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Neuroscience Research 22 (1995) 203–207

NEUROSCIENCE RESEARCH

High-frequency stimulation of the basolateral amygdala facilitates the induction of long-term potentiation in the dentate gyrus in vivo

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Received 5 December 1994; accepted 10 February 1995

Abstract

We investigated the effect of high-frequency stimulation of the basolateral amygdala (BLA) on the induction of long-term potentiation (LTP) in the medial perforant path (PP)-dentate gyrus (DG) synapses of anesthetized rats. A conditioning stimulation (100 pulses at 100 Hz) of the ipsilateral BLA did not change the DG synaptic potential. However, when the BLA conditioning stimulation was applied at the same time as a weak tetanic stimulation of PP (20 pulses at 20 Hz) which alone did not induce LTP, robust DG LTP was induced. Simultaneous application of contralateral BLA stimulation and PP weak tetanus did not induce LTP. Moreover, the ipsilateral BLA stimulation enhanced the magnitude of LTP induced by a moderate tetanic stimulation of PP (30 pulses at 60 Hz), but did not further enhance the LTP induced by a strong tetanic stimulation of PP (100 pulses at 100 Hz). These results suggest that the ipsilateral BLA neurons modulate the induction of DG LTP in vivo.

Keywords: Long-term potentiation; Hippocampus; Basolateral amygdala; Amygdalo-hippocampal interaction; Synaptic plasticity

1. Introduction

Long-term potentiation (LTP) of evoked potentials in the hippocampus is a form of activity-dependent synaptic plasticity which may underlie learning and memory (Berger, 1984; Skelton et al., 1987; Bliss and Collingridge, 1993). LTP has been studied extensively as part of investigations into synaptic mechanisms between intrinsic hippocampal neurons, but modulation of LTP by extrinsic inputs is also an important subject for elucidating the nature of LTP in vivo.

Neural projections from the amygdala to the hippocampus have been confirmed by several physiological and anatomical studies (Thomas et al., 1984; Aggleton, 1986; Saunders et al., 1988). Furthermore, we have recently found that LTP of population spikes in the dentate gyrus (DG) of anesthetized rats is attenuated by surgical lesion of the basolateral amygdala (BLA) (Ikegaya et al., 1994). These observations suggest that the BLA neurons participate in the formation of hippocampal LTP. To test this hypothesis, in the present study, we investigated the effect of high-frequency stimulation of the BLA on the induction of LTP in the DG of anesthetized rats.

2. Materials and methods

Evoked potentials were recorded as described in our previous paper (Ikegaya et al., 1994). Briefly, male Wistar rats, 8–9, weeks old were anesthetized with a combination of urethane (1 g/kg, i.p.) and α-chloralose (25 mg/kg, i.p.), and fixed in a stereotaxic frame. In this anesthetic condition, we could record stable field potentials for more than 2 h. A bipolar electrode was placed in the left entorhinal cortex to stimulate the medial perforant path (PP), and the evoked potential was extracellularly recorded with a monopolar electrode positioned at the granule cell layer of the ipsilateral DG. A single test stimulus (0.08 ms duration) was applied at intervals of 30 s and the stimulus intensity was set to a level which produced a population spike of about 50%

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0168-0102/95/$09.50
SSDI 0168-0102(95)00894-Y

of the maximum. Since we have previously observed that BLA lesion resulted in attenuation of LTP of population spikes, changes in evoked potential were evaluated by measuring the population spike amplitude. The population spike amplitude was defined as shown in Fig. 1A. To induce LTP in the PP-DG synapses, a tetanic stimulation was applied to the PP at the same stimulus intensity through the same electrode as used for test stimulation. Another stimulating electrode was inserted into the left (ipsilateral to the recording site) or right (contralateral) BLA (2.8 mm posterior to bregma, 5.2 mm lateral to midline, 7.6 mm ventral to dura) or left (ipsilateral) central amygdala (CeA) (2.8 mm posterior to bregma, 4.2 mm lateral to midline, 6.9 mm ventral to dura), and a conditioning stimulation (0.08 ms duration, 10 V, 100 pulses at 100 Hz) was applied. This condition of BLA or CeA stimulation alone evoked no apparent field potentials in the DG.

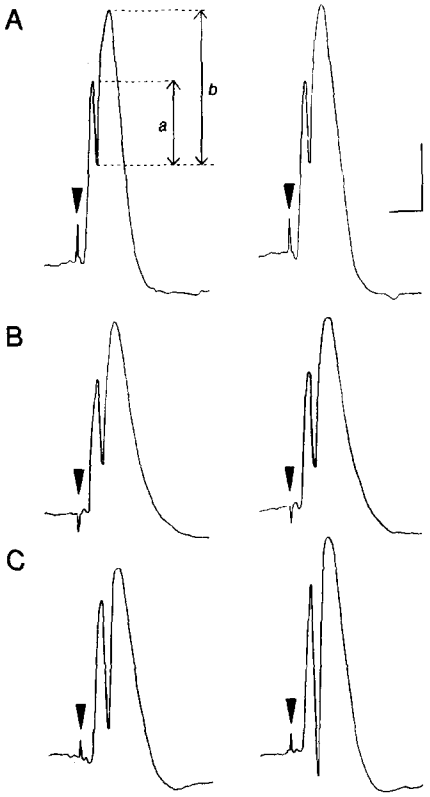

Fig. 1. Typical evoked potentials recorded from the DG cell layer of anesthetized rats immediately before (left) and 10 min after (right) the BLA stimulation or PP tetanic stimulation, or both. A: ipsilateral BLA stimulation (100 pulses at 100 Hz) was applied. B: weak tetanic stimulation (20 pulses at 20 Hz) was applied to PP. C: the ipsilateral BLA stimulation and the PP weak tetanic stimulation were simultaneously applied. Test stimulation was delivered at the time indicated by arrowheads. Calibration bars: vertical 5 mV, horizontal 10 ms. The amplitude of a population spike was defined as the average of the amplitude from the first positive peak to the succeeding negative peak (a) and the amplitude from the negative peak to the second positive peak (b), i.e., $(a + b)/2$.

At the end of each experiment, stimulating sites were marked by small lesions (40 μA of current passed across the tips, 10 s of each polarity). The rats were perfused with ice-cold phosphate-buffered saline (pH 7.4) containing 8% paraformaldehyde. The brains were removed and soaked in the same fixative for 24 h. After freezing, each brain was coronally sliced at 20 mm thickness with a microtome and stained with cresyl violet. Microscopic examination of the histological sections confirmed that we had stimulated precisely the intended amygdaloid nuclei in all rats tested.

3. Results

A conditioning stimulation (100 pulses at 100 Hz) of the ipsilateral BLA did not affect the synaptic responses in the PP-DG synapses (Figs. 1A and 2A). A weak tetanic stimulation (20 pulses at 20 Hz) of PP did not induce LTP in the PP-DG synapses (Figs. 1B and 2B);

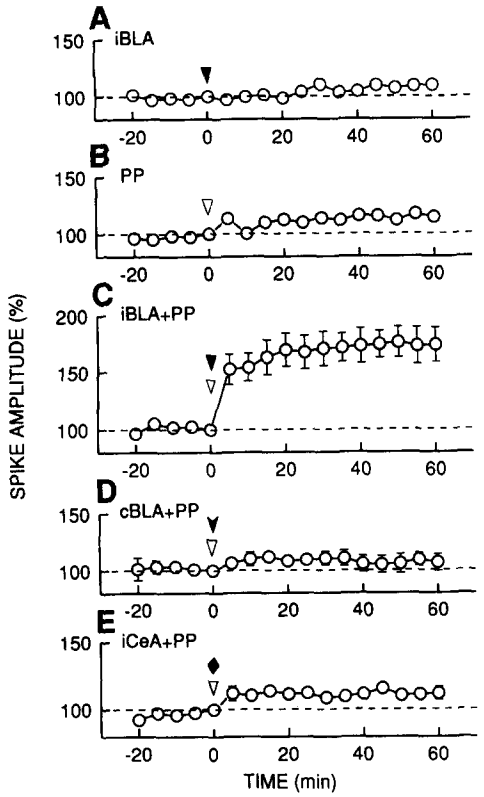

Fig. 2. Changes in the population spike amplitude following the PP tetanic stimulation and the BLA or CeA stimulation. All tetanic stimulation was applied at time 0 and population spike amplitude is expressed as a percentage of baseline value immediately before tetanus. A: ipsilateral BLA stimulation (100 pulses at 100 Hz) was applied ($n = 5$). B: PP weak tetanic stimulation (20 pulses at 20 Hz) was applied ($n = 5$). C: the ipsilateral BLA stimulation and PP weak tetanic stimulation were simultaneously applied ($n = 6$). D: contralateral BLA stimulation (100 pulses at 100 Hz) and the PP tetanus were simultaneously applied ($n = 4$). E: ipsilateral CeA stimulation (100 pulses at 100 Hz) and the PP weak tetanus were simultaneously applied ($n = 4$). Vertical bars on data points represent the S.E.M.; when not indicated, the S.E.M. fell within the data point.

however, when the ipsilateral BLA stimulation was applied at the same time as a weak tetanic stimulation of PP, robust LTP was induced (Figs. 1C and 2C). On the other hand, simultaneous application of the contralateral BLA stimulation and the PP weak tetanic stimulation did not induce LTP (Fig. 2D). Furthermore, simultaneous application of the ipsilateral CeA stimulation and the PP weak tetanus did not induce LTP (Fig. 2E).

To examine if the ipsilateral BLA stimulation affects the responses during tetanic stimulation, we investigated the effect of the BLA stimulation within the same rats, because the responses to tetanic stimulation varied considerably among rats. In control experiments, when a weak tetanic stimulation of PP was repeatedly applied at intervals of 30 min, LTP was not induced (Fig. 3A). The response during the second weak tetanic stimulation was the same as that during the first trial (Fig. 3A). When the ipsilateral BLA stimulation was coupled with the second PP weak tetanus, LTP was induced (Fig. 3B). At this time, the BLA stimulation showed no effect on the response during PP tetanic stimulation (Fig. 3B).

We also investigated the effect of ipsilateral BLA stimulation when coupled with different patterns of PP tetanic stimulation. A strong tetanic stimulation (100 pulses at 100 Hz) of PP induced LTP in the PP-DG synapses. A moderate tetanic stimulation (30 pulses at 60 Hz) of PP also induced LTP, but the magnitude of LTP induced by a 30-pulse, 60-Hz tetanus was smaller than that induced by a 100-pulse, 100-Hz tetanus (Figs. 4A and B). The BLA stimulation enhanced the magnitude of LTP induced by a moderate tetanic stimulation of PP (Fig. 3A), but did not further enhance the LTP induced by a strong tetanic stimulation of PP (Fig. 4B).

To determine if the ipsilateral BLA stimulation is required at the same time as PP tetanic stimulation, the BLA stimulation was applied prior to the PP tetanic stimulation (30 pulses at 60 Hz). In this experiment, the moderate tetanic stimulation of PP was applied to test the possibility that the preceding BLA stimulation enhances or suppresses the PP tetanus-induced LTP. As shown in Fig. 5, the BLA stimulation applied 1 s, 5 s or 10 min prior to the moderate PP tetanic stimulation neither enhanced nor suppressed DG LTP, suggesting that the timing of BLA stimulation is critical.

4. Discussion

We found that the conditioning stimulation of ipsilateral BLA facilitates the induction of LTP in the PP-DG synapses, consistent with our previous observation that DG LTP is attenuated by lesion of the ipsilateral BLA. In addition, the same conditioning stimulation of contralateral BLA or ipsilateral CeA, which neighbors the BLA, did not facilitate DG LTP, indicating that the facilitation of DG LTP is due to a specific effect of the ipsilateral BLA stimulation and not to some general, indirect effect. These results strongly support the idea that the ipsilateral BLA neurons modulate hippocampal synaptic plasticity.

LTP is saturable; if the synapses are fully strengthened by sufficient activation of afferents, no further LTP

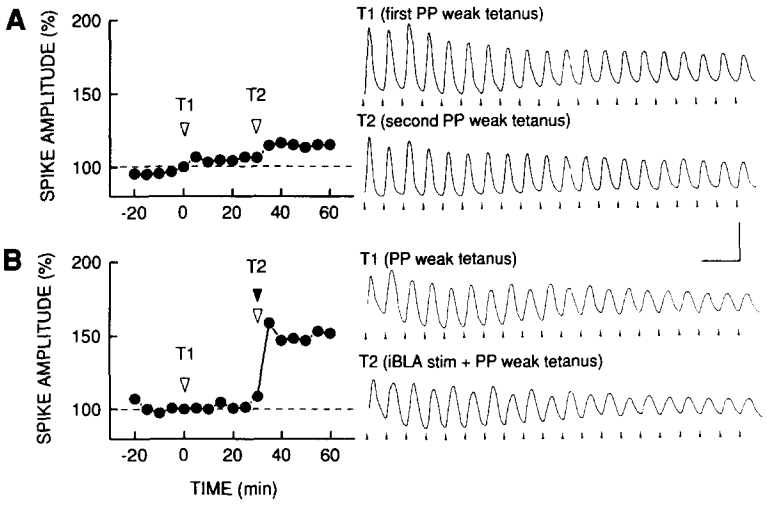

Fig. 3. Effect of the ipsilateral BLA stimulation on synaptic responses during PP weak tetanic stimulation. The PP weak tetanic stimulation (20 pulses at 20 Hz) was repeatedly applied within the same rat, and the synaptic responses during the first and second tetani were compared. A: the PP weak tetanus was applied twice at an interval of 30 min (T1 and T2). In the example shown here, the responses seem to be slightly potentiated after T2, but the changes are not statistically significant. The mean population spike amplitude 30 min after T2 of five rats was 107.7 ± 2.6%. B: at the first trial (T1, 0 min), the PP weak tetanus alone was applied, and at the second trial (T2, 30 min), the ipsilateral BLA stimulation (100 pulses at 100 Hz) and the PP weak tetanus were simultaneously applied. The right traces are the synaptic responses during T1 and T2. Calibration bars: vertical 5 mV, horizontal 100 ms.

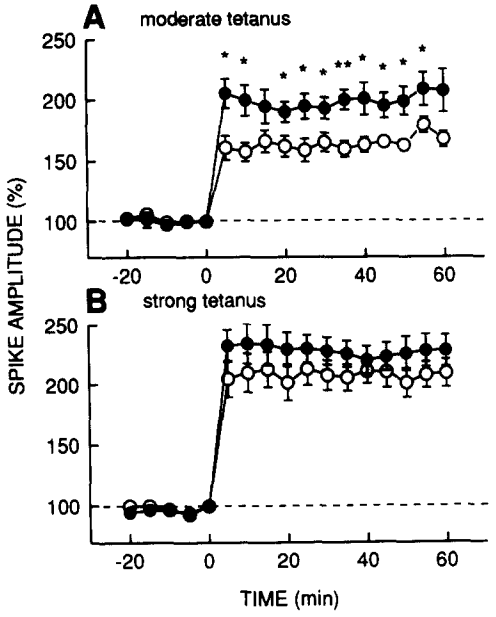

Fig. 4. Effects of the ipsilateral BLA stimulation on DG LTP induced by different conditions of PP tetanic stimulation. A: moderate tetanic stimulation of PP (30 pulses at 60 Hz) was applied at time 0 (○, $n = 6$). The BLA stimulation was applied with PP moderate tetanus (●, $n = 5$). B: strong tetanic stimulation of PP (100 pulses at 100 Hz) was applied alone (○, $n = 5$) or at the same time as the BLA stimulation (●, $n = 5$). The data are shown as the mean ± S.E.M. $^*P < 0.05$; $^{**}P < 0.01$; Student's t-test.

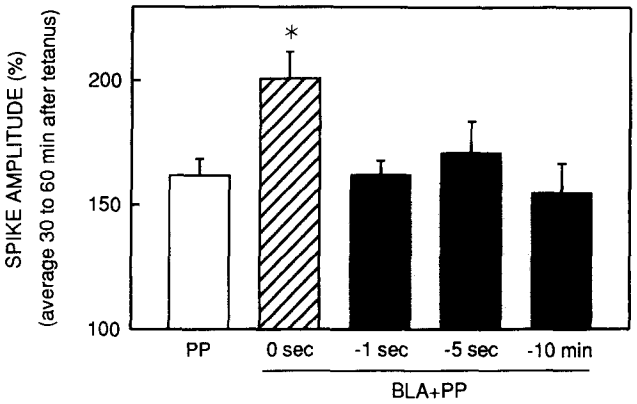

Fig. 5. Effects of the ipsilateral BLA stimulation paired or unpaired with PP tetanic stimulation on DG LTP. An open column indicates the magnitude of LTP induced by PP moderate tetanus (30 pulses at 60 Hz; $n = 6$). A hatched column indicates the magnitude of LTP induced by simultaneous application of the ipsilateral BLA stimulation and the PP moderate tetanus ($n = 5$). Solid black columns indicate the magnitude of LTP in the groups which received the BLA stimulation 1 s ($n = 5$), 5 s ($n = 6$) or 10 min ($n = 6$) prior to the PP moderate tetanus ($n = 6$). The average percent amplitude of population spikes 30–60 min after tetanus was calculated to compare the magnitude of LTP in each group. All data are represented as the means ± S.E.M. $^*P < 0.05$ vs. the control group (open column); Duncan's multiple range test following analysis of variance (ANOVA).

can be produced (Mulkey and Malenka, 1992). The BLA stimulation enhanced the LTP induced by weak or moderate tetanic stimulation of PP, but did not further enhance the LTP induced by strong tetanus. It is therefore probable that the BLA stimulation modulates the mechanisms involved in the tetanus-induced LTP. For example, the BLA neurons may play a role in lowering the threshold of hippocampal LTP induction.

Hippocampal LTP is triggered by the action of glutamate and postsynaptic membrane depolarization, which allows Ca^{2+} influx through postsynaptic NMDA receptor channels, and is expressed as a persistent increase in transmitter release from a presynaptic terminal (Bekkers and Stevens, 1990; Malinow and Tsien, 1990; Bliss and Collingridge, 1993). Since the BLA stimulation alone produced no apparent DG field potentials and did not affect the responses of DG neurons to PP tetanic stimulation, it is unlikely that the BLA stimulation facilitates the induction of LTP by increasing the DG neuron excitability during tetanic stimulation. The BLA neurons may modulate the LTP induction mechanism downstream of the NMDA receptor activation or may promote the expression of LTP in presynaptic terminals. Neural projection pathways from the BLA to the DG remain to be investigated. To clarify the mechanism by which the BLA neurons modulate hippocampal LTP, the following issues must be solved: where do the BLA neurons terminate; and what neurotransmitter(s) is released from the BLA neuron terminals? Further investigations are underway in our laboratory.

The BLA stimulation facilitates the induction of hippocampal LTP only when coupled with the PP tetanic stimulation. In other words, the BLA neurons facilitate potentiation at active, but not quiescent, hippocampal synapses. This suggests that BLA neurons contribute to associative memory processing in the hippocampus. Recent behavioral studies have provided evidence that the amygdala modulates hippocampal-dependent memory (McGaugh et al., 1990, 1993; Parent et al., 1992; Packard et al., 1994). Activity-dependent facilitation of hippocampal LTP by BLA neurons is a particularly useful model for studying synaptic mechanisms underlying memory enhancement associated with emotional arousal (McGaugh, 1989).

References

Aggleton, J.P. (1986) A description of the amygdalo-hippocampal interconnections in the macaque monkey. Exp. Brain Res., 64: 515–526.

Bekkers, J. and Stevens, C. (1990) Presynaptic mechanism of long-term potentiation. Nature, 346: 724–729.

Berger, T.W. (1984) Long-term potentiation of hippocampal synaptic transmission affects rate of behavioral learning. Science, 224: 627–630.

Bliss, T.V.P. and Collingridge, G.L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature, 361: 31–39.

Collingridge, G.L. (1983) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J. Physiol., 334: 33–46.

Ikegaya, Y., Saito, H. and Abe, K. (1994) Attenuated hippocampal long-term potentiation in basolateral amygdala-lesioned rats. Brain Res., 656: 157–164.

Malinow, R. and Tsien, R.W. (1990) Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices. Nature, 346: 177–180.

McGaugh, J.L. (1989) Involvement of hormonal and neuromodulatory systems in the regulation of memory storage. Annu. Rev. Neurosci., 12: 255–287.

McGaugh, J.L., Introini-Collison, I.B., Nagahara, A.H. and Cahill, L.F. (1990) Involvement of the amygdaloid complex in neuromodulatory influences on memory storage. Neurosci. Biobehav. Rev., 14: 425–431.

McGaugh, J.L., Introini-Collison, I.B., Cahill, L.F., Castellano, C., Dalmaz, C., Parent, M.B. and Williams, C.L. (1993) Neuromodulatory systems and memory storage: role of the amygdala. Behav. Brain. Res., 58: 81–90.

Mulkey, R.M. and Malenka, R.C. (1992) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron, 9: 967–975.

Packard, M.G., Cahill, L. and McGaugh, J.L. (1994) Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. Proc. Natl. Acad. Sci. USA, 91: 8477–8481.

Parent, M.B., McGaugh, J.L. and Tomaz, C. (1992) Increased training in an aversively motivated task attenuates the memory-impairing effects of posttraining *N*-methyl-D-aspartate-induced amygdala lesions. Behav. Neurosci., 106: 789–797.

Saunders, R.C., Rosen, D.L. and Van Hosen, G.W. (1988) Comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey. II. Reciprocal and nonreciprocal connections. J. Comp. Neurol., 271: 185–207.

Skelton, R.W., Scarth, A.S, Willie, D.M., Miller, J.J. and Phillips, A.G. (1987) Long-term increases in dentate granule cell responsivity accompany operant conditioning. J. Neurosci., 7: 3081–3087.

Thomas, S.R., Assaf, S.Y. and Iversen, S.D. (1984) Amygdaloid complex modulates neurotransmission from the entorhinal cortex to the dentate gyrus of the rat. Brain Res., 307: 363–365.