduction from the magnitude of potentiation by PP tetanus paired with MeA stimulation (*B*) to the magnitude of enhancement by MeA stimulation alone (Fig. 2*B*). *C*: contralateral MeA stimulation and PP tetanus were simultaneously applied.

## DISCUSSION

High-frequency stimulation of the MeA induced long-lasting synaptic enhancement in the PP-DG synapses, whereas single-pulse stimulation of the MeA facilitated neurotransmission only transiently. Moreover, high-frequency stimulation of the MeA facilitated LTP induction in the DG. These results suggest that neuron activities of the MeA augment both the basal synaptic strength and the formation of synaptic plasticity in the PP-DG synapses ipsilaterally and unilaterally.

High-frequency stimulation of the MeA induced long-lasting enhancement of synaptic efficacy in the PP-DG synapses without requiring PP tetanus, which made contrast with the basolateral amygdala and the central amygdala, whose stimulations did not affect basal synaptic transmission (Ikegaya et al. 1995). This result is somewhat surprising because it is well known that hippocampal synapses that are quiescent during activation of postsynaptic neurons display long-lasting decrement of efficacy of neurotransmission that is termed heterosynaptic long-term depression (Linden 1994). This synaptic enhancement induced by MeA stimulation was not occluded by saturated LTP produced by a strong tetanus. Moreover, synaptic efficacy after MeA stimulation gradually increased in 20 min, which is conspicuously different from the time course of LTP. It is therefore probable that the mechanism of MeA stimulation-induced synaptic enhancement is essentially different from the mechanism of LTP in PP-DG synapses. Unclear as this mechanism is in detail, it is possible to interpret this result as a function of vasopressin, because it is known to be a transmitter of projections from the MeA to the hippocampus (Albeck et al. 1990; Caffe et al. 1987) that induces long-lasting synaptic potentiation in the PP-DG synapses (Chen et al. 1993). A contribution of the peptidergic projections may explain different effects of stimulations between the MeA and the other amygdaloid subnuclei on the PP-DG synaptic transmission. Further pharmacological approaches are underway in our laboratory.

A conditioning stimulation of the MeA, similar to the basolateral amygdala (Ikegaya et al. 1995), facilitates the induction of hippocampal LTP when coupled with PP tetanus. This satisfies "associativity," which is an important property of synaptic plasticity (Bliss and Collingridge 1993). Consequently, MeA neurons may contribute to associative memory processing in the hippocampus. Recent behavioral studies have provided evidence that the amygdala modulates hippocampal-dependent memory (McGaugh et al. 1992; Packard et al. 1994). Activity-dependent facilitation of hippocampal LTP by MeA neurons is a particular useful model for studying synaptic mechanisms underlying memory enhancement by emotional arousal (LeDoux 1993; McGaugh 1989).

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