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Activity-dependent alteration of the morphology of a hippocampal giant synapse

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ABSTRACT

Activity-dependent synaptic plasticity is a fundamental cellular process for learning and memory. While electrophysiological plasticity has been intensively studied, morphological plasticity is less clearly understood. This study investigated the effect of presynaptic stimulation on the morphology of a giant mossy fiber-CA3 pyramidal cell synapse, and found that the mossy fiber bouton altered its morphology with an increase in the number of segments. This activity-dependent alteration in morphology required the activation of glutamate receptors and an increase in postsynaptic calcium concentration. In addition, the intercellular retrograde messengers nitric oxide and arachidonic acid were necessary. Simultaneous recordings demonstrated that the morphological complexity of the presynaptic bouton and the amplitude of excitatory postsynaptic currents were well correlated. Thus, a single mossy fiber synapse has the potential for activity-dependent morphological plasticity at the presynaptic bouton, which may be important for learning and memory.

1. Introduction

Changes in synaptic efficacy are essential for information processing in the brain, and underlie mechanisms required for learning and memory. Activity-dependent electrophysiological synaptic plasticity, including long-term potentiation (LTP) and long-term depression, has been intensively studied, and knowledge of the cellular and molecular mechanisms has been accumulated (Kim and Linden, 2007; Malenka and Bear, 2004). In contrast, activity-dependent morphological synaptic plasticity, which is typically expressed as sprouting and remodeling of synapses, has been characterized to a lesser extent. Morphological changes in pre- and post-synaptic structures following various types of stimulation, including tetanus and intense experience, were first observed by electron microscopy in several brain regions (Bell et al., 2014; Fifková and Van Harreveld, 1977; Globus et al., 1973; Toni et al., 1999; Zhao et al., 2012). Subsequently, time-lapse imaging was introduced for the observation of synapses in the hippocampus and the cerebral cortex (De Paola et al., 2003; Engert and Bonhoeffer, 1999; Grutzendler et al., 2002; Kwon and Sabatini, 2011; Maletic-Savatic et al., 1999; Trachtenberg et al., 2002; Upreti et al., 2012). However, the majority of studies have evaluated morphological plasticity by stimulating multiple presynaptic inputs, and the specificity and reliability of the stimulations were not precisely assessed. Therefore, it remains unclear whether morphological changes are induced in a specific manner, directly at the stimulated synapse/s. Furthermore, the contribution of activity-dependent morphological plasticity to synaptic function is yet to be fully understood. A recent study used uncaging of caged glutamate to stimulate postsynaptic spines: repetitive stimulation induced rapid and persistent spine enlargement, which was associated with LTP at the CA1 Schaffer collateral synapses of the hippocampus (Matsuzaki et al., 2004). In contrast, morphological changes at presynaptic boutons in response to action potential firing have not been analyzed by time-lapse imaging. To elucidate the mechanisms of activity-dependent synaptic plasticity, detailed analyses of morphological plasticity at pre- and post-synapses are awaited.

The mossy fibers, which are the axons of dentate granule cells, form giant synapses with the dendrites of CA3 pyramidal cells. Each mossy fiber bouton is large, having a diameter of 4–8 μm, approximately 25 neurotransmitter release sites, and approximately 20,000 vesicles (Rollenhagen et al., 2007). Because of its enormous size, location on the proximal dendrite, and large synaptic responses (Bischofberger et al., 2006; Henze et al., 2000), each synaptic transmission via a single

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mossy fiber is thought to reliably evoke an action potential in a CA3 pyramidal cell, allowing information to flow efficiently. Despite the functional importance of mossy fiber synapses for information transfer in the hippocampus, dynamic changes in their morphology have not been studied, especially in response to presynaptic stimulation.

To obtain further insight into activity-dependent synaptic plasticity of mossy fiber synapses, we stimulated a single dentate granule cell reliably with a patch pipette under time-lapse imaging, and demonstrated for the first time activity-dependent morphological plasticity of a presynaptic bouton at a mossy fiber synapse, which was characterized by segmentation of the presynaptic bouton. Moreover, the signaling mechanisms for this morphological plasticity were determined, and a correlation was identified between the morphological and electrophysiological properties of the mossy fiber synapses, as evaluated by simultaneous recording of the morphology of a single bouton and its unitary excitatory postsynaptic currents (EPSCs).

2. Materials and methods

2.1. Mice

Wild-type C57BL/6J mice were obtained from CLEA Japan, Inc. (Tokyo, Japan) (http://www.clea-japan.com/en/animals/animal_f/f_02.html). The transgenic mouse line SLICK (line V) is described elsewhere (Young et al., 2008), and was obtained from Jackson Laboratory (Bar Harbor, ME) (007610 B6;SJL-Tg(Thy1-cre/ERT2,-EYFP)VGfng/J, http://jaxmice.jax.org/strain/007610.html). The day of birth was defined as P0. All animal procedures were approved by the Animal Care and Use Committee at Kobe University Graduate School of Medicine (Permit Numbers: P110802 and P140614).

2.2. Slice culture preparation

All experiments were conducted with hippocampal slice cultures prepared from P5–6 mouse pups. Hippocampal slice cultures were prepared as previously described (Gähwiler et al., 1997). Briefly, hippocampal slices were sectioned at 400 μm, attached to glass coverslips using clotted chicken plasma (Japan Biotest, Saitama, Japan), placed in sealed test tubes with serum-containing medium, and placed in a roller-drum incubator at 36 °C for 14–18 days.

2.3. Drugs and chemicals

D-aminophosphonovaleric acid (D-APV), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 1-(4-Aminophenyl)-3-methylcarbamyl-4-methyl-3,4-dihydro-7,8-methylenedioxy-5*H*-2,3-benzodiazepine hydrochloride (GYKI 53655), α-methyl-4-carboxyphenylglycine (MCPG), and DL-*threo*-β-benzyloxyaspartate (TBOA) were purchased from Tocris Cookson (Bristol, UK). NG-nitro-L-arginine methyl ester (L-NAME) was from Dojindo Laboratories (Kumamoto, Japan). (5*S*,10*R*)-(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (MK-801), ***N***-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251) and arachidonyl trifluoromethyl ketone (AACOCF3) were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Alexa Fluor 594 was from Thermo Fisher Scientific (Waltham, MA). ATP, CrP, EGTA, and GTP were from Sigma-Aldrich (St. Louis, MO). BAPTA was from Cayman Chemicals Co. (Ann Arbor, MI).

2.4. Imaging

Cultures were transferred to a recording chamber mounted on a two-photon laser-scanning microscope, FV1000 (Olympus, Tokyo, Japan). To visualize the mossy fiber boutons, Alexa Fluor 594 (40 μM) was added to the pipette solution. Twenty-five minutes after the whole-cell recording of a granule cell was obtained, several mossy fiber boutons of the granule cells were identified, and their morphology was imaged using the two-photon laser-scanning microscope system with a 25× water immersion objective specially designed for the system (XLPLN25XWMP, NA:1.05). Non-saturating imaging parameters were set on each time-lapse series. To appropriately compare images at different time points, mossy fiber axonal shafts were set as internal references and laser intensities were adjusted to obtain the identical signal intensity throughout a time-lapse series.

2.5. Electrophysiology

Slices were superfused with an external solution (pH 7.4) containing 148.8 mM Na^+, 2.7 mM K^+, 149.2 mM Cl^-, 2.8 mM Ca^{2+}, 2.0 mM Mg^{2+}, 11.6 mM HCO_3^-, 0.4 mM $H_2PO_4^-$, and 5.6 mM D-glucose (pH 7.4). All experiments were performed at 34 °C. Recordings were obtained from dentate granule cells and CA3 pyramidal cells of the hippocampus with patch pipettes (2–5 MΩ) using an EPC 10 amplifier (HEKA Elektronik, Lambrecht, Germany). For granule cells, pipettes were filled with a solution containing 135 mM K-gluconate, 5 mM KCl, 10 mM Hepes, 1 mM EGTA, 2 mM Mg-ATP, 5 mM creatine phosphate (CrP), 0.4 mM GTP, 0.07 mM $CaCl_2$, pH 7.2. For CA3 pyramidal cells, pipettes were filled with a solution containing 121.6 mM CsF, 8.4 mM CsCl, 10 mM Hepes, 1 mM EGTA, 1 mM picrotoxin, 2 mM Mg-ATP, 5 mM CrP, 0.4 mM GTP, 0.07 mM $CaCl_2$, pH 7.2. Alexa Fluor 594 (40 μM) was included for imaging the mossy fiber boutons of the granule cell recorded in slices from wild-type mice. Tetramethyl rhodamine dextran (0.1%) was included in the pipette solution to visualize a postsynaptic CA3 pyramidal cell. The actual membrane potentials were corrected for the liquid junction potential. Presynaptic action potentials were evoked by injecting a depolarizing current (1 ms, 1.5–2.5 nA) at 0.05 Hz, unless otherwise specified. TBOA was dissolved in Tyrode's solution. D-APV, GYKI 53655, CNQX, and L-NAME were dissolved in water and diluted in a ratio of 1 to 1000 in Tyrode's solution at the use. MCPG was first dissolved at 100 mM in 1 M NaOH solution and then diluted in a ratio of 1 to 100 in Tyrode's solution at the use. AACOCF3 and AM251 were dissolved in DMSO and diluted in a ratio of 1 to 2500 in Tyrode's solution at the use. L-NAME, AACOCF3, or AM251 was applied 1.5 h prior to stimulation and was included in the external solution throughout the experiments. Series resistance (typically between 5 and 15 MΩ) was regularly monitored, and the cells were excluded if a change of more than 20% occurred.

2.6. Data acquisition and analysis

Electrophysiological signals were filtered at 5 or 10 kHz, digitally recorded using PATCHMASTER software (HEKA Elektronik) and stored on hard disks for later analysis. Numerical data in the text were expressed as mean ± S.E.M. The two-tailed paired or unpaired Student's *t*-test was used to compare values when appropriate. Morphological signals obtained by the two-photon laser-scanning microscope were deconvolved using AutoQuant X3.0 software (Media Cybernetics) and analyzed with Imaris software (Bitplane AG). The complexity of morphology of the boutons was quantified by two methods: (1) semi-automatic counting of the number of seed points using the imaging software Imaris, which represented the number of segments of a bouton (Kremer et al., 2010) (Fig. 1C, D); and (2) two-dimensional (2D) analysis of Z-axis maximum projection images for the perimeter and area of the mossy fiber boutons. HFS in the presence of TBOA induced a significant increase in the perimeter, but not in the area of the mossy fiber boutons, indicating an increase in complexity consistent with the above segment analyses (Fig. 1E, F). Segmentation analysis was conducted as follows: first, surface-rendered three-dimensional (3D) volumes were constructed using identical settings, and expanded to just mask the original images of the boutons. Second, seed points at a minimum of 0.6 μm were