Neuropharmacology 64 (2013) 65–73

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Hippocampal long-term depression mediates spatial reversal learning in the Morris water maze

Zhifang Dong [a,b,**], Yanrui Bai [a,b], Xiaoyan Wu [a,b], Hongjie Li [a,b], Bo Gong [c], John G. Howland [d], Yan Huang [a,b], Wenting He [a,b], Tingyu Li [a,b], Yu Tian Wang [a,b,c,*]

[a] Ministry of Education Key Laboratory of Child Development and Disorders, Children's Hospital of Chongqing Medical University, Chongqing 400014, PR China
[b] Chongqing Key Laboratory of Translational Medical Research in Cognitive Development and Learning and Memory Disorders, Children's Hospital of Chongqing Medical University, Chongqing 400014, PR China
[c] Brain Research Centre, University of British Columbia, Vancouver, BC V6T 2B5, Canada
[d] Neural Systems and Plasticity Research Group, Department of Physiology, University of Saskatchewan, Saskatoon, SK S7N 3R2, Canada

ARTICLE INFO

Article history:
Received 22 March 2012
Received in revised form
29 May 2012
Accepted 14 June 2012

Keywords:
Hippocampus
NMDA receptor
AMPA receptor
Stress
Interference peptide

ABSTRACT

Synaptic plasticity at hippocampal excitatory synapses has been proposed as the cellular mechanism underlying spatial learning and memory. However, most previous studies have focused on the role of long-term potentiation (LTP) in learning and memory, and much less is known about the role of long-term depression (LTD). Here, we report that hippocampal-dependent spatial learning in the Morris water maze facilitated hippocampal CA1 LTD induction *in vivo*. The LTD can be blocked by systemic application of the selective GluN2B antagonist Ro25-6981 (6 mg/kg, i.p.) or a synthetic peptide Tat-GluA2$_{3Y}$ (3 μmol/kg, i.p.) that interferes with the endocytosis of AMPA receptors. In addition, systemic or intrahippocampal administration of these two mechanistically and structurally distinct inhibitors impaired spatial reversal learning of a novel target location, when the hidden platform was moved to the quadrant opposite the initial target location. Notably, acute elevated-platform stress, which facilitates hippocampal LTD induction, enhanced both acquisition and retrieval of spatial reversal memory. The present study demonstrates that reversal learning is impaired by blocking hippocampal LTD, and enhanced by facilitating hippocampal LTD, suggesting that hippocampal LTD may be necessary and sufficient to mediate new information processing.

This article is part of a Special Issue entitled 'Cognitive Enhancers'.

1. Introduction

It is widely held that the cellular mechanism underlying learning and memory in the brain is activity-dependent synaptic plasticity, e.g., N-methyl-D-aspartate receptor (NMDAR)-dependent long-term potentiation (LTP) and long-term depression (LTD) (Bliss and Collingridge, 1993; Collingridge et al., 2010; Malenka and Nicoll, 1999; Martin et al., 2000). Behavioral experience may generate endogenous synaptic plasticity to dynamically modulate the induction threshold for subsequent hippocampal LTP and LTD (Kemp and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 1999). However, most previous experiments have focused on the potential role of LTP in learning and memory (Lynch, 2004; Malenka and Nicoll, 1999; Martin et al., 2000). Correlations between behavioral experience and LTD modulation have not been extensively investigated. Early speculation that LTD may serve as a reversal mechanism for LTP, or a forgetting mechanism, assumed that LTP encodes memories (Stanton, 1996; Tsumoto, 1993). However, recent reports also indicate that LTD plays important roles in processing new information. For example, hippocampal LTD is facilitated by exposure to a novel environment with novel objects or novel configuration of objects (Kemp and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 1999) and we have recently found that induction of hippocampal CA1 LTD promotes the consolidation of spatial learning in freely moving rats (Ge et al., 2010).

The exact mechanisms underlying the involvement of hippocampal LTD in learning and memory are still not clear, partially due

* Corresponding author. Brain Research Centre, University of British Columbia, F117-2211 Wesbrook Mall, Vancouver, BC V6T 2B5, Canada. Tel.: +1 604 822 0398.
** Corresponding author. Children's Hospital of Chongqing Medical University, 136 Zhongshan Er Road, Yuzhong District, Chongqing 400014, PR China. Tel.: +86 23 63637857.
E-mail addresses: zhifang.dong@gmail.com (Z. Dong), ytwang@brain.ubc.ca, ytwang@interchange.ubc.ca (Y.T. Wang).

0028-3908/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuropharm.2012.06.027

to the difficulty of inducing LTD with classical low frequency stimulation (LFS) protocols in adult animals (Staubli and Scafidi, 1997; Wong et al., 2007; Xu et al., 1998b). To better understand the role of hippocampal LTD in learning and memory, it is necessary to use a behavioral model in which experience may decrease the induction threshold of hippocampal LTD so that the subsequent classical LFS induces LTD. Several groups have recently investigated the potential role of hippocampal LTD in spatial reversal learning in the Morris water maze. Reversal learning occurs following initial spatial learning by changing the location of hidden platform in water maze. A recent report shows that exogenous D-serine enhances GluN2B-dependent LTD as well as reversal learning in mice, although it has no effect on normal water maze memory acquisition (Duffy et al., 2008). Another report shows that transgenic mice lacking NMDAR-dependent LTD display normal spatial learning ability, but exhibit both delayed acquisition of reversal learning and perseveration for the previous location during reversal learning in the water maze (Nicholls et al., 2008). Although these results suggest that NMDAR-dependent LTD may be involved in spatial reversal learning, it has been difficult to obtain a direct causal link between hippocampal LTD and spatial reversal learning. Thus, the use of specific LTD inhibitors to examine the precise contribution of hippocampal LTD in spatial reversal learning is necessary.

In the present study, we use two mechanistically and structurally distinct LTD inhibitors to investigate the role of hippocampal LTD in reversal learning with a combination of electrophysiological and behavioral assessments. We found initial spatial learning in the water maze facilitates hippocampal CA1 LTD induced by classical LFS, which can be blocked by systemic administration of Ro25-6981 and Tat-GluA2$_{3Y}$ peptide. Furthermore, spatial reversal learning is impaired by Ro25-6981 and Tat-GluA2$_{3Y}$ peptide, and enhanced by facilitating hippocampal CA1 LTD with acute stress. These results suggest that hippocampal CA1 LTD is critical for spatial reversal learning.

2. Materials and methods

2.1. Subjects

Adult male Sprague–Dawley rats (300–350 g; obtained from University of British Columbia Animal Care Centre and Chongqing Medical University Animal House Center) were pair-housed in plastic cages in a temperature-controlled (21 °C) colony room on a 12/12 h light/dark cycle. Food and water were available ad libitum. All experiment protocols were approved by the University of British Columbia Animal Care Committee and Chongqing Medical University Animal House Center. All efforts were made to minimize the number of animals used.

2.2. Drugs and treatment procedures

The selective GluN2B antagonist Ro25-6981 was purchased from Sigma–Aldrich Inc. (St Louis, MO, USA). GluA2$_{3Y}$ ($_{869}$YKEGYNVYG$_{877}$ in the carboxyl-terminal region of the AMPA GluA2 subunit) and scrambled ($_{869}$VYKYGGYNE$_{877}$) peptide were synthesized by GL Biochem Ltd. (Shanghai, China). To render the peptide membrane permeable and allow it to be applied systemically, we fused the peptide to the cell membrane transduction domain of the HIV-1 protein to make a Tat-GluA2$_{3Y}$ peptide (YGRKKRRQRRR-$_{869}$YKEGYNVYG$_{877}$) or scrambled Tat-GluA2$_{3Y}$ peptide (YGRKKRRQRRR-$_{869}$VYKYGGYNE$_{877}$). Ro25-6981 (6.0 mg/kg for i.p. and 0.5 nmol/μl per side for intrahippocampal infusion) and the peptides (3.0 μmol/kg, i.p. and 100 pmol/μl per side for intrahippocampal infusion) were dissolved in 0.9% sterile saline and applied before the first trial of each reversal training day or low frequency stimulation (LFS) in electrophysiological recording.

2.3. Electrophysiological recording

Field EPSPs from the CA1 region of the hippocampus were recorded using techniques described previously (Dong et al., 2006b; Wong et al., 2007). Rats were anesthetized with urethane (1.5 g/kg, i.p.; Sigma, St. Louis, MO) and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). Rectal temperature was maintained at 36.5 ± 0.5 °C over the course of the experiment with a homeothermic blanket (Harvard Apparatus, Holliston, MA). The scalp was opened, and trephine holes were drilled for the recording (AP −3.5 mm, ML 2.8 mm) and stimulating electrodes (AP −4.2 mm, ML 3.5 mm). A third hole was drilled anterior and lateral to bregma for the recording electrode ground wire. The recording electrode (130 μm Teflon-coated platinum iridium wire; A-M Systems Inc., Carlsborg, WA) was lowered into the CA1 region, and the stimulating electrodes (tungsten bipolar-stimulating electrode; FHC Inc., Bowdoin, ME) were placed in the Schaffer collaterals of the dorsal hippocampus through the holes. Stimulation pulses (0.12 ms) were generated with an A/D analog digital converter (BNC-2090; National Instruments, Austin, TX) and a digital stimulus isolation unit (Getting Instruments, San Diego, CA). Field EPSPs were recorded with a differential AC preamplifier (Model P55, Grass Technologies, Astro-Med, Inc.) and LabVIEW data acquisition system (National Instruments, Austin, TX). Test EPSPs were evoked at a frequency of 0.033 Hz and at a stimulus intensity adjusted to around 50% of the maximal response size. Low frequency stimulation protocol (LFS) consisted of 900 pulses at 1 Hz. EPSP amplitude was expressed as mean ± SEM % of the baseline EPSP slope recorded over at least a 40-min baseline period, and those in the last 5 min recordings were averaged in one animal and then across animals to give baseline response for the group. In the control group (untrained, Fig. 1A), rats were subjected to swimming in the pool with the hidden platform absent for 4 trials per day with 10 min inter-trial intervals for 4 consecutive days. Swim time was matched to the platform search time in the Morris water maze learning group (MWM, Fig. 1A). Immediately after the last trial of spatial learning, rats were anaesthetized with urethane for fEPSP recording. Following surgery and baseline recording (~1.5 h), LFS was delivered. When necessary, Ro25-6981 or Tat-GluA2$_{3Y}$ peptide was injected 1 h before delivery of LFS (Fig. 1C).

2.4. Bilateral hippocampal microinjection

Rats were chronically implanted with cannulae above dorsal hippocampus as previously described (Dong et al., 2006a; Ge et al., 2010). Briefly, under sodium pentobarbital anesthesia (60 mg/kg, i.p.), rats were implanted with two 22 Ga stainless steel guide cannulae (10 mm; Plastics One Inc., Roanoke, VA) above the dorsal hippocampus (3.8 mm posterior to bregma, 2.5 mm lateral to the midline and 2.5 mm below the surface of the dura) that were fixed to the skull with four jeweler's screws and dental cement. Sterile dummy cannula (30 Ga stainless steel rod, 10 mm, Plastics One Inc.) was inserted into guide cannula to avoid bacterial infection and cerebral spinal fluid leakage through the cannula. All rats were allowed to recover for 7 days before behavioral experiments.

On the day before experiments, the animals were placed in the experiment room and given a sham intrahippocampal injection to get acclimatized to the injection procedure. Dummy cannulas were removed and the rats were placed into a Plexiglas injection box (25 × 45 × 25 cm, same as homecage) with 30 Ga injection cannulas in their guide cannulas. Injection cannulae (11 mm, Plastics One Inc., Roanoke, VA) were connected to a microsyringe pump (Harvard Apparatus) by PE-50 tubing, extended 1 mm beyond the tip of the guide cannulae.

Drugs were injected with 10-μl Hamilton syringes and a microsyringe pump at 0.5 μl/min for 2 min. After injection, the injection cannulas were left in place for an additional minute to allow the diffusion of the drug away from the cannula tips. The rats were then removed from the injection box, their dummy cannulae replaced, and they were placed back in their home cages. Cannulae placement was verified by histological examination of the brain after methylene blue injection (1 μl per side), and only data obtained from rats with correctly inserted cannulas were included in statistical analysis.

2.5. Water maze tests

Spatial learning and memory was performed in the Morris water maze using procedures similar to those described previously (Ge et al., 2010; Wong et al., 2007). The Morris water maze consisted of a circular fiberglass pool (200 cm diameter) filled with water (25 ± 1 °C) made opaque with black non-toxic paint. The pool was surrounded by light blue curtains, and three distal visual cues were fixed to the curtains. Four floor light sources of equal power provided uniform illumination to the pool and testing room. A CCD camera suspended above the pool center recorded the swim paths of the animals and video output was digitized by an EthoVision tracking system (Noldus, Leesburg, VA). The water maze tests include 3 periods: initial spatial training, spatial reversal training and probe test.

Initial spatial training: Twenty-four hours before spatial training, animals were allowed to adapt to the maze for a 60 s free swim. Rats were then required to swim to find a hidden platform (15 cm × 15 cm, located at NW) submerged 1 cm below the water surface. Rats were placed into the water facing the pool wall at four starting positions (N, S, E, and W). Animals were then trained in the initial spatial learning task for 4 trials per day with 10 min inter-trial intervals for 4 consecutive days. During each trial, rats were allowed to swim until they found the hidden platform where they remained for 20 s before being returned to a holding cage. Rats that failed to find the hidden platform in 60 s were guided to the platform where they remained for 20 s.

Spatial reversal training: After the initial spatial learning task, a reversal learning protocol was conducted with some rats. During reversal learning, the hidden platform was moved to the opposite quadrant (e.g., from NW to SE). Reversal learning entailed 3 additional days with 4 trials per day, similar to initial training. Rats exposed