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SHORT COMMUNICATION
Hippocampal long-term depression as an index of spatial working memory

Kazuhito Nakao, Yuji Ikegaya, Maki K. Yamada, Nobuyoshi Nishiyama and Norio Matsuki
Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113–0033, Japan

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Abstract

Long-term potentiation (LTP), a form of synaptic plasticity in the hippocampus, is a cellular model for the neural basis of learning and memory, but few studies have investigated the contribution of long-term depression (LTD), a counterpart of LTP. To address the possible relationship between hippocampal LTD and spatial performance, the spatial cognitive ability of a rat was assessed in a spontaneous alternation test and, thereafter, LTD in response to low-frequency burst stimulation (LFBS) was monitored in the dentate gyrus of the same rat under anaesthesia. To enhance a divergence in the ability for spatial performance, some of the animals received fimbria–fornix (FF) transection 14 days before the experiments. LTD was reliably induced by application of LFBS to the medial perforant path of intact rats, while no apparent LTD was elicited in rats with FF lesions. The behavioural parameters of spatial memory showed a significant correlation with the magnitude of LTD. We found no evidence that the cognitive ability correlated with other electrophysiological parameters, e.g. basal synaptic responses, stimulus intensity to produce half-maximal responses, paired-pulse facilitation or paired-pulse depression. These results suggest that the magnitude of LTD in the dentate gyrus serves as a reliable index of spatial cognitive ability, providing insights into the functional significance of hippocampal LTD.

Introduction

Persistent activity-dependent changes in a synaptic strength of excitatory transmission are believed to be a component of the neural substrates of learning and memory. Most past studies have focused on hippocampal long-term potentiation (LTP), which is a long-lasting increase in synaptic efficacy following brief high-frequency stimulation of afferent fibers in the hippocampus (Bliss & Collingridge, 1993). However, much less is known about the role of long-term depression (LTD) of synaptic strength, which is usually induced by lower-frequency stimulation protocols (Bear & Abraham, 1996). This is partly because homosynaptic LTD could not be reliably induced in acute hippocampal slices prepared from adult animals or *in vivo* preparations (Errington *et al*., 1995). The early evidence for no LTD in adults appeared to cast doubt on the involvement of hippocampal LTD in learning and memory. However, recent studies with substantial improvement of LTD-inducing protocols demonstrated that robust LTD is readily induced in either anaesthetized (Izaki *et al*., 2000) or awake (Manahan-Vaughan, 1997) adult rats, suggesting that information can be stored as LTD-relevant synaptic changes. This idea may be supported by a report from Manahan-Vaughan & Braunewell (1999), in which they show that at hippocampal CA1 synapses the magnitude of LTD is enlarged when low-frequency stimulation is given to a rat during exploration of a novel environment. This LTD-enhancing effect was not obtained for re-exposure to the same environment, which suggests that the induction of LTD is associated with novelty acquisition. In spite of such advances in the reliable induction of LTD *in vivo*, however, no direct comparison of LTD with behavioural parameters has yet been conducted. To elucidate the role of LTD, we therefore investigated the relationship between spatial learning ability and the magnitude of LTD in a rat *in vivo*.

Correspondence: Dr Yuji Ikegaya, as above.
E-mail: ikegaya@tk.airnet.ne.jp

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Materials and methods

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 250 to 300 g at the time of surgery. The animals were deeply anaesthetized with intraperitoneal injection of 50 mg/kg pentobarbital, and then placed in a stereotaxic headholder. The fimbria–fornix (FF) is one of the principal fibre tracts in the central nervous system, and reciprocally connects the hippocampus with the cortical and subcortical areas. Bilateral FF lesions in animals are known to produce impairments of spatial working memory and are thus widely used as a model of dementia (Cassel *et al*., 1997). In the present study therefore some of the animals received FF transection in order to enhance a divergence in the ability for spatial performance. The 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). Sham operation was performed with the same surgical procedure except for the blade insertion to 1.0 mm in depth. Intact rats did not receive any surgery.

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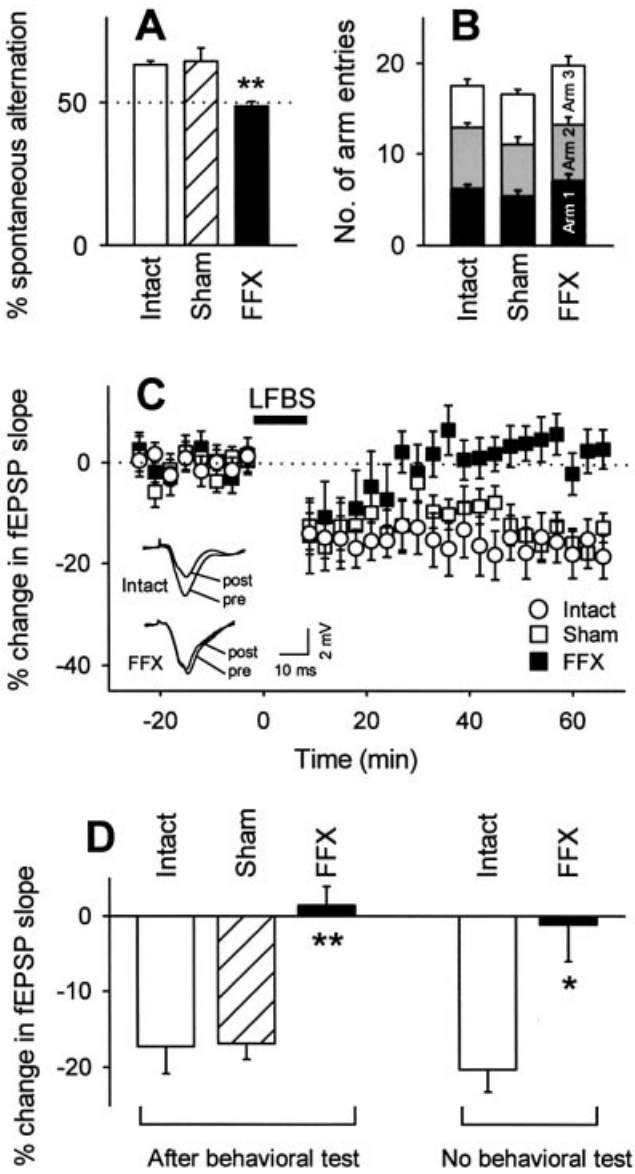

FIG. 1. Impairment of spatial working memory and LTD in the dentate gyrus of rats with FF lesions. (A) Ratio of spontaneous alternation behaviours was measured in intact rats ($n = 8$), sham-operated rats ($n = 7$) or rats with FF lesions (FFX, $n = 9$). The chance level of alternation ratio is assumed to be 50%, indicated by the dotted line. (B) The number of entries into each arm was measured during the behavioural test. (C) Time course of changes in fEPSP slopes in anaesthetized rats of intact (open circles), sham operation (open squares) and FF lesion (closed squares). LFBS was applied to the medial perforant pathway from time 0 min to time 10 min. The insets show typical field potentials immediately before (pre) and 60 min after (post) LFBS application. The ordinate is expressed as a percentage of the average baseline values at time –20 to 0 min. (D) Average changes in fEPSP slopes from 50–60 min after LFBS application was summarized in groups of intact, sham operation or FF lesion that received spontaneous alternation tests (after behavioural test) or without the prior test (no behavioural test). $*P < 0.05$, $**P < 0.01$ vs. Intact or Sham; Tukey's test after one-way ANOVA. Data show the means ± SEM of n cases.

Behaviour experiments were conducted in a Y-shaped maze > 14 days after the surgery (Ikegaya *et al*., 2001). The three trough-shaped arms (190 mm in width, 672 mm in length, and 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. Entry was counted when the hind limbs had completely entered the arm of the maze. 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 2. The memory component in this task is that the rat must remember which arm is more recently visited in order to alternate. This spontaneous alternation behaviour was implicated in spatial working memory (Lalonde, 2002).

After the behavioural experiments, the rats were allowed free access to pellet chow and water for 60 min. They were then anaesthetized 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 inner molecular layer (3.5 mm posterior, 2.0 mm lateral to bregma), and bipolar stainless steel stimulating electrodes were placed along the medial perforant pathway (8.1 mm posterior, 4.0 mm lateral to bregma). Test stimulation (80-μs duration) was applied at intervals of 30 s, and its intensity was adjusted to produce fEPSP with a slope that was ≈ 50% of maximum. In order to induce LTD, low-frequency burst stimulation (LFBS) consisting of 600 bursts (each four pulses at 250 Hz) at 1 Hz was delivered to the medial perforant pathway.

After the electrophysiological experiments, the lesion procedure was validated with Nissl staining and histochemical detection of acetylcholine esterase activity (Nakao *et al*., 2001). The enzyme activity was detected according to a modification of the direct colouring thiocholine method of Di Patre *et al*. (1993). We confirmed that surgical transection of the FF tract induced a decrease in acetylcholine esterase activity in the hippocampus without obvious changes in the other regions (data not shown).

Results

In a Y-shaped maze, intact rats demonstrated an excellent alteration ratio in arm choices (Fig. 1A). Similar results were obtained for rats given sham operations. However, animals with FF lesions displayed a low ratio, which was close to a putative chance level of 50% ($F_{2,21} = 11.02$, $Q_{3,21} = 5.31$, $P < 0.01$ vs. the intact group; Tukey's test after one-way ANOVA) (Fig. 1A). Because it was possible that changes in locomotor activity or preference for a particular arm affected task performance, we simultaneously monitored the number of entries into each arm. No groups displayed a preference in arm choices ($F_{2,42} = 1.76$, $P = 0.19$; two-way ANOVA), nor did the total numbers of arm entries differ significantly among the groups ($F_{2,22} = 1.84$, $P = 0.19$; one-way ANOVA) (Fig. 1B). These results suggest that FF lesions cause deterioration in spontaneous alternation behaviours through impairing spatial working memory rather than changing locomotor characteristics or activity.

After the behavioural test, the animal was anaesthetized and the fEPSPs evoked by stimulation of the medial perforant pathway were recorded from the dentate molecular layer (Fig. 1C). Baseline fEPSP slopes prior to the induction of LTD were not significantly different among the groups *in vivo*; the average slopes 25–0 min before LFBS were 1.89 ± 0.20 mV/ms (intact), 1.78 ± 0.44 mV/ms (sham operation) and 1.91 ± 0.64 mV/ms (FF lesion) (means ± SEM, $F_{2,21} = 1.04$, $P = 0.37$; one-way ANOVA).

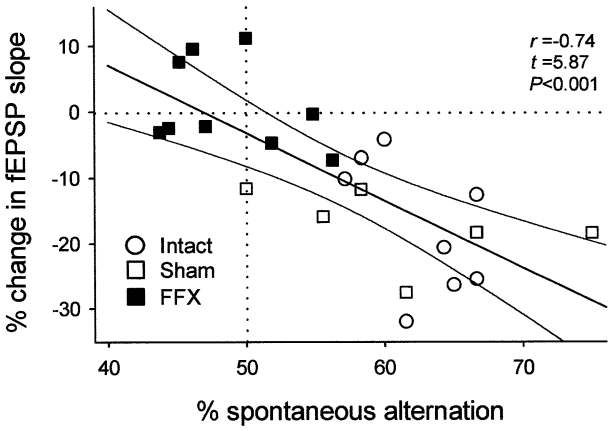

FIG. 2. Correlation between spatial performance and LTD in the dentate gyrus. Scatter plots of correlations between alternation ratios and average changes in fEPSP slopes from 50–60 min after LFBS application. Solid lines are the best linear fit to the data and the 95% confidence bands of the fit ($r = –0.74$, d.f. = 22, $P < 0.01$). Each symbol represents one animal. FFX, FF lesions.

When LFBS was applied to the medial perforant pathway of intact or sham-operated rats, the fEPSPs were immediately reduced, and homosynaptic LTD was induced. The average percentage changes in fEPSP slopes 50–60 min after LFBS stimulation was –18.8 ± 3.9% in the intact group and –17.1 ± 3.3% in the sham-operation group. In rats with FF lesions, the fEPSPs were decreased after the same application of LFBS but gradually returned to baseline within 30 min The average change in fEPSP slopes at 50–60 min was 0.4 ± 3.6%, which was significantly less than that of intact rats ($F_{2,21} = 19.2$, $Q_{3,21} = 7.74$, $P < 0.01$; Tukey's test after one-way ANOVA). Therefore, FF lesions impaired the induction of LTD in the dentate gyrus. The magnitude of LTD observed above was almost the same as that in naïve rats that had not experienced the preceding behavioural test (Fig. 1D).

Correlation coefficients between alternation ratios and mean fEPSP slopes at 50–60 min post-LFBS were calculated using individual data. The correlation for combined data collapsing across all three groups showed a good correlation between spatial cognitive ability and the magnitude of LTD ($r = –0.74$, d.f. = 22, $P < 0.001$) (Figs 2 and 3). However, the LTD magnitude did not correlate with any other behavioural parameters assessed, i.e. the total numbers of arm entries (Fig. 3A), the latencies of the first arm choice (Fig. 3B), the total numbers of rearing behaviours during the test (Fig. 3C), and body weights (Fig. 3D). Likewise, there were no correlations between the alternation ratios and other electrophysiological parameters, i.e. basal synaptic responses (half-maximal fEPSP slopes) (Fig. 3E) or stimulus intensities to produce the half-maximal fEPSP slopes (Fig. 3F). Although the correlation between the alternation ratios and stimulus intensities seemed nearly significant, the LTD magnitudes did not show a good correlation with basal responses (Fig. 3G). Therefore, we do not believe that the correlation of LTD with spatial performance was a secondary consequence of the variety of baseline responses. Paired-pulse facilitation and paired-pulse depression of synaptic transmission are main forms of short-term synaptic plasticity. At perforant path–dentate synapses, they could be evoked predominantly by two succeeding stimuli at intervals of 30 and 400 ms, respectively, but neither of them correlated with the alternation ratios when measured 30 min before (Fig. 3H and I) or 60 min after (Fig. 3J and K) LFBS application.

Discussion

Although LTD is one of the major forms of hippocampal synaptic plasticity, its functional significance in learning and memory remains unclear. Here we have made a direct comparison between LTD and behavioural parameters and have shown for the first time that spatial performance tightly correlates with the magnitude of LTD but not with other electrophysiological parameters, suggesting that LTD of the dentate gyrus could serve as a reliable index of spatial working memory in rats.

Like LTP, homosynaptic LTD in the hippocampus fulfils several crucial criteria for a neuronal basis that is essential for learning and memory, e.g. persistence (Manahan-Vaughan, 1997), synaptic input specificity (Dudek & Bear, 1992), and pre/postsynaptic association (Stanton & Sejnowski, 1989). In earlier neuroscience, nonetheless, LTD was apt to be simply considered a cellular mechanism to extinguish once-established memory (Tsumoto, 1993) or to reverse LTP (Wagner & Alger, 1996). However, Migaud *et al.* (1998) indicated that PSD-95 knockout mice, in which a leftward shift in the frequency–response curve of synaptic plasticity produced a considerable enhancement of LTP and resulted in virtually no induction of LTD, displayed a severe impairment of spatial cognitive ability. More recently, Zeng *et al.* (2001) established transgenic mice in which the activity of the serine/threonine phosphatase calcineurin was disrupted specifically in the adult forebrain. These mutant mice showed a decrease in the magnitude of LTD and an impairment of hippocampus-dependent working memory. These findings suggest that LTD does not merely act to extinguish previous memory or preexisting LTP but does play an active role in memory acquisition, probably by coworking with LTP (Bienenstock *et al.*, 1982; Martin *et al.*, 2000). In this respect, even if the present study does not provide direct evidence for a causal relationship between LTD and learning ability, it does provide another line of support for the association of LTD with learning and memory.

Because brief exposure of animals to novel environments facilitates the induction of LTD, one role of LTD may be relevant to novelty acquisition and object recognition (Braunewell & Manahan-Vaughan, 2001). Considering our finding that LTD correlated with spontaneous alteration ratios without a significant difference in the LTD magnitude between naïve rats and the rats that had received the behavioural test, our data imply an additional role of LTD, i.e. the involvement in spatial working memory. Although the present study did not examine the correlation with reference memory, it is possible that LTD contributes predominantly to working memory. Indeed, forebrain-specific calcineurin knockout impairs LTD and working memory without affecting reference memory (Zeng *et al.*, 2001). Further investigation will be needed to clarify how hippocampal LTD plays a role in working memory.

In conclusion, the present study has demonstrated that the facility to induce LTD at perforant path–dentate synapses is statistically linked to spatial learning ability of animals. Our previous study showed that spatial cognitive ability also correlates with LTP (Nakao *et al.*, 2001), but the coefficient of correlation with LTP ($r = 0.57$) was less excellent than that of LTD ($r = –0.74$). At least in a spontaneous alteration task, therefore, LTD serves as a better index of cognitive ability than does LTP, although most previous studies have dealt mainly with LTP as a cellular basis of learning and memory.

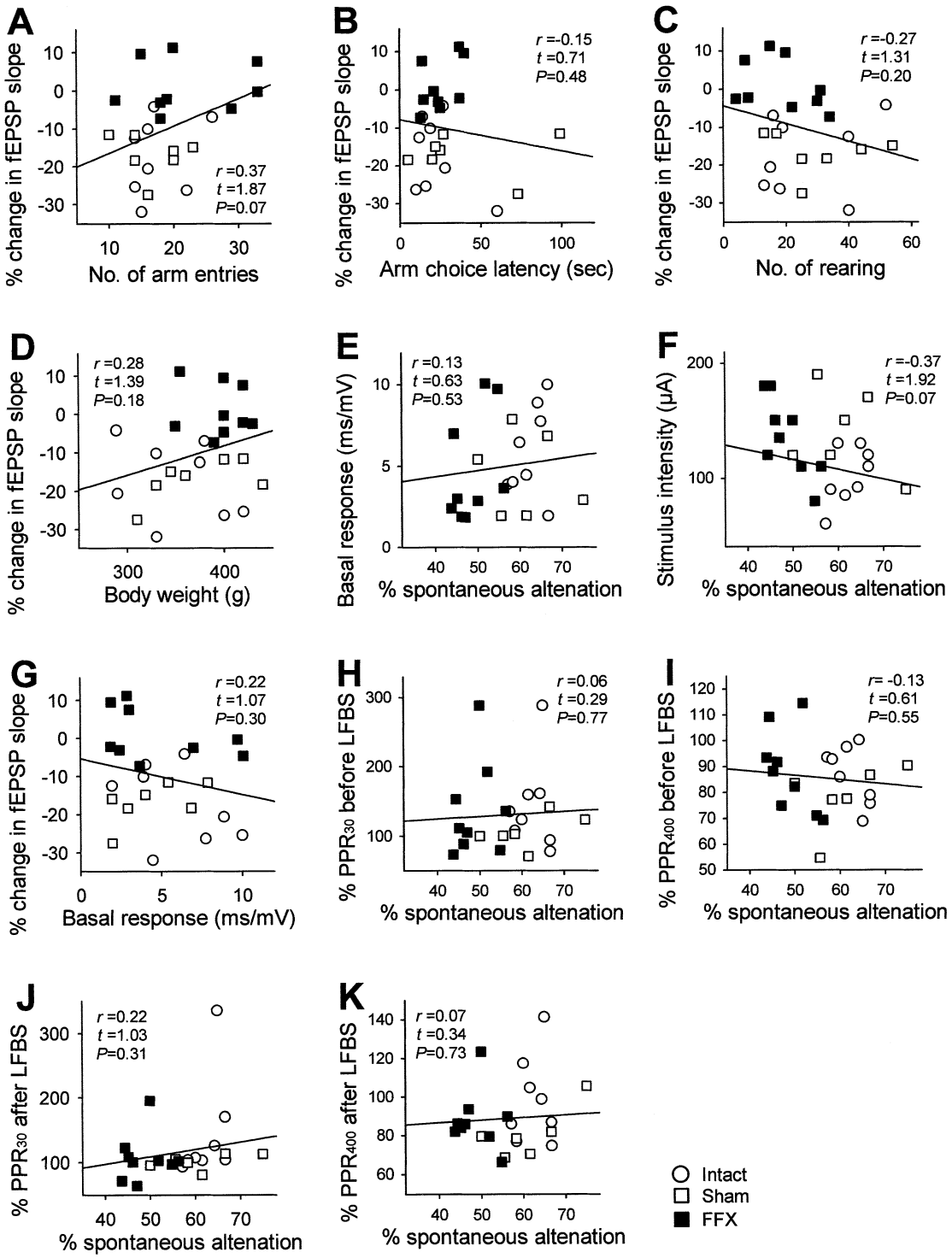

FIG. 3. Neither LTD nor spatial cognitive ability correlates with other parameters. Scatter plots of correlations between behavioural and electrophysiological parameters. Paired-pulse responses (PPR) were measured at paired-pulse intervals of 30 (PPR30) and 400 ms (PPR400), respectively, 30 min before or 60 min after LFBS application and indicated as a ratio of the second fEPSP slope to the first fEPSP slope. Solid lines show linear regression for the experimental data. Each symbol represents one animal. FFX, FF lesions. The values of r, t and P (d.f. = 22) were calculated using Pearson's linear regression test.

Thus, the present study provides new insights into the functional significance of hippocampal LTD and may necessitate revision of interpretations of the role of bidirectional synaptic modification in hippocampal information processing.

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Abbreviations

fEPSP, field excitatory postsynaptic potentials; FF, fimbria–fornix; LFBS, low-frequency burst stimulation; LTD, long-term depression; LTP, long-term potentiation.

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