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Phase-dependent synaptic changes in the hippocampal CA1 field underlying extinction processes in freely moving rats

Yasuhiro Saito [a], Machiko Matsumoto [a,*], Satoru Otani [b], Yoshiki Yanagawa [a], Sachiko Hiraide [a], Shuhei Ishikawa [a], Shin-ichi Kimura [a], Kei-ichi Shimamura [a], Hiroko Togashi [a]

[a] Department of Pharmacology, School of Pharmaceutical Science, Health Sciences University of Hokkaido, Ishikari-Tobetsu 061-0293, Japan
[b] Physiopathology of Central Nervous System Diseases, Universite Pierre et Marie Curie-Paris 6, Paris, France

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ABSTRACT

Recent studies focus on the functional significance of a novel form of synaptic plasticity, low-frequency stimulation (LFS)-induced synaptic potentiation in the hippocampal CA1 area. In the present study, we elucidated dynamic changes in synaptic function in the CA1 field during extinction processes associated with context-dependent fear memory in freely moving rats, with a focus on LFS-induced synaptic plasticity. Synaptic transmission in the CA1 field was transiently depressed during each extinction trial, but synaptic efficacy was gradually enhanced by repeated extinction trials, accompanied by decreases in freezing. On the day following the extinction training, synaptic transmission did not show further changes during extinction retrieval, suggesting that the hippocampal synaptic transmission that underlies extinction processes changes in a phase-dependent manner. The synaptic potentiation produced by extinction training was mimicked by synaptic changes induced by LFS (0.5 Hz) in the group that previously received footshock conditioning. Furthermore, the expression of freezing during re-exposure to footshock box was significantly reduced in the LFS application group in a manner similar to the extinction group. These results suggest that LFS-induced synaptic plasticity may be associated with the extinction processes that underlie context-dependent fear memory. This hypothesis was supported by the fact that synaptic potentiation induced by extinction training did not occur in a juvenile stress model that exhibited extinction deficits. Given the similarity between these electrophysiological and behavioral data, LFS-induced synaptic plasticity may be related to extinction learning, with some aspects of neuronal oscillations, during the acquisition and/or consolidation of extinction memory.

1. Introduction

Numerous studies have provided evidence that synaptic efficacy in the hippocampus is modulated by contextual elements of fear memory (Eldridge, Knowlton, Furmanski, Bookheimer, & Engel, 2000; Kim & Jung, 2006). For example, synaptic transmission including synaptic plasticity in the hippocampal CA1 field was inhibited by contextual fear conditioning (CFC) based on fear memory (Hirata et al., 2009; Koseki et al., 2007). Given the involvement of the hippocampus in contextual encoding, lesion studies have focused on the functional role of the hippocampus in the contextual modulation of fear extinction (Corcoran, Desmond, Frey, & Maren, 2005; Corcoran & Quirk, 2007; Ji & Maren, 2005). However, to our knowledge, no studies have examined dynamic changes in hippocampal function that underlie the extinction processes.

Fear extinction is thought to consist of a learning process based on the formation of safety memory, which can be termed as extinction memory formed through extinction training. Extinction, therefore, is characterized by the acquisition, consolidation, and retrieval phases of extinction memory, similar to the general form of learning process (Abel & Lattal, 2001). We recently reported that synaptic transmission in the hippocampus-medial prefrontal cortex (mPFC) pathway was enhanced by retrieval of context-dependent fear extinction (Judo et al., 2010). Thus, the hippocampus-mPFC network appears to be crucial for the retrieval phase after extinction training. Indeed, several studies have provided evidence that synaptic potentiation in the mPFC occurred after extinction training (Farinelli, Deschaux, Hugues, Thevenet, & Garcia, 2006; Herry & Garcia, 2002; Hugus & Garcia, 2007). Furthermore, the synaptic potentiation in the mPFC responsible for the retrieval phase was suppressed by low-frequency stimulation (LFS; 2 Hz for 25 min) of the hippocampus, which impaired extinction (Farinelli, 2006; Hugus & Garcia, 2007; Garcia, Spennato, Nilsson-Todd, Moreau, & Deschaux, 2008). These findings suggest that the synaptic changes in the mPFC are regulated by processes that occur in the hippocampus and that dynamic changes in the hippocampal function are important mediators of extinction training.

* Corresponding author. Fax: +81 133 23 1379.
E-mail address: mbird@hoku-iryo-u.ac.jp (M. Matsumoto).

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Recently, *in vivo* and *in vitro* studies revealed the existence of a new form of synaptic plasticity in the hippocampal formation, in which the delivery of LFS (0.5–1 Hz) for a brief period of time (1–5 min) produced a gradual and long-lasting synaptic enhancement in the CA1 field but not in the CA3 or dentate gyrus (Habib & Dringenberg, 2009, 2010a, 2010b; Lanté, Cavalier, Cohen-Solal, Guiramand, & Vignes, 2006; Lanté, de Jésus Ferreira, Guiramand, Récasens, & Vignes, 2006). Moreover, *in vivo* experiments demonstrated that the LFS-induced synaptic potentiation in the CA1 field in anesthetized rats was prevented by systemic or local administration of *N*-methyl-D-aspartate (NMDA) receptor antagonists (Habib & Dringenberg, 2009). Thus, this new form of synaptic plasticity appears to be mediated by mechanisms that are similar to those involved in tetanus-induced long-term potentiation (LTP), which is the electrophysiological basis of learning and memory. Although the physiological significance has not been clarified, Habib and Dringenberg (2010a) suggested that the LFS-induced LTP-like changes in the CA1 field may be a neural mechanism of state-dependent memory processes that involve neuronal oscillations occurring during the acquisition or consolidation phase. This notion led us to speculate that the long-lasting synaptic enhancement observed in response to LFS may be related to fear extinction processes, including the acquisition, consolidation, or retrieval phase.

Based on this hypothesis, the present study elucidated the dynamic changes in hippocampal function that may occur during extinction processes, focusing on LFS-induced synaptic plasticity. Schaffer collaterals stimulation-evoked potential in the CA1 field was monitored in freely moving rats under CFC paradigms. Synaptic changes associated with extinction processes were also examined in a juvenile stress model that exhibits extinction deficits (Judo et al., 2010; Matsumoto et al., 2008). Furthermore, pharmacological approaches were used to examine the possible involvement of NMDA receptors. Finally, to clarify the functional significance of LFS-induced synaptic plasticity, experiments were performed to elucidate the effects of LFS on synaptic transmission and behavioral responses in rats that previously underwent CFC.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (11–14 weeks old) were used. Rats were housed in a room with a 12 h light/dark cycle with constant temperature (21 ± 2 °C). Rat pups were divided into two groups, i.e. non-footshock (Non-FS) control group and a juvenile stress model (Judo et al., 2010). Briefly, pups received an aversive FS (shock intensity, 1.0 mA; intershock interval, 28 s; shock duration, 2 s) for 5 days using FS box (Freeze Frame-41, Neuroscience Co. Ltd., Tokyo, Japan) combined with an automated analysis system (LimeLight-1, Neuroscience Co. Ltd.) during the third postnatal week (PND 21–25; 3wFS group). We chose at least two pups from each litter to serve as controls. Non-FS control group was remained in the FS box for the same time period (12.5 min) without FS stimuli for 5 days. All animal procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Animal Research Committee of Health Sciences University of Hokkaido and were in accordance with National Institutes of Health Guidelines.

2.2. Experimental protocol

During the post-adolescent period (11–14 weeks old), rats were subjected to the contextual fear conditioning (CFC) paradigm (Fig. 1A). Briefly, rats were acclimated to the FS box for 5 min and subjected to five FS (shock intensity, 1 mA; intershock interval, 28 s; shock duration, 2 s) and remained in the FS box for further 5 min without FS stimuli, and then returned to their home cages. The FS box (i.e., conditioning chamber; 50 × 16 × 25 cm [height]; grid floor [diameter of rods, 0.5 cm; spacing, 1.0 cm]) was composed of opaque acrylic. The box was specially constructed by Nihon Koden Co. Ltd. (Tokyo, Japan) for simultaneous determination of electrophysiological and behavioral parameters. The FS conditioning was reinforced by conducting FS stimuli for 2 days (Day 1 and Day 2). Three hours after the second FS stimuli, rats were exposed to the FS box without FS stimuli for 10 min to verify the acquisition of fear memory (Acquisition [Acq]). Twenty-four hours later, rats were re-exposed to the FS box without FS stimuli for 10 min (Extinction trial [Ext]). This trial was repeated three times with 3 h interval (Ext-1, Ext-2, and Ext-3) (Extinction training on Day 3). On the day following extinction training, rats were re-exposed to the FS box for 10 min as extinction retrieval (Retrieval on Day 4). Non-FS control group was divided as follows: No-conditioning group [No-Cond]; Rats that were not subjected to fear conditioning, No-extinction group [Ext(−)]; Conditioned rats that did not undergo extinction training, Extinction group [Ext(+)]; Conditioned rats that underwent extinction training, and LFS applied group [LFS]; Conditioned rats that received LFS in the absence of extinction training on Day 3. Fear related behavior was evaluated by measuring the presence or absence of freezing behavior every 5 s.

2.3. Electrophysiological experiments

Under pentobarbital anesthesia (60 mg/kg, i.p.), a recording electrode was stereotaxically lowered into the CA1 field (5.0 mm posterior, 3.0 mm lateral to bregma, approximately 2.3 mm ventral to dura), and a bipolar stimulating electrode constructed of stainless steel with a tip separation of 500 μm (diameter, 100 μm) was placed in the Schaffer collaterals (3.0 mm posterior, 1.5 mm lateral to bregma, 2.8 mm ventral to dura) via holes drilled on the skull according to the atlas of Paxinos and Watson (1986). The recording electrode and stimulating electrode were implanted in the right ipsilateral side. The potential evoked by test stimulation (frequency, 0.1 Hz; pulse duration, 250 μs) was monitored with an oscilloscope (VC-10, Nihon Koden). The peak population spike amplitude (PSA) was defined following the electrophysiological criteria: (*i*) the peak latency of the PSA is 8–10 ms, (*ii*) the amplitude of evoked potential depends on stimulation intensity (input–output curves), and (*iii*) the negative-trending field potential continues at least 500 μm downward after the first appearance. The evoked potential in the CA1 field disappeared if the stimulating electrode was positioned outside of the Schaffer collaterals. After confirming the appropriate evoked potential, the stimulating and recording electrodes were affixed to the skull with dental cement and quick, self-curing acrylic resin (UNIFAST, gc Corp., Tokyo, Japan). The stimulating electrodes were anchored by small, electrically grounded screws and affixed to the skull as described above.

The electrophysiological experiments combined with behavioral analysis under freely moving conditions were carried out 5 days after surgery. Briefly, 3 h after the second FS stimulus (see Section 2.2), the rats were exposed to the FS box without FS stimuli as the acquisition session, and then they were returned to their homecage. On the next day (Day 3), the stimulating electrode was connected to an electric constant-current stimulator (SEN-3301 or SEN-8203, Nihon Koden, Tokyo, Japan) and an isolator (SS-202J or SS-203J, Nihon Koden) using a cable. Another cable from the recording electrode was connected to a junction box (JH-220H, Nihon Koden) via a transistor connector (JB-220J, Nihon Koden) and an amplifier (gain 1000×, bandpass 0.08–10KHz,