Neurobiology of Learning and Memory 123 (2015) 35–42

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Latent memory facilitates relearning through molecular signaling mechanisms that are distinct from original learning

Steven A. Menges [a,1], Joshua R. Riepe [b], Gary T. Philips [b,*]

[a] Department of Ophthalmology, University of California, Irvine, 845 Health Sciences Rd, Room 1241, Irvine, CA 92697, United States
[b] Center for Neural Science, New York University, 4 Washington Place, Room 809, New York, NY 10003, United States

ARTICLE INFO

Article history:
Received 17 March 2015
Revised 25 April 2015
Accepted 27 April 2015
Available online 6 May 2015

Keywords:
Forgetting
Training pattern
MAPK
ERK
Translation

ABSTRACT

A highly conserved feature of memory is that it can exist in a latent, non-expressed state which is revealed during subsequent learning by its ability to significantly facilitate (savings) or inhibit (latent inhibition) subsequent memory formation. Despite the ubiquitous nature of latent memory, the mechanistic nature of the latent memory trace and its ability to influence subsequent learning remains unclear. The model organism *Aplysia californica* provides the unique opportunity to make strong links between behavior and underlying cellular and molecular mechanisms. Using *Aplysia*, we have studied the mechanisms of savings due to latent memory for a prior, forgotten experience. We previously reported savings in the induction of three distinct temporal domains of memory: short-term (10 min), intermediate-term (2 h) and long-term (24 h). Here we report that savings memory formation utilizes molecular signaling pathways that are distinct from original learning: whereas the induction of both original intermediate- and long-term memory in naïve animals requires mitogen activated protein kinase (MAPK) activation and ongoing protein synthesis, 2 h savings memory is not disrupted by inhibitors of MAPK or protein synthesis, and 24 h savings memory is not dependent on MAPK activation. Collectively, these findings reveal that during forgetting, latent memory for the original experience can facilitate relearning through molecular signaling mechanisms that are distinct from original learning.

1. Introduction

Memory is typically assessed through the measurement of overt changes in behavior. However, studies of learning and memory have also clearly established that memory can also develop in a latent, non-expressed form following a learning experience and, although inaccessible through tests of recall, is revealed through the ability to influence (promote or inhibit) subsequent learning and memory formation in a wide variety of paradigms (e.g., priming (Antzoulatos, Wainwright, Cleary, & Byrne, 2006; Parsons & Davis, 2012; Philips, Tzvetkova, Marinesco, & Carew, 2006), savings (Ebbinghaus, 1885/1913), latent inhibition (Tolman & Honzik, 1930)). In many instances, latent memory can outlast the overt expression of memory for an experience to provide a platform for "savings" (a reduction in the number of study trials or time required for relearning; Ebbinghaus, 1885/1913; Nelson, 1971). While well established as a behavioral feature of memory, the mechanistic nature of the latent memory trace and the means by which savings occurs during relearning remains unclear.

Despite its ubiquitous nature, very few studies of latent memory and savings have been initiated in invertebrate model organisms (Antzoulatos et al., 2006; Matzel, Collin, & Alkon, 1992; Parvez, Stewart, Sangha, & Lukowiak, 2005; Philips et al., 2006; Susswein & Schwarz, 1983), wherein strong links can be made between behavior and the underlying cellular and molecular mechanisms. With its large, readily identifiable neurons and simple nervous system, the invertebrate mollusk *Aplysia californica* has proven to be an advantageous model for elucidating the cellular and molecular mechanisms of memory formation. Moreover, learning in *Aplysia* recapitulates critical features of the human savings phenomenon (Antzoulatos et al., 2006; Philips et al., 2006; Susswein & Schwarz, 1983). We previously showed that a latent memory outlasts the initial forgetting of long-term memory (LTM) for sensitization of the tail-elicited siphon withdrawal reflex (T-SWR) for at least two days, and supports the facilitated induction of three distinct temporal phases of memory during relearning: short-term (10 min), intermediate-term (2 h) and long-term savings memories (24 h) (Philips et al., 2006). Importantly, savings was not observed when retraining was delayed for four days after

* Corresponding author.
E-mail address: gp60@nyu.edu (G.T. Philips).
[1] Present address.

http://dx.doi.org/10.1016/j.nlm.2015.04.013
1074-7427/

initial signs of forgetting, identifying a strong parallel to the human learning phenomenon, in which the benefit of prior experience is time-limited (Ebbinghaus, 1885/1913). The demonstration of savings in *Aplysia* has established a unique opportunity to study the cellular and molecular features of the latent memory trace and its facilitation of subsequent memory formation in simple neural circuits.

In the present study, we examined the molecular features of savings in *Aplysia*. First, we replicated our earlier observation of savings for the induction of sensitization memories within the T-SWR (Philips et al., 2006). We then showed that the facilitated induction of 2 h and 24 h memory requires the interaction between two spaced training trials and is supported by plasticity in the previously described narrow temporal window for two-trial LTM formation in naïve animals (Philips, Tzvetkova, & Carew, 2007). Savings could also be observed when retraining reduced behaving preparations of previously trained *Aplysia*. In molecular studies, we found that the induction of 2 h savings memory was unique from the induction requirements for a comparable phase of ITM formation in naïve animals, in that (i) it no longer required activation of the highly conserved mitogen activated protein kinase (MAPK) signaling pathway and (ii) it was not disrupted by the inhibition of ongoing protein synthesis. We additionally showed that 24 h savings memory was distinct from the LTM induced in naïve animals, in that its induction was independent of MAPK activation, but still required protein synthesis. Collectively, these findings indicate that latent memory for prior sensitization facilitates relearning across multiple temporal phases of memory by engaging distinct molecular rules for subsequent memory formation.

2. Materials and methods

2.1. Animals

Wild-caught *A. californica* (250–400 g; Marinus Scientific, Long Beach, CA and South Coast Bio-Marine, San Pedro, CA) were housed in a 200 gallon tank of artificial seawater (Reef Crystals) at 15 °C. To facilitate monitoring of the tail-elicited siphon withdrawal reflex (T-SWR), animals were anesthetized in ice-cooled seawater and the parapodia around the siphon was surgically removed. The ink gland was also removed to permit training in the absence of conspecific signaling through ink release (Stopfer, Chen, & Carew, 1993). Animals recovered for 4–5 days in the home tank before training.

2.2. Behavioral procedures

The T-SWR was initiated by stimulating the posterior tip of the tail midline with a pulsed water jet (0.4 s, 45 psi, Teledyne Water Pik; Philips et al., 2006). The duration of the tail-elicited siphon withdrawal responses was measured from the onset of the stimulus to the initial relaxation of the neck of the siphon. Baseline T-SWR duration was established using the average of three tests (inter-test interval [ITI] = 15 min).

After establishing baseline, animals were randomly assigned into either experimental or control groups. The experimental group received *Phase I* sensitization training (four midline tail shocks [TSs; 1 shock: 2 s train of 10 ms, 15 mA DC pulses at 50 Hz] inter-shock interval [ISI] = 15 min; Philips et al., 2006). Control animals were not trained, but were tested and housed with trained animals.

Twenty-four hours following training, memory was assessed with the average of two tests of the T-SWR (ITI = 30 min). These posttests at 24 h were used to group animals according to previously established criteria (Philips et al., 2006). Trained animals with average responding below 120% pre-training levels were removed from further study (42% of trained animals) because we previously reported that these weakly sensitized animals do not demonstrate long-term savings memory induction with retraining (Philips et al., 2006). Thus, for our studies of short-, intermediate- and long-term savings memory induction mechanisms we only continued with animals whose responses were greater than or equal to 120% pre-training levels at 24 h. Importantly, the lack of robust memory expression in a significant fraction of trained animals was by design. *Phase I* training uses weak training stimuli so that long-term memory duration does not persist longer than a week. Animals expressing $\geqslant$120% baseline T-SWR behavior at the initial 24 h test demonstrated an average LTM duration of two days.

LTM expressing animals (and matched naïve controls) were subsequently tested every 24 h to describe the forgetting curve for each trained animal. Day 1 of "forgetting" was identified as the first day when average responding of trained animals fell below 120% of the baseline average (Philips et al., 2006), and was always confirmed by additional tests 24 h later (Day 2 of "forgetting"). Thus, all trained animals demonstrated two consecutive days of apparent forgetting before they were given *Phase II* training (see Fig. 1A). Whereas *Phase I* training used 4 training trials to establish an original LTM, in *Phase II* we tested for latent memory for the *Phase I* experience by using a savings test of retraining with fewer trials (2 spaced midline tail shocks [1 shock: 2 s train of 10 ms, 15 mA DC pulses at 50 Hz], ISI = 15 min; Philips et al., 2006). In *Phase II*, both experimental and control animals received the two training shocks and posttests were conducted at 10 min following the first *Phase II* training shock, at 2 h after the second shock, and at 24 h (two tests, ITI = 30 min) to assess short, intermediate- and long-term phases of memory induction, respectively.

2.3. Reduced T-SWR preparation

For the mechanistic analysis of savings memory induction, we administered *Phase II* training in reduced preparations of the tail-elicited siphon withdrawal reflex (T-SWR) of a second cohort of *Aplysia*. In these experiments, *Phase I* training and post-tests were conducted in intact *Aplysia* as described above. However, immediately following tests on Day 2 of forgetting, experimental and matched control animals were anesthetized using isotonic $MgCl_2$ and reduced behaving preparations of the T-SWR were generated (Fig. 2A) (Sutton, Masters, Bagnall, & Carew, 2001).

Following a 2 h recovery, baseline T-SWR was re-established (RP baseline; 3 tests, ITI = 15 min). We tested the same site as was used in the intact, freely behaving animal. Ten minutes after baseline establishment, *Phase II* training was administered. Post-tests were conducted at 10 min after the first TS, and at 2 h and 24 h (3 tests, ITI = 15 min) after the second TS. Memory expression was determined by comparison to the baseline T-SWR re-established in the reduced behavioral preparation, since an overall reduction in the T-SWR response duration was observed after surgery (intact vs reduced preparation scores on day of surgery, $p < .05$ paired t test; Fig. 2B).

2.4. Drug treatments

To test the requirement for protein synthesis in savings learning, we used the translation inhibitor emetine dihydrochloride (Sigma) at a concentration (100 μM in ASW) that blocks >95% of protein synthesis and disrupts memory formation in the T-SWR reduced behavioral preparations from naïve animals (Sutton et al., 2001). An identical testing and training protocol was used