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Research report

Early life stress alters synaptic modification range in the rat lateral amygdala

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HIGHLIGHTS

- Saturating levels of LTP and LTD were induced in slices of the lateral amygdala.
- Maximum LTP and LTD determine the extent of the synaptic modification range.
- Maternal separation stress shrank the modification range in the thalamic input.
- Maternal separation enhanced LTD in the cortical input.
- Altered synaptic plasticity will have consequences for amygdala-dependent learning.

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ABSTRACT

The influence of exposure to early adversity on emotional learning later in life remains poorly understood. Long-term potentiation (LTP) in the cortico-amygdalar and thalamo-amygdalar pathways has been postulated to provide a mechanism of synaptic modifications underlying fear learning and memory. These synapses also express homosynaptic long-term depression (LTD). Here we examined the effects of maternal separation stress on the extent of LTP and LTD which could be induced in the lateral amygdala (LA) of adolescent rats. Rat pups were subjected to maternal separation (MS, 3 h/day) on post-natal days 1–21. Field potentials were recorded *ex vivo* from slices containing the LA, which were prepared from adolescent males. Saturating levels of LTP and LTD were induced using repeated sequences of theta-burst stimulation and low frequency stimulation, respectively. An impairment of the maximum LTP and an enhancement of the maximum LTD were observed in the cortical input in slices prepared from MS-subjected rats. In the thalamic input, both the maximum LTP and the maximum LTD were reduced when compared to control animals. Thus, in the cortico-amygdalar pathway MS stress shifted the potential for bidirectional synaptic modification toward LTD but it shrank the synaptic modification range in the thalamo-amygdalar pathway.

1. Introduction

Exposure to adversity in early life has been linked to the pathogenesis of psychiatric disorders and may act as a predisposing factor for substance abuse and altered social behavior in adult humans (reviewed in [1–3]). Early life stress has been shown to induce persistent alterations in anxious behavior and function of amygdala [4], which plays a central role in the acquisition, consolidation and retrieval of fear-related memories (reviewed in [5,6]). Maternal separation (MS) of rat pups has been widely used to study the mechanisms underlying the effects of early adversity on the adult organism. Several studies indicated that later in life, MS results in an increased level of fear and anxiety [7,8]. Some studies reported that MS impairs the acquisition of fear conditioning to a cue, fear memory consolidation and extinction [9–13].

In Pavlovian auditory fear conditioning, an essential event for occurrence of the conditioned reaction is the association between the activity of inputs converging in the lateral nucleus (LA) of the amygdala which deliver information related to auditory conditioned stimulus and to nociceptive unconditioned stimulus (reviewed in [14–16]). A large body of experimental evidence from pharmacological, electrophysiological and behavioral studies sug-

Abbreviations: ACSF, artificial cerebrospinal fluid; FP, field potential; LA, lateral amygdala; LFS, low frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; MS, maternal separation; PND, postnatal day; TBS, theta burst stimulation.

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gests that long-term synaptic plasticity at auditory inputs to the LA plays a critical role in the formation and storage of auditory fear memories (reviewed in [15,17,18]). The LA receives auditory information from the thalamus [19] and cerebral cortex [20]. Fear conditioning can be mediated via either the thalamic or the cortical input to the LA [21]. Homosynaptic long-term potentiation (LTP) has been postulated to provide a mechanism of synaptic modifications underlying fear conditioning [22–25]. The efficacy of synaptic connections formed by the cortical and thalamic afferent inputs to the LA can be modified bidirectionally as these synapses also express homosynaptic long-term depression (LTD) [25–27].

As plastic synapses appear to express upper and lower limit of plasticity, saturating levels of LTP and LTD can be used to determine the synaptic modification range (*e.g.* [28]). While it has been shown that MS stress modifies fear learning, the effects of MS on synaptic plasticity in the inputs to the amygdala have not been investigated yet. In this study we sought to determine the effects of MS on synaptic transmission and plasticity in the LA. To this end, we examined the effect of MS on single- and paired-pulse evoked field potentials and the influence of MS on the synaptic modification range at the cortical and the thalamic input to the LA of adolescent rats, using saturating amounts of repeated conditioning stimulation inducing LTP and LTD.

2. Materials and methods

2.1. Animals and treatment

The experimental protocols were approved by the local Animal Care and Use Committee and were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) guidelines for the use of experimental animals, and the national law. All efforts were made to minimize the number of animals used and their suffering.

Wistar dams with their offspring were kept under the controlled light/dark cycle (light: 7.00–19.00) with standard food and water available *ad libitum*. On each of postnatal days (PNDs) 1–21 the dams were removed from maternity cages for 3 h and placed individually in holding cages, while the litter stayed in home cages. Then, the dams were returned to the maternity cages. MS-subjected animals, as well as control rats, were weaned at PND 28 and housed in groups (4–6 animals per cage).

2.2. Slice preparation and field potential recording

Male rats (PND 35–PND 55) were anesthetized with isoflurane and decapitated. Then their brains were removed from the skull and immersed in an ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 4 $MgSO_4$, 4.5 KCl, 2 $CaCl_2$ and 10 D-glucose, saturated with a mixture of 95% O_2 and 5% CO_2. The brains were cut into slices (thickness: 450 μm) in the coronal plane using the vibrating microtome (VT 1000 Leica Microsystems, Wetzlar, Germany). Slices containing the LA were transferred to the interface-type recording chamber and perfused at 2 ml/min with a modified ACSF (33.5 ± 0.2 °C) in which $MgSO_4$ concentration was lowered to 1 mM. One slice per animal was investigated. Recordings began approx. 2.5 h after slice preparation and LTP/LTD induction was attempted approx. 1 h later. Field potentials (FPs) were evoked using the concentric platinum/stainless steel electrode (FHC, USA) by the stimulation (frequency: 0.05 Hz, duration: 100 μs) of external or internal capsule to stimulate cortico-amygdalar or thalamo-amygdalar connections, respectively (reviewed in [24]). FPs were recorded using ACSF-filled glass micropipettes (1–3 MΩ) placed in the LA. Signals were amplified (Axoprobe 2, Axon Instruments, USA), band-pass filtered (1 Hz–5 kHz), A/D converted (micro1401 interface, CED, UK) and analyzed on- and off-line (Signal 2 software, CED, UK). Since the initial slope measure of the FP in the LA is more sensitive to variability and noise in the signal [23], we analyzed the amplitude of FPs. After initial stabilization of the responses, input–output curves were constructed and then the stimulation intensity was adjusted to evoke FPs of 50% of the maximum amplitude.

After 15 min of baseline recording, LTP was induced by the theta burst stimulation (TBS) protocol consisting of three trains of stimuli delivered every 5 min. Each train was composed of six sequences of pulses delivered at frequency of 5 Hz [29]. Each sequence consisted of eight pulses delivered at 100 Hz. TBS stimulation pattern was used since LA projection neurons have an intrinsic propensity to generate voltage-dependent membrane potential oscillations in the theta frequency range [15] and stimulation of the LA at theta frequency induces local activity that resembles that occurring during fear [30].

In a separate set of experiments, after 15 min of baseline recording, LTD was induced by low frequency stimulation (LFS, 900 pulses at 1 Hz) [26]. Both TBS and LFS were repeated every 45 min in order to reach the saturation level of LTP and LTD, respectively [28]. LTP/LTD was considered saturated if the difference between the amplitudes of FPs after consecutive TBS/LFS delivery was not significant.

To examine short-term plasticity, paired-pulse stimulation protocols were used. The stimulation intensity was set so that the first stimulus of a pair evoked a response that was 50% of the maximum. Paired stimuli were delivered every 20 s to both inputs to the LA in an interleaved fashion. Inter-stimulus intervals (ISIs) of 25, 50, 100, 200, 600 and 1000 ms were tested in an ascending order and at each interval four successive FPs were recorded.

2.3. Statistical analysis

Statistical analysis was performed using the Statistica v. 9.1 software (StatSoft Inc., USA). The paired-pulse ratio was calculated by expressing the amplitude of the response to the second stimulus of a pair relative to the response to the first stimulus. The amplitude of the first FP was taken as 100% and one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was applied for group comparisons. To determine the effects of TBS or LFS, the mean amplitude responses recorded after application of last TBS/LFS stimulation were compared to the preceding pre-TBS or pre-LFS baseline values and expressed as percentage of change. Repeated measures ANOVA followed by Tukey's post-hoc test was applied for group comparisons; $p < 0.05$ was taken as significant. Data are presented as group means ± SEM.

3. Results

3.1. The effects of maternal separation on basal synaptic transmission and short-term plasticity at the cortical and thalamic inputs

Analyses of responses recorded before the delivery of TBS or LFS revealed no significant group differences in the relation between the stimulation intensity and the amplitude of FPs evoked in the cortico-amygdalar pathway (Fig. 1A). In contrast, FPs in the thalamo-amygdalar pathways were smaller over a wide range of stimulation intensities in slices obtained from MS rats compared to controls (Fig. 1B).

Paired-pulse stimulation was used to examine the effect of MS on short-term plasticity at both inputs to the LA. While paired-pulse facilitation was evident at ISIs of 25 and 50 ms, with longer ISIs, paired-pulse inhibition was observed. No significant differ-