Manipulation of hippocampal CA3 firing via luminopsins modulates spatial and episodic short-term memory, especially working memory, but not long-term memory

Da Song[a,1], Qinghu Yang[a,b,1], Yiran Lang[c], Zhaosen Wen[a], Zhen Xie[a], Da Zheng[a], Tianyi Yan[a], Yujun Deng[a], Hiroshi Nakanishi[d], Zhenzhen Quan[a,*], Hong Qing[a,*]

[a] School of Life Science, Beijing Institute of Technology, Beijing 100081, China
[b] College of Life Sciences & Research Center for Resource Peptide Drugs, Shaanxi Engineering & Technological Research Center for Conversation & Utilization of Regional Biological Resources, Yanan University, Yanan 716000, China
[c] Beijing Advanced Innovation Center for Intelligent Robots and System, Beijing Institute of Technology, Beijing 100081, China
[d] Laboratory of Oral Aging Science, Faculty of Dental Sciences, Kyushu University, Fukuoka 812-8582, Japan

ARTICLE INFO

ABSTRACT

The CA3 subregion of the hippocampus is important for rapid encoding, storage and retrieval of associative memories. Lesions and pharmacological inhibitions of hippocampal CA3 suggest that it is essential for different memories. However, how CA3 functions in spatial and episodic memory in different time scales (i.e. short-term versus long term) without permanent lesions has not been systematically investigated yet. Taking advantage of the chemogenetic access to opsins, this study used luminopsins, fusion proteins of luciferase and optogenetic elements, to manipulate neuronal activity in CA3 during memory tasks over a range of spatial and temporal scales. In this study, we found that excitation or inhibition of CA3 neurons had no significant effects on long-term spatial or episodic memory, but had remarkable effects on spatial working memory, spatial short-term memory as well as episodic short-term memory. In addition, stimulation of CA3 neurons altered the expression levels of NR2A. Intracerebral injection of receptor inhibitors further confirmed that NR2A is crucial to spatial working memory, which is consistent with the luminopsins experiments. These findings indicate that CA3 maintains a specific role on spatial and episodic memory over a short period of time.

1. Introduction

Hippocampus is a locus for learning and memory, which is composed of various complex neural circuits. The hippocampal CA3 neurons are characterized by their ability to connect to themselves and receive less than one-third of the inputs from other cell populations, forming an auto-associative network, known as associative/commissural (A/C) loop (Amaral, Ishizuka, & Claiborne, 1990; Rebola, Carta, & Mulle, 2017). This recurrent network is supposed to modulate associative memories that are stored and recalled through pattern completion (Mcnaughton and Morris, 1987; Rebola et al., 2017; Rolls, 2007), and also for episodic and event memory (Schapiro, Turk-Browne, Botvinick, & Norman, 2017; Schwindel, Navratilova, Ali, Tatsuno, & McNaughton, 2016). In addition, sparse and powerful excitatory inputs from the dentate gyrus to CA3 by the mossy fiber

pathway are considered to assist in the new memory encoding in CA3 through pattern separation. The direct connections from the entorhinal cortex to CA3 are purposed to contribute to the retrieval of information from CA3, when incomplete information is provided (Leutgeb and Leutgeb, 2007; Rebola et al., 2017; Treves and Rolls, 1992). According to the computational theories, CA3 is responsible for spatial rapid one-trial learning, arbitrary association learning where space is a component, pattern completion, and spatial short-term memory (Rolls and Kesner, 2006, 2016; Rolls, 2007). However, whether CA3 plays any role in spatial and episodic memories in different time scales has not been systematically studied.

Recently, optogenetics has revolutionized the study of neural circuits and is being widely used in neuroscience to selectively modulate neuronal activity through short-term light stimulation of specifically expressed channelrhodopsins (ChR) (Yamamoto et al., 2015; Yizhar

---

et al., 2011). The optogenetic silencing of CA3 pyramidal cells impairs spatial short-term memory, but does not affect hippocampus-independent visual (non-spatial) long-term memory (Shipton et al., 2014). However, optogenetics has significant limitations for clinical applications due to the implantation of invasive optical fibers (Fenno, Yizhar, & Deisseroth, 2011). Luminopsins were developed via integration of opto- and chemogenetics that fuses bright *Gaussia* luciferase variants with ChR to stimulate neurons (Luminescent opsin, LMO3) or a proton pump to inhibit neurons (inhibitory LMO, iLMO), which can be manipulated by emission of bioluminescence through substrate application (Coelenterazine, CTZ). Furthermore, it has the advantage that all neurons expressing LMOs can be completely engaged by using CTZ rather than the limited number within reach of a light fiber with limited reach. This has been confirmed to be able to manipulate the activity of neurons over a range of spatial and temporal scales (Berglund et al., 2016). Several studies have used LMOs to manipulate rotational behavior in rodents (Berglund et al., 2016; Berglund, Birkner, Augustine, & Hochgeschwender, 2013; Park et al., 2017; Tung, Gutekunst, & Gross, 2015). However, this technique has not yet been employed for the analysis of memory, which motivated us to manipulate the activity of CA3 neurons in memory tasks to assay the potential changes in tasks performance.

Glutamatergic receptors are thought to mediate long-term synaptic plasticity, which is crucial to learning and memory (Liu et al., 2004; Malinow and Malenka, 2002). Stimulation of neurons would change the synaptic plasticity, along with the expressional alterations of synaptic related proteins, especially the excitatory synaptic receptors, such as AMPA (ARs) and NMDA receptors (NRs) (Liu et al., 2004; Malinow and Malenka, 2002). Deletions of NRs restricted to CA3 pyramidal cells of adult mice, were reported to impair short-term memory tasks but were found to cause no changes in long-term memory tests (Nakazawa et al., 2003). Due to the structural particularity of CA3, the input from left CA3 pyramidal neurons forms NR2 synapses on the apical dendrites but the input from right CA3 pyramidal neurons forms NR2 synapses on the basal dendrites (Kawakami et al., 2003). Therefore, it is indispensable to illustrate the function of different NR2 subunits in CA3 during memory tasks. And it is intriguing to speculate if stimulation of CA3 neurons via LMOs could alter the NR2 expression levels.

In this study, we employed excitatory and inhibitory luminopsins as powerful tools for studying neuronal networks, particularly the function of CA3 in spatial memory and episodic memory. Experiments herein show that excitation or inhibition of CA3 neurons by luminopsins seldom affect either spatial or episodic long-term memory, but strongly influence spatial working and short-term memory and episodic short-term memory. Furthermore, activation of CA3 neurons significantly increases the mRNA levels of NR2A and NR2B. However, inhibition of CA3 neurons only changes the expression of NR2A. Lastly, the pharmacological experiment suggested that NR2A is critical for spatial working memory in CA3. It reveals that interference of CA3 neurons, either activated or inhibited, would disturb the neural network in CA3 and thereby influences short-term memories, rather long-term memories.

2. Materials and methods

2.1. Animals

C57BL/6 mice (2–3 months old) housed in a vivarium with a 12 h light, 12 h dark cycle (with no more than four mice per cage) were used for analysis in accordance with protocols approved by the Beijing Institute of Technology, Beijing, and by the governmental regulations of China.

2.2. Virus construct and preparation

LMO3 and iLMO plasmids were generous gifts from Ute

Hochgeschwender (Central Michigan University) (Berglund et al., 2013, 2016). LMO3 consists of *Gaussia* luciferase variant (sbGLuc) and Volvox channelrhodopsin 1 (vChR1) (also named pAAV-hSyn-sbGLuc-vChR1-EYFP), which can excite neurons. iLMO consists of *Gaussia* luciferase variant (slGLuc) and proton pump from the fungus Leptosphaeria maculans (Mac) (also named pAAV-hSyn-slGLuc-Mac-EGFP), which can suppress neuronal activity.

LMO3 and iLMO Virus were prepared as follows: LMO3 and iLMO vectors were co-transfected with pH22 and pfd6 into 293T Cells by Calcium Phosphate Cell Transfection Kit (C0508, Beyotime). 72 h later, cells were collected, lysed by sodium deoxycholate, and purified by HiTrap™ Heparin HP (17-0406-01, GE healthcare). The titer of virus was measured by Quantitative Real-time PCR (RT-qPCR), and titers of virus ranging from 2×10^{12} to 3×10^{12} v.g./ml were used in this study.

2.3. Surgery and virus injections

Mice were anesthetized with sodium pentobarbital (50 mg/kg) for stereotaxis. Injections were targeted bilaterally to the CA3 (-2.06 mm anteroposterior (AP), ± 2.35 mm mediolateral (ML), -2.35 mm dorsoventral (DV)). Injection volumes of virus (LMO3, iLMO) and saline (vehicle) were 400 nl. Viruses were injected with a 100 nl/min rate by using a 10 µl Hamilton microsyringe. The needle was lowered to the target site and remained for 2 min before injection and for 5 min after injection. Mice were allowed to recover for 4 weeks before behavioral experiments. After 1 h of behavioral tests, the mice were anesthetized with sodium pentobarbital (50 mg/kg) and perfused with saline. Brains were then taken and placed in 4% paraformaldehyde (PFA) for two days, and transferred into 30% sucrose at 4 °C. Brains were sectioned with a width of 30 µm using a cryostat (Leica), incubated with DAPI, and imaged using a fluorescent microscope (Olympus).

2.4. Electrophysiology recording in vivo

Five weeks after injection of LMO3 or iLMO into CA3, animals were anesthetized with isoflurane (2% for induction and 0.5% for maintenance) and the heads were fixed in a stereotaxic frame. Single electrode (Cat. No. 366-130605-00, Alpha Omega, USA) was implanted in the CA3. Then the spike and local field potential were recorded via Blackrock Microsystems. Before applying CTZ by intraperitoneal injection (i.p., 150 µg/mouse), baseline spiking was recorded for 10 min, and then continued for 30 min. The raw signals of neurons were firstly denoised and sorted with offline sorter (v4.2, plexon, USA).

2.5. Drug preparation and administration

Coelenterazine, shortened as CTZ, was dissolved in 10% ethanol in saline to a final concentration of 2.36 mM. Acting as a substrate of *Gaussia* luciferase, it has the ability to cross the blood brain barrier, thus being selected to inject in the periphery and generating bioluminescence that is capable of activating opsins and initiating specific motor behavior in mice (Berglund et al., 2016). D-AP5 (D (−)-2-Amino-5-phosphonopentanoic acid), a general NR antagonist, was purchased from Abcam (Cat. No. ab120003). D-AP5 was dissolved in sterilized saline with a final concentration of 38 mM. PEAQX tetrasodium hydrate ([[(1S)-1-(4-bromophenyl) ethyl] amino] (1,2,3,4-tetrahydro-2,3-dioxo-5-quinoxalinyl) methyl] phosphonic acid tetrasodium hydrate), a competitive NR2A-preferring antagonist, was obtained from Sigma-Aldrich (Cat. No. P1999). PEAQX was dissolved in 10% DMSO in saline with a final concentration of 3.7 mM. Ro 25–6981 maleate ((αR, βS)-α-(4-Hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinepropanol maleate), a noncompetitive NR2B-selective antagonist, was obtained from Tocris Bioscience (Cat. No. 1594) and dissolved in saline to a final concentration of 43.9 mM (Felix-Ortiz and Tye, 2014; McQuail et al., 2016). The cannula was implanted in CA3 and mice were sent back to