Neurobiology of Learning and Memory 115 (2014) 86–94

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

C. elegans positive olfactory associative memory is a molecularly conserved behavioral paradigm

Geneva M. Stein, Coleen T. Murphy *

Lewis-Sigler Institute for Integrative Genomics, Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA

ARTICLE INFO

Article history:
Received 10 April 2014
Revised 17 July 2014
Accepted 30 July 2014
Available online 7 August 2014

Keywords:
Short-term memory
Intermediate-term memory
C. elegans
cAMP
Adaptation

ABSTRACT

While it is thought that short-term memory arises from changes in protein dynamics that increase the strength of synaptic signaling, many of the underlying fundamental molecular mechanisms remain unknown. Our lab developed a *Caenorhabditis elegans* assay of positive olfactory short-term associative memory (STAM), in which worms learn to associate food with an odor and can remember this association for over 1 h. Here we use this massed olfactory associative assay to identify regulators of *C. elegans* short-term and intermediate-term associative memory (ITAM) processes. We show that there are unique molecular characteristics for different temporal phases of STAM, which include: learning, which is tested immediately after training, short-term memory, tested 30 min after training, intermediate-term memory, tested 1 h after training, and forgetting, tested 2 h after training. We find that, as in higher organisms, *C. elegans* STAM requires calcium and cAMP signaling, and ITAM requires protein translation. Additionally, we found that STAM and ITAM are distinct from olfactory adaptation, an associative paradigm in which worms learn to disregard an inherently attractive odor after starvation in the presence of that odor. Adaptation mutants show variable responses to short-term associative memory training. Our data distinguish between shorter forms of a positive associative memory in *C. elegans* that require canonical memory pathways. Study of STAM and ITAM in *C. elegans* could lead to a more general understanding of the distinctions between these important processes and also to the discovery of novel conserved memory regulators.

1. Introduction

Learning and memory allow animals to navigate, find food, and survive in a changing environment. In humans, memory declines with age, and memory deficits are often a hallmark of neurodegenerative disorders, such as Alzheimer's Disease (Hodges & Patterson, 1995; Salthouse, 1991). Therefore, an understanding of the molecular bases of different forms of memory is essential to develop treatments for memory loss.

Short-term memory lasts from minutes to hours, resulting from changes in synaptic strength mediated by modifications at the synapse in the appropriate neurons (Kandel, 2001). cAMP signaling and calcium signaling are required for short-term memory in many organisms (Hawkins, Kandel, & Bailey, 2006; Kandel, 2001). These pathways are activated in response to neurotransmitter receptor activity, can modulate receptor activity, and are thought to activate synaptic proteins to facilitate memory formation and maintenance. Several kinases (Giese & Mizuno, 2013; Kandel, 2012) and synaptic cellular adhesion proteins (Cheng et al., 2001; Grotewiel, Beck, Wu, Zhu, & Davis, 1998) have also been identified as regulators of short and intermediate-term memory in higher organisms. However, downstream targets or parallel regulatory pathways remain largely unknown. Therefore, it is important to establish a model system in which regulation of short-term memory is conserved and new genetic regulators of short-term memory can be rapidly identified.

Caenorhabditis elegans has a simple nervous system comprised of just 302 neurons, and its stereotypic neural connections (the "connectome") have been mapped (Varshney, Chen, Paniagua, Hall, & Chklovskii, 2011; White, Southgate, Thomson, & Brenner, 1986). This small nematode can learn and form both associative and non-associative memories, lasting as long as 24 h (Ardiel & Rankin, 2010; Kauffman, Ashraf, Corces-Zimmerman, Landis, & Murphy, 2010). We developed a protocol that pairs a relatively neutral odor (butanone at a specific concentration) with food to create a positive association, resulting in strong attraction to the odor (Kauffman et al., 2010). Short-term associative memory (STAM) after one conditioning period decays by 2 h after training,

Abbreviations: STAM, short-term associative memory; ITAM, intermediate-term associative memory.

* Corresponding author. Address: Lewis-Sigler Institute for Integrative Genomics, Department of Molecular Biology, 148 Carl Icahn Laboratory, Washington Rd., Princeton, NJ 08544, USA.

E-mail address: ctmurphy@princeton.edu (C.T. Murphy).

http://dx.doi.org/10.1016/j.nlm.2014.07.011
1074-7427/

while long-term memory after spaced training (7 conditioning cycles) lasts more than 16 h (Kauffman et al., 2010). Importantly, because the worms display an increased response to odor after training, our positive associative assay allows clear distinction from non-associative forms of learning, such as habituation and adaptation, which repress behavioral response to a stimulant and therefore could be interpreted as negative associative learning.

We previously found that positive associative learning requires conserved receptors, and that long-term associative memory requires transcription as well as the activity of the Zn finger transcription factor CREB (Kauffman et al., 2010). Here we show that *C. elegans* STAM requires cAMP and calcium signaling pathways. We also find that protein translation is required at two different steps: it is first required during massed associative memory training to extend memory past 30 min, and subsequently after memory training to ensure proper decay of the associative memory, or forgetting. Intermediate-term memory requires translation but not transcription (Ashraf, McLoon, Sclarsic, & Kunes, 2006; Ghirardi, Montarolo, & Kandel, 1995); our results show that massed training results in both short-term (30 min) and intermediate-term (1 h) associative memory (STAM and ITAM). Our data show that shorter-term memory mechanisms in *C. elegans* are conserved with higher organisms and further establish *C. elegans* as a good model to study the genetic regulation of short-term and intermediate-term associative memory.

C. elegans has been shown to associate starvation with a myriad of sensory cues including olfactory, gustatory, thermosensory, and pathogenic cues (Colbert & Bargmann, 1995; Mohri et al., 2005; Saeki, Yamamoto, & Iino, 2001; Zhang, Lu, & Bargmann, 2005). Adaptation is a behavior in which worms display reduced responsiveness to an odor after a one-hour odor exposure in the absence of food (Colbert & Bargmann, 1995). Stetak, Horndli, Maricq, van den Heuvel, and Hajnal (2009) use the same odor/starvation conditioning paradigm to induce what they refer to as a negative associative short-term memory. Our massed associative memory assay also involves a one-hour exposure to an odor, but in the presence of food rather than the absence of food, resulting in a positive association between food and odor. Therefore, one hypothesis that we set out to test is whether STAM is simply a response occurring in the opposite direction of adaptation that requires the same molecular machinery. However, we find that mutants that are defective for adaptation have varying STAM/ITAM phenotypes, including prolonged, reduced, and normal memory, establishing positive olfactory STAM/ITAM as a distinct memory process in *C. elegans*.

2. Methods

2.1. Worm cultivation

C. elegans were cultivated at 20 °C on High Growth Media (HG) or Nematode Growth Media (NGM) seeded with OP50 *E. coli* using standard methods (Brenner, 1974). Animals were synchronized by hypochlorite treatment and tested for learning and memory at Day 1 of adulthood at room temperature.

2.2. Strains

Wild type: (N2 Bristol); mutant strains: KP1182 (*acy-1(nu329)*), KG518 (*acy-1(ce2)*), KG532 (*kin-2(ce179)*), KG744 (*pde-4(ce268)*), VC1052 (*unc-43(gk452)*), VC1408 (*magi-1(gk657)*), JH1270 (*nos-1(gv5)*), JK3022 (*fbf-1(ok91)*), CX20 (*adp-1(ky20)*), RB995 (*hpl-2(ok916)*), NL917 (*mut-7(pk204)*), and DR466 (*him-5(e1490)*) were obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, Minneapolis, MN). *egl-4(ky95)*; *him-5(e1490)* was a kind gift from N. L'Etoile (University of California, San Francisco, San Francisco, CA).

2.3. Behavioral assays

2.3.1. Short-term associative memory training

STAM assays were performed as previously described (Kauffman et al., 2010, 2011). Briefly, synchronized day 1 hermaphrodites were starved for 1 h in M9 buffer. Worms were then transferred to 6 cm NGM plates with 500 μL of OP50 and 2 μL of 10% butanone in ethanol on the lid and conditioned for 1 h. After conditioning, 100–500 worms from the trained population of worms were tested once for chemotaxis to butanone either immediately (0 h) or after being transferred to 10 cm NGM plates with 900 μL fresh OP50 for specified intervals before testing (30 min–4 h). Graphs display the average chemotaxis index at each time-point for 3 or more separate biological replicate experiments, with each experiment displayed as a pale colored curve behind this average line. Each experiment included at least 3 technical replicates per time-point, with the exception of one of four biological replicate experiments for *acy-1(nu329)* and *kin-2(ce179)* mutants that had only one technical replicate per time-point. Significance was calculated by comparing the experimental and control groups using two-way ANOVAs ($p \leqslant 0.05$ for significant experiments) followed by Bonferroni-corrected post hoc unpaired *t*-tests comparing the experimental and control at each time-point (Bonferroni-correction per *t*-test: $p_{bonf} \leqslant 0.05$).

2.3.2. Chemotaxis assay

Chemotaxis assays were performed as previously described (Bargmann, Hartwieg, & Horvitz, 1993). Briefly, 100–500 day 1 adult worms were placed at the origin on a 10 cm NGM plate with butanone (1 μL 10% butanone in ethanol +1 μL NaN_3) and ethanol control (+1 μL NaN_3) equidistant from the origin. After 1 h., black and white images of each plate were taken using a Basler A fire wire camera (Basler AG, Ahrensburg, Germany) using "Measurement and Automation" software (National Instruments) to capture images. Images were analyzed using count_worms_v7.3 (Kauffman et al., 2011). The chemotaxis index = $[(n_{\text{attractant}}) - (n_{\text{control}})]/[(\text{Total} - n_{\text{origin}})]$.

2.3.3. Drug treatments

200 μg/mL Actinomycin $D \geqslant 95\%$ (Sigma Aldrich, Saint Louis, Missouri) was added to M9 buffer for the final 30 min of starvation and added to S basal during conditioning along with 1:1000 Butanone and OP50 *Escherichia coli* bacteria that had been grown overnight. Cycloheximide ⩾ 94% (Sigma Aldrich, Saint Louis, Missouri) was added to NGM at 0.8 mg/mL. Plates were poured and solidified overnight at 4 °C, seeded with OP50 *E. coli*, and then used for Short-term associative memory training either during conditioning or after training. To ensure 1 h of drug treatment for 30 min conditioning, worms were added to cycloheximide plates with no food for the last 30 min of starvation followed by 30 min of conditioning on cycloheximide with OP50 and 10% butanone.

3. Results

3.1. C. elegans STAM and ITAM require conserved memory mechanisms

3.1.1. Blocking translation, but not transcription, inhibits both memory maintenance and forgetting

We and others have shown that long-term memory in *C. elegans* requires both transcription and translation (Kauffman et al., 2010; Timbers & Rankin, 2011; Vukojevic et al., 2012), as is required for