Neurobiology of Learning and Memory 138 (2017) 182–197

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Persistent modifications of hippocampal synaptic function during remote spatial memory

Alice Pavlowsky [a,b,1,2], Emma Wallace [c,2], André A. Fenton [b,d,e,*], Juan Marcos Alarcon [a,b,*]

[a] Department of Pathology, The Robert F. Furchgott Center for Neural and Behavioral Science, SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, USA
[b] The Robert F. Furchgott Center for Neural and Behavioral, SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, USA
[c] Graduate Program in Neural and Behavioral Science, SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, USA
[d] Department of Physiology and Pharmacology, SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, USA
[e] Center for Neural Science, New York University, 4 Washington Place, New York, NY 10003, USA

ARTICLE INFO

Article history:
Received 6 April 2016
Revised 15 August 2016
Accepted 19 August 2016
Available online 26 August 2016

Keywords:
Hippocampus
Memory
CA1
Synaptic circuits
Remote memory
Schema
Active place avoidance
Synaptic plasticity

ABSTRACT

A widely accepted notion for a process underlying memory formation is that learning changes the efficacy of synapses by the mechanism of synaptic plasticity. While there is compelling evidence of changes in synaptic efficacy observed after learning, demonstration of persistent synaptic changes accompanying memory has been elusive. We report that acquisition of a hippocampus and long-term potentiation dependent place memory persistently changes the function of CA1 synapses. Using extracellular recordings we measured CA3-CA1 and EC-CA1 synaptic responses and found robust changes in the CA3-CA1 pathway after memory training. Crucially, these changes in synaptic function lasted at least a month and coincided with the persistence of long-term place memories; the changes were only observed in animals that expressed robust memory, and not in animals with poor memory recall. Interestingly, our findings were observed at the level of populations of synapses; suggesting that memory formation recruits widespread synaptic circuits and persistently reorganizes their function to store information.

1. Introduction

The synaptic plasticity and memory hypothesis is recognized as the most influential proposition for the mechanisms that underlie learning and memory. It asserts that the neural activity that underlies experience changes the efficacy of appropriate synapses to create and store memory (Martin & Morris, 2002; Neves, Cooke, & Bliss, 2008). To support the hypothesis, an extensive body of work has focused on investigating the functional and mechanistic properties of synaptic plasticity elicited by artificial manipulations and how these properties correlate with altered learning and memory expression in, typically, genetically manipulated animals (Abraham, 2008; Frick, Magee, & Johnston, 2004; Malenka & Bear, 2004; Mayford, 2014; Takeuchi, Duszkiewicz, & Morris, 2014). Indeed, selective modifications of gene expression or protein activity have provided tremendous insight into the molecular mechanisms that underlie synaptic plasticity and to a lesser extent, learning and memory processes (Malenka & Bear, 2004). In contrast, relatively few studies have investigated whether learning changes synaptic function and whether these changes maintain with the persistence of memory (Mayford, 2014; Takeuchi et al., 2014).

There is compelling evidence of changes in neural and synaptic function in neocortex with sensory stimulation (Barth & Poulet, 2012; Wen, DeBlois, & Barth, 2013) and dendritic spine structure changes in prefrontal cortex and hippocampus with environmental enrichment (Kozorovitskiy et al., 2005; Makara, Losonczy, Wen, & Magee, 2009), however, it has been difficult to reliably show that persistent memory storage is accompanied by persistent changes in synaptic function. In the hippocampus, the structure that is central to our concepts of memory for places and events (Garner et al., 2012; Mayford, 2014; Takeuchi et al., 2014), and even procedural learning (Micheau, Riedel, Roloff, Inglis, & Morris, 2004), recent studies show evidence of changed hippocampal neural and synaptic function after the acquisition of a hippocampus-dependent

* Corresponding authors at: Center for Neural Science, New York University, 4 Washington Place, New York, NY 10003, USA (A. A. Fenton). SUNY Downstate Medical Center, Department of Pathology (MSC 25), 450 Clarkson Avenue, Brooklyn, NY 11203, USA (J. M. Alarcon).
E-mail addresses: afenton@nyu.edu (A.A. Fenton), juanmarcos.alarcon@downstate.edu (J.M. Alarcon).
[1] Present address: Brain Plasticity Unit, CNRS UMR 8249, Ecole Supérieure de Physique et de Chimie Industrielles, 10 rue Vauquelin, 75005 Paris, France.
[2] Equal contributions.

http://dx.doi.org/10.1016/j.nlm.2016.08.015
1074-7427/

memory (Gruart, Munoz, & Delgado-Garcia, 2006; Matsuo, Reijmers, & Mayford, 2008; McKay, Oh, & Disterhoft, 2013; Park, Burghardt, Dvorak, Hen, & Fenton, 2015; Whitlock, Heynen, Shuler, & Bear, 2006). While these studies support the synaptic plasticity and memory hypothesis, there still is an absence of direct evidence that persistently changed hippocampal synaptic function accompanies long-term hippocampus-dependent memory.

Detecting a memory trace in hippocampus synapses has long been a subject of intense investigation because it is a key prediction of the synaptic plasticity and memory hypothesis. Prior work showed that learning can change hippocampus excitability (McKay et al., 2013; Oh, Kuo, Wu, Sametsky, & Disterhoft, 2003) as well as synapses (Green, McNaughton, & Barnes, 1990; Gruart et al., 2006; Sacchetti et al., 2001; Whitlock et al., 2006) but the memory persisted much longer than the changes in synapse function (Sacchetti et al., 2001; Whitlock et al., 2006). This discrepancy in duration has raised the question of whether the experience-driven synaptic alterations were indeed due to memory storage, instead of due to transient changes in synaptic homeostasis (Kirkwood, Rioult, & Bear, 1996; Turrigiano & Nelson, 2000), or other confounding features of the experience (Moser, Mathiesen, & Andersen, 1993). Hence, demonstrating that changes in synaptic circuit function during learning persist with memory, still remains a challenge for the synaptic plasticity and memory hypothesis. In particular, morphological changes of putative learning-recruited CA1 synapses have been reported to last only three days after contextual fear conditioning (Matsuo et al., 2008). Enhancement of the post-synaptic response induced during passive avoidance learning could only be observed for a few hours (Whitlock et al., 2006). Encouragingly, changes in CA1 synaptic function have been observed up to seven days after contextual fear conditioning, although the memory could be expressed for at least a month (Sacchetti et al., 2001). In addition to the technical challenge, the lack of correspondence between the persistence of synaptic plasticity changes and the persistence of memory may also constitute an important conceptual challenge for the synaptic plasticity and memory hypothesis. It is largely assumed by the community that synaptic changes should persist with memory; however a demonstration of this is lacking.

Here we report the use of a robust experimental system to investigate memory associated functional changes in hippocampus CA1 synaptic inputs that mediate spatial information. Our findings demonstrate that long-term traces of a spatial experience can be detected as persistent modifications in the function of the CA1 hippocampal circuitry lasting at least a month. Remarkably, these changes in synaptic function coincided with the persistence of long-term place memories; the changes were only observed in animals that expressed robust memory, and not in animals with poor memory recall. Notably, these changes were detected in the extracellular synaptic potentials recorded from the CA1 region of *ex vivo* slices, indicating a widespread change in the function of the CA1 synaptic network with memory. We speculate that widespread synaptic circuit changes at the level of hippocampal microcircuits include the embedding of explicit memory information at a particular set of synapses within a broader synaptic network that contains related information to which the newly acquired memory is associated.

2. Materials and methods

2.1. Behavior

All procedures were performed in compliance with the Institutional Animal Care and Use Committee of the State University of New York, Downstate Medical Center and New York University. C57BL/6 male mice (3–4 months old) were trained in a hippocampus-dependent two-frame active place avoidance task. The place avoidance system consisted of a 40-cm diameter arena with a parallel rod floor that could rotate at 1 rpm. The position of the animal was tracked using PC-based software (Tracker, Bio-Signal Group Corp., Brooklyn, NY) that analyzed 30-Hz digital video images from an overhead camera. Mice in the trained condition learned the "Room+Arena-" task variant. Place avoidance of a 60° zone was reinforced by a constant current foot shock (60 Hz, 500 ms, 0.2 mA) that was scrambled (5-poles) across pairs of the floor rods. Rotation of the arena would carry the mouse into the shock zone unless the animal actively avoided the zone. Entering the shock zone for more than 500 ms triggered shock. Additional shocks occurred every 1.5 s until the animal left the shock zone. Measures of place avoidance were computed by TrackAnalysis software (Bio-Signal Group Corp., Brooklyn, NY). The behavioral protocol began with a 10-min session with shock off to habituate the mice to the rotating arena. Twelve training trials followed, with three trials occurring each day across a period of four days. Within a day, the mice were returned to the home cage for 40-min between trials. Except for the shock being always off, the conditions were identical for pretraining and retention sessions, as well as for the control mice (untrained mice). The conditions were identical for the yoked group of mice except that these mice received the same time series of shocks as a mouse from the trained group. The times that shocks were delivered to the trained mice over the 4-day training period were recorded and used to deliver shocks to the yoked group of mice (yoked-group) who could not avoid or otherwise control the delivery of shock. Typically, the number of shocks trained mice received rapidly decreased over the training period due to place avoidance learning. On Day 1, mice experienced the bulk of shocks during the first trial (20–25 shocks, most of them during the first 5 min); then the number of shocks reduces in half during the second trial of Day 1, to only a few shocks in the third and last trial of Day 1. As learning progressed from Day 2 to Day 4, trained mice received very few to no shocks. For yoked-conditioning, we replayed the precise timing of the shock sequence from a trained mouse so that the yoked mouse received the identical time order of shocks. Hence, the yoked group of mice is exposed to the rotating arena and receives shocks just like the trained mice, but the shocks are uncorrelated to a specific location and cannot be avoided.

Long-term and remote memory was tested either 24 h or 30 days after the final training session with the shock off, respectively.

2.2. Electrophysiology

One or thirty days after the last training day, mice were tested for retention memory and then returned to their home-cage for 20 min (some mice were not memory tested but they were handled at the same times as the mice that had the memory retention test). Then, mice were transferred into an induction (anesthetizing) chamber and kept there to habituate to the chamber for 10 min. Next, mice were deeply anesthetized with vaporized Isoflurane (5% in 100% oxygen) for 3 min, and immediately euthanized by rapid decapitation. Transverse slices (400 μm) from the right dorsal hippocampus were obtained for *ex vivo* electrophysiology experiments. Slices were cut (Neurolab tissue chopper) in ice cold artificial cerebrospinal fluid (ACSF containing: (mM) 119 NaCl, 4.0 KCl, 1.5 MgSO4, 2.5 CaCl2, 26.2 NaHCO3, 1 NaH2PO4 and 11 Glucose saturated with 95% O2, 5% CO2) and then warmed in oxygenated ACSF to 35 °C for 45 min. Slices were thereafter allowed to equilibrate for at least 60 min in oxygenated ACSF at room temperature. For experiments, slices were immersed in a submerged recording chamber subfused with oxygenated ACSF at 35–36 °C.