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Spatial performance correlates with long-term potentiation of the dentate gyrus but not of the CA1 region in rats with fimbria–fornix lesions

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Abstract

Although hippocampal long-term potentiation (LTP) is generally assumed to be a cellular mechanism of learning and memory, there has not been definitive evidence for this hypothesis. In the present study, therefore, we addressed the possible relationship between spatial learning ability and LTP by using rats with bilateral fimbria–fornix lesions. The animals were tested for spatial performance in spontaneous alternation behaviors with further in vivo investigation of LTP. The behavioral parameters of spatial memory showed a significant correlation with LTP in the dentate gyrus, but we found no evidence for a linkage with LTP in the CA1 region. Thus, LTP in the dentate gyrus may be important for spatial cognitive ability.

Keywords: Long-term potentiation; Fimbria–fornix; CA1; Dentate gyrus; Learning and memory; Spontaneous alternation behavior; Rat

Long-term potentiation (LTP), which is a long-lasting increase in synaptic strength following brief high-frequency stimulation of afferent fibers in the hippocampus, is the primary experimental model of synaptic plasticity that may underlie learning and memory [12]. Although a wide variety of pharmacologic and genetic studies have implied a linkage of cognitive ability with changes in hippocampal LTP, loss or enhancement of LTP is not universally correlated with learning deficits or surplus [10,13,16]. This discrepancy may be attributable to simple comparisons between different species of animals that received the same treatment. Indeed, most studies using the same animals confirmed a significant correlation between task performance and LTP induction [5,15]. However, these studies employed isolated hippocampal slices to assess LTP. In the in vitro conditions, the behaviors of LTP may not naturally reflect the hippocampal physiology. Furthermore, most of previous studies did not address the regional difference in LTP contributions, while several distinct excitatory pathways in the hippocampus display LTP. To ensure a reliable comparison between learning ability and LTP, therefore, the present study assessed in vivo LTP of the dentate gyrus and the CA1 region soon after measuring task performance of rats.

The fimbria/fornix (FF) is one of the principal fiber tracts in the brain and provides the major afferent and efferent systems for the hippocampus, connecting it with the diencephalon, striatum, basal forebrain, and prefrontal cortex [4]. Bilateral FF lesions in animals are known to produce impairments of spatial working memory [3,14]. Therefore, to enhance a divergence in the ability for spatial performance in the present study, some of the animals received treatment of FF transection before the experiments.

The animal experiments were performed according to the Japanese Pharmacological Society guide for the care and use of laboratory animal. Subjects were male Wistar/ST rats (SLC, Shizuoka, Japan) weighing from 210 to 250 g at the time of surgery. The animals were deeply anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital, and then placed in a stereotaxic headholder. FF lesions were made bilaterally by inserting a razor blade (6.0 mm in width) into the brain (5.0 mm in depth at a position 1.1 mm posterior to bregma). The sham operation was performed with the same surgical procedure except for the blade insertion of 1.0 mm in depth. The intact rats did not receive any surgery.

The behavior experiment was conducted in a Y-shaped

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maze 14 ± 5 days after the surgery. At this time, body weights of the animals were not different among groups: 303.9 ± 8.9 g in intact rats, 295.5 ± 13.1 g in Sham-operated rats, and 279.3 ± 8.5 g in rats with FF lesions ($F(2,44) = 1.37$, $P = 0.3$: one-way analysis of variance (ANOVA)). The three trough-shaped arms (190 mm in width, 672 mm in length, 240 mm in depth) were separated by angles of 120°. Following a 12-h fast, a rat naive to the maze was placed at the end of one arm of the apparatus and allowed to explore the maze for a period of 10 min or up to 12 entries into the arms. Entry was counted when the hind limbs completely entered the arm. Any three consecutive choices of three different arms were considered as an alternation. The percentage of alternation was determined by dividing the total number of alternations by the total number of choices minus two. The memory component in this task is that the rat must remember which arm is more recently visited in order to alternate.

After the behavioral experiments, the rats were allowed free access to pellet chow and water for 60 min. They were then anesthetized with urethane (1 g/kg, i.p.) and α-chloralose (25 mg/kg, i.p.), and fixed in a stereotaxic frame. To record field excitatory postsynaptic potentials (fEPSP), a tungsten recording electrode was inserted into the dentate molecular layer (3.5 mm posterior, 2.0 mm lateral to bregma) or the CA1 stratum radiatum (3.8 mm posterior, 3.0 mm lateral to bregma), and a bipolar stainless steel stimulating electrode was placed along the perforant pathway (8.1 mm posterior, 4.4 mm lateral to bregma) or the Schaffer collaterals (3.8 mm posterior, 2.2 mm lateral to bregma) [11]. Test stimulation (80-μs duration) was applied at intervals of 30 s, and its intensity was adjusted to produce an fEPSP with a slope that was about 50% of maximum. In order to induce LTP, theta-burst stimulation consisting of five burst-like trains (ten pulses at 200 Hz) at 5 Hz was applied four times every 30 s to the perforant pathway or the Schaffer collaterals.

After the electrophysiological experiments, the lesion procedure was validated with Nissl staining and histochemical detection of acetylcholine esterase (AChE) activity. The brains were sagittally cryosectioned at 20-μm thickness. The sections were fixed with 4% paraformaldehyde and 0.2% picric acid for 24 h. Nissl staining was conducted with 10-min incubation with 0.1% cresyl fast violet. AChE activity was detected by a modified thiocholine method of Patre et al. [6]. We found a successful transection of the FF tract (Fig. 1A,B), which always resulted in severely decreased AChE activity in the hippocampus without obvious changes in other regions such as the striatum, the thalamus, or the cerebral cortex (Fig. 1C,D) whereas FF lesions per se induced no apparent loss of hippocampal neurons (Fig. 1A,B).

Intact rats placed in Y-shaped apparatus displayed an excellent alteration ratio in arm choices (Fig. 2A). Similar results were obtained for rats that received sham operations. In rats with FF lesions, however, the alternation behaviors

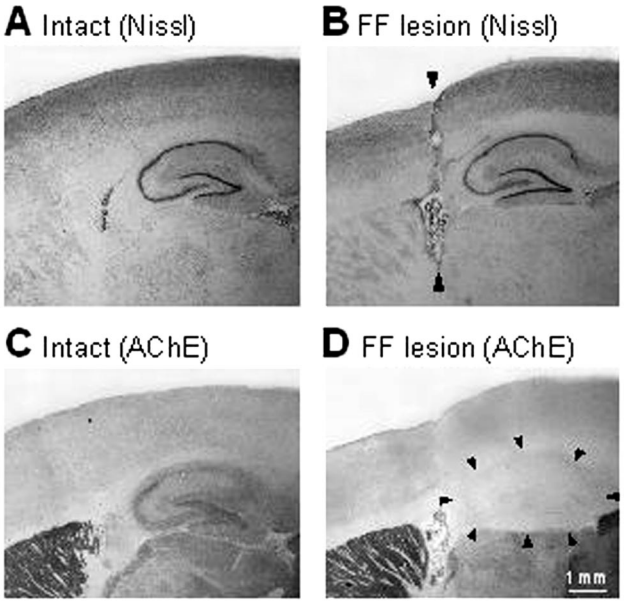

Fig. 1. Representative bright-field microscopic images of sagittal brain sections with Nissl staining (A,B) and histochemical AChE staining (C,D) in intact rats (A,C) and rats with FF lesions (B,D). Arrowheads in the panel B indicate the lesioned site. Arrowheads in the panel D enclose the hippocampus, which does not show evident AChE activity following the FF lesions.

were almost collapsed, the ratio of which was close to a putative chance level of 50% ($F(2,44) = 9.37$, $Q(3,44) = 5.85$, $P < 0.01$ vs. intact group: Tukey's test following one-way ANOVA) (Fig. 2A). No difference in total moving distance during the test was found among group ($F(2,44) = 0.86$, $P = 0.4$: one-way ANOVA). Any groups of rats did not display either preference for a particular arm or variance in the number of arm choices ($F(2,44) = 0.41$, $P = 0.7$: two-way ANOVA). These results suggest that the FF lesions impair task performance relevant to spatial working memory without affecting sensorimotor functions.

To determine whether LTP is altered in rats with FF lesions, fEPSPs evoked in the perforant pathway-DG synapses were recorded (Fig. 2B). The baseline fEPSP slopes before LTP induction were not significantly different among the groups in vivo ($F(2,44) = 2.56$, $P = 0.1$: one-way ANOVA). When theta-burst stimulation was applied to the perforant pathway of intact rats, fEPSP was abruptly enhanced, and LTP was induced in the all cases tested. Similar results were obtained for sham-operated rats. The average percentage of fEPSP slope at 50–60 min after theta-burst stimulation was $132.7 \pm 6.97\%$ in intact rats and $129.7 \pm 7.08\%$ in sham rats. In rats with FF lesions, fEPSPs were facilitated following the same theta-burst stimulation but gradually returned to baseline by 40 min. The average of fEPSP slope at 50–60 min was $104.2 \pm 7.36\%$, which was significantly less than that of intact rats ($F(2,22) = 4.70$, $Q(3,44) = 3.97$, $P < 0.05$: Tukey's test following one-way ANOVA). Using animals that had not received the

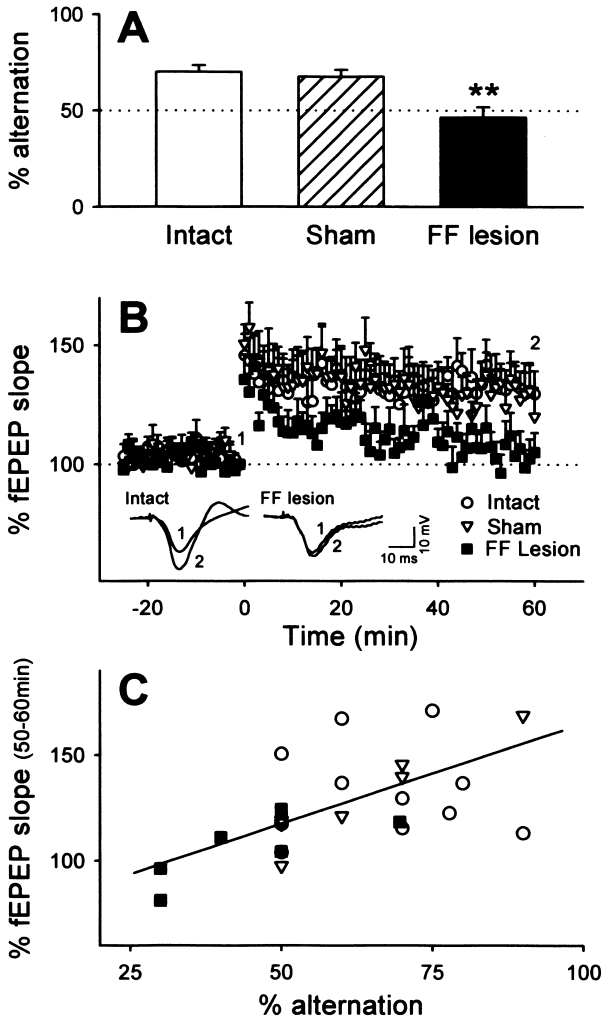

Fig. 2. Correlation between spatial performance and DG LTP. (A) Ratio of spontaneous alternation behaviors was measured in intact rats (open column, $N = 11$), sham-operated rats (hatched column, $N = 7$), or rats with FF lesions (closed column, $N = 7$). A horizontal dashed line shows an expected level for random arm choice (50%). $^{**}P < 0.01$ vs. Intact: Tukey's test following one-way ANOVA. (B) Time course of changes in fEPSP slope in anesthetized rats of intact (open circles), sham operation (open triangles), and FF lesions (closed squares). The fEPSPs evoked by stimulation of the perforant pathway were recorded from the dentate molecular layer. The insets show typical field potentials immediately before (1) and 60 min after theta-burst stimulation (2). The ordinate is expressed as a percentage of baseline values immediately prior to theta-burst stimulation. Data represents means ± SEM of N cases. (C) Scatter plots of correlations between alternation ratio and averaged fEPSP slope (50–60 min following theta-burst stimulation). A solid line shows linear regression for the experimental data ($r = 0.57$, d.f. $= 23$, $P < 0.01$). The same symbols are used as the panel B, and each symbol represents one animal.

Y-maze test, our previous study showed a similar result; DG LTP was attenuated in rats with FF lesions [12]. Therefore, the impaired LTP is not due to the preceding test for spatial performance.

Correlation coefficients between alternation ratios and averaged fEPSP slopes at 50–60 min were calculated. The correlation for combined data collapsing across all three groups showed a good positive correlation between spatial cognitive ability and LTP magnitude ($r = 0.57$, d.f. $= 23$, $P < 0.01$: Pearson correlation coefficients) (Fig. 2C).

When fEPSPs evoked in the Schaffer collateral-CA1 synapses were recorded in rats with FF lesions, robust LTP was induced following theta-burst stimulation (Fig. 3). The averaged fEPSP slopes at 50–60 min were 128.2 ± 3.42% in intact rats, 130.1 ± 7.64% in sham rats, and 134.7 ± 5.7% in rats with FF lesions ($F(2, 15) = 2.68$, $P = 0.2$: one-way ANOVA). There was no significant correlation between spatial learning ability and CA1 LTP across three groups ($r = 0.04$, d.f. $= 13$, $P = 0.9$: Pearson correlation coefficients).

The present study aimed to explore a possible link between spatial memory and LTP in the two major hippocampal subregions, i.e. the DG and the CA1 region. Although a number of studies have addressed the hypothesis that LTP represents a cellular basis of certain types of learning and memory, our study has at least four advantages as follows; (i) the cognitive ability and LTP were both measured in the same animals; (ii) LTP was monitored in vivo; (iii) LTP was assessed in both the DG and the CA1 region; (iv) the assessment of learning ability and LTP was performed on the same day (within a few hours). Using this system, we have shown for the first time that FF lesions causes a selective loss of DG LTP without affecting CA1 LTP, and further that DG LTP is well correlated with behavioral parameters of spatial working memory in spontaneous alternation behaviors. Although our finding does not completely exclude the possibility that CA1 LTP is involved in spatial memory, we consider that LTP induction in the DG may primarily be associated with the cognitive ability.

Consistent with numerous experimental and clinical

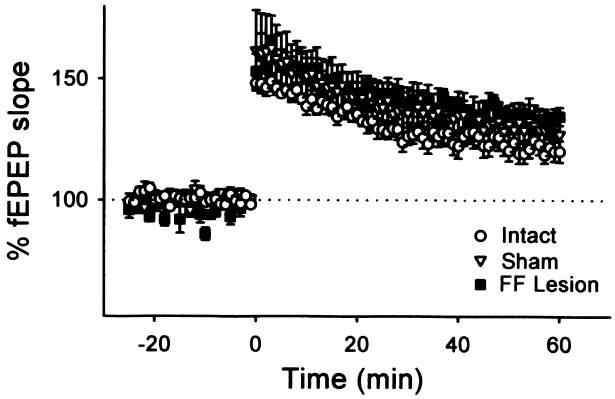

Fig. 3. Normal LTP of the CA1 region in rats with FF lesions. The fEPSPs evoked by stimulation of the Schaffer collaterals were recorded from the CA1 stratum radiatum in anesthetized rats of intact (open circles, $N = 5$), sham operation (open triangles, $N = 6$), and FF lesions (closed squares, $N = 7$). The ordinate is expressed as a percentage of baseline values at time 0. Data are means ± SEM of N cases.

observations that the integrity of the FF tract is important for learning and memory [3,7,8,14], several previous reports showed that the FF lesioning abolishes DG LTP of the population spike in vivo [1,18]. In addition, the present study provided further evidence that DG LTP of the fEPSP is also attenuated after the FF transection. Therefore, FF-mediated modulation of DG LTP is likely to depend on a change in synaptic efficacy of dentate synapses rather than that in the cell excitability (i.e. the firing threshold) of dentate granule cells.

The cognitive deterioration following the FF lesions is generally thought to result from a decrease in cholinergic innervation from the medial septum [4]. Indeed, we confirmed the massive loss of AChE activity in the hippocampus. Hence, the collapsed DG LTP in rats with FF lesions may also be explained by missing cholinergic afferents. From this point of view, the lack of the effect of FF lesions on CA1 LTP is of particular interest because AChE activity was severely reduced in the CA1 region as well as the DG. Considering that the induction of in vitro CA1 LTP depends on the cholinergic neuromodulatory system [2,9,17,19], it is feasible that the in vivo cholinergic effect can be compensated by other mechanisms to support CA1 LTP.

In conclusion, the present study indicates that in vivo DG LTP is a good index of spatial learning ability of animals. Thus, this experimental system provides a unique opportunity to study possible relationships between behavioral, physiological, and pharmacological processes intimately associated with the hippocampal formation.

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