Neurobiology of Learning and Memory 123 (2015) 217–224

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Place memory retention in *Drosophila*

Daniela Ostrowski, Lily Kahsai, Elizabeth F. Kramer, Patrick Knutson, Troy Zars *

114 Lefevre Hall, Division of Biological Sciences, University of Missouri, Columbia, MO 65211, United States

ARTICLE INFO

Article history:
Received 18 May 2015
Revised 25 June 2015
Accepted 26 June 2015
Available online 2 July 2015

Keywords:
Place memory
Memory phases
High temperature
Intermittent training
Genetic mutation
Drosophila

ABSTRACT

Some memories last longer than others, with some lasting a lifetime. Using several approaches memory phases have been identified. How are these different phases encoded, and do these different phases have similar temporal properties across learning situations? Place memory in *Drosophila* using the heat-box provides an excellent opportunity to examine the commonalities of genetically-defined memory phases across learning contexts. Here we determine optimal conditions to test place memories that last up to three hours. An aversive temperature of 41 °C was identified as critical for establishing a long-lasting place memory. Interestingly, adding an intermittent-training protocol only slightly increased place memory when intermediate aversive temperatures were used, and slightly extended the stability of a memory. Genetic analysis of this memory identified four genes as critical for place memory within minutes of training. The role of the *rutabaga* type I adenylyl cyclase was confirmed, and the *latheo* Orc3 origin of recognition complex component, the novel gene encoded by *pastrel*, and the small GTPase *rac* were all identified as essential for normal place memory. Examination of the dopamine and ecdysone receptor (*DopEcR*) did not reveal a function for this gene in place memory. When compared to the role of these genes in other memory types, these results suggest that there are genes that have both common and specific roles in memory formation across learning contexts. Importantly, contrasting the timing for the function of these four genes, plus a previously described role of the *radish* gene, in place memory with the temporal requirement of these genes in classical olfactory conditioning reveals variability in the timing of genetically-defined memory phases depending on the type of learning.

1. Introduction

Some memories last a lifetime, while others are already forgotten within a few seconds. By altering learning conditions and molecular genetic manipulations, memory phase complexity can be revealed. In addition to humans, animals ranging in nervous system complexity from primates and rodents to simpler organisms like the fly *Drosophila* and the nematode *Caenorhabditis elegans* are capable of forming memories with various levels of temporal complexity (Burne et al., 2011; Glanzman, 2010). Our understanding of how different memories are more or less stable is far from complete.

Drosophila can be operantly trained to learn and remember a safe place (Foucaud, Burns, & Mery, 2010; Ofstad, Zuker, & Reiser, 2011; Zars, 2010). The heat box provides a relatively simple learning task to study place memory dynamics (Kahsai & Zars, 2011; Ostrowski & Zars, 2014; Wustmann, Rein, Wolf, & Heisenberg, 1996; Zars, Wolf, Davis, & Heisenberg, 2000). In this apparatus, a fly is placed in an elongated rectangular chamber in which the temperature is regulated by the behavior of the fly. Flies can be conditioned within minutes in this paradigm by pairing a rising temperature with a part of the chamber. If a fly chooses to spend time in a safe part of the chamber, the temperature falls to a preferred temperature of 24 °C (Hamada et al., 2008; Kahsai & Zars, 2011; Sayeed & Benzer, 1996; Zars, 2001). So far the memory dynamics have not been extensively studied in this learning paradigm (LaFerriere, Speichinger, Stromhaug, & Zars, 2011; Putz & Heisenberg, 2002). With the advent of identifying potential common and unique mechanisms that are important for maintaining memory performance in the fly, we characterized multiple training and genetic parameters that influence place memory stability.

We trained flies with reinforcing temperatures of 33°, 37° and 41 °C with training durations of 4, 10, 15 and 20 min. Memory performance was tested up to several hours after training. Our results show that conditioning with 37 °C does not result in any measurable lasting place memory. Conditioning using 41 °C for 4 min results in place memory that lasts for at least one hour. However, simply extending the training duration did not delay memory decay. Additionally, intermittent training, which usually results in more stable memories, only slightly improved memory stability. Finally, the roles of the *rutabaga* type I adenylyl cyclase,

* Corresponding author.
E-mail address: zarst@missouri.edu (T. Zars).

http://dx.doi.org/10.1016/j.nlm.2015.06.015
1074-7427/

the *latheo* Orc3 origin of recognition complex component, the novel gene encoded by *pastrel*, the small GTPase *rac*, and the dopamine and ecdysone receptor (*DopEcR*) in promoting the stability of a place memory were examined.

2. Material and methods

2.1. Animals

Drosophila melanogaster were raised on cornmeal-based fly food media and maintained on a 12 h/12 h day/night cycle at 24 °C and 60% relative humidity. For behavioral experiments wild-type Canton S (CS) flies at the age of 2–5 days were used. Prior to the behavioral experiments flies were provided 16–24 h on new fly food. The Canton S and cantonized w^{1118} mutant flies stem from the Martin Heisenberg laboratory stocks. The *elav-C155*, *tublin-Gal80^{ts}* and *UAS-Drac1(N17)* stocks were obtained from the Bloomington Stock Center. These experimental lines were outcrossed to the cantonized w^{1118} line for six generations and had the first chromosomes replaced with a wild-type version. The *rutabaga* adenylyl cyclase (rut^{2080}), *latheo* (lat^{6}), *pastrel* (pst^{1}), and the *dopamine/ecdysone receptor* ($DopEcR^{PB1}$), provided by Drs. Paul Shaw (Washington University in St. Louis) and Toshihiro Kitamoto (University of Iowa), are in a Canton S background (Boynton & Tully, 1992; Dubnau et al., 2003; Ishimoto, Wang, Rao, Wu, & Kitamoto, 2013; Levin et al., 1992). Standard genetic crosses were used to generate experimental groups. Female flies were used for all *rac* experiments. Crosses for the *rac* experiments were raised at 18 °C and progeny were divided into two groups. The induced group was transferred to a 30 °C incubator for 3 days, whereas the control group was kept at 18 °C. Both groups were allowed to recover at 24 °C for at least 3 h before behavioral experiments. Heterozygous controls were raised under identical conditions and in parallel to exclude influence of the temperature shift on fly performance.

2.2. Behavioral experiments

Place memory was tested using the heat-box apparatus. The heat-box consists of multiple rectangular chambers in which single flies are allowed to walk freely back and forth (Ostrowski & Zars, 2014; Zars, 2009, 2010). The position of a single fly within each chamber is recorded throughout an experiment. Fast temperature changes within the chambers are provided by Peltier-elements on top and bottom. A computer coordinates rising temperatures with position of the fly. Before each training session flies are provided a pre-test phase (30 s) at constant 24 °C to determine any potential spontaneous side preference. During conditioning (the training phases) one chamber half is defined as the side associated with high temperature and the other as not. Every time the fly enters the high temperature associated side the whole chamber heats up to an aversive temperature (33–41 °C). The return of the fly to the other side quickly cools down the chamber to a non-aversive temperature (24 °C) (Sayeed & Benzer, 1996; Zars, 2001). The following 3 min post-test measures place preference while the chamber is kept at the same non-aversive temperature. A performance index (PI) is calculated by the difference in time a fly spent in either chamber half (unpunished side vs. punished side) divided by the total time within a session. The PI can vary from 1.0 to −1.0. Zero indicates that on average the flies spent equal time on both sides of the chamber, whereas 1.0 shows a perfect side preference of the fly for the unpunished chamber half.

Flies were conditioned with different reinforcing temperatures (33, 37 and 41 °C) and training durations of 4, 10, 15 or 20 min. Training duration was either massed or intermittent with intervals of rest. For intermittent training each 2 min training interval was followed by a 1 min rest period; e.g., a 20 min training was interrupted by 9 × 1 min rest periods. During the rest periods flies remained within the chambers that were kept at 24 °C. Memory performance was either tested immediately after training or after time intervals as listed in Section 3. During the time intervals flies were taken out of the heat-box chambers and put together in a tube with fresh food. The time intervals include handling of flies, so that the rest period starts with the end of the training period and ends with the beginning of the post-test. Post-tests included short reminder training for 30 s using 41 °C as the reinforcing temperature.

Olfactory Memory: Undiluted 4-methylcyclohexanol (MCH) and 3-octanol (OCT) were used as odorants with protocols previously described (Krashes & Waddell, 2008; Zars, Fischer, Schulz, & Heisenberg, 2000). To test for aversive olfactory memories, memory was tested after training as described in Section 3. Flies were held in fly food vials in the longer retention intervals. Flies were trained by pairing either MCH or OCT with 12, 100 V electric shocks. Flies were given 1 min to choose between converging odorant streams in a T-maze for the memory tests. A PI was calculated for the memory experiments. This score was calculated by subtracting the number of flies choosing the control odorant from the number of flies choosing the shock-associated odorant, divided by the total number of flies in a "half test." An average PI was calculated from a pair of half-test PIs, where each half came from conditioning of one of the two odors.

2.3. Data analysis

Position of flies within the chambers is recorded by a custom made program and spatial preference (PI) of individual flies during post-test is automatically calculated. Flies that were inactive during pre-test or did not experience heat during training were automatically discarded. Data are shown as mean ± SEM. Unless otherwise noted each group consists of at least 120 flies. The olfactory memory experiments used at least 5 experiments per genotype. For statistical analysis Sigmaplot 12 or Statistica was used. Data were analyzed for their normal distribution using Shapiro–Wilk test (data not shown). Results from tests of normal distribution varied. Therefore, non-parametric tests have been used to test for significant differences in place memory. Parametric ANOVA were used for the olfactory memory tests. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Place memory performance

Flies were trained with reinforcing temperatures of 33, 37 or 41 °C for training durations of 4, 10, 15 or 20 min (Fig. 1A). A low reinforcing temperature of 33 °C with extended training of at least 10 min revealed a change in place preference of flies when tested during the post-test phase (Fig. 1A). Prolonged training duration with higher reinforcing temperatures strengthened place memory performance. For each training session of 4, 10 or 20 min, higher temperatures induced higher memory scores (Fig. 1A), largely consistent with previous results (Diegelmann, Zars, & Zars, 2006; Zars & Zars, 2006). However, while it was previously shown that 4 min of training at 33 °C can induce a place memory (Diegelmann et al., 2006), the experiments described here did not induce a significant place memory. This difference might reflect variability in the ability to detect a low-level place memory under these modest conditioning parameters. Nevertheless, the general relationship of higher temperature and increased training duration giving rise to higher levels of place memory is maintained.

There is a maximum in memory performance level flies reach with extended training duration and temperatures. For