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Competition between two memory traces for long-term recognition memory ☆

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This paper is dedicated to Prof. Dr. Gerald Wolf who celebrated his 65th birthday in February 2008.

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ABSTRACT

Previous studies investigating the processes which underlie memory consolidation focused almost exclusively on isolated learning events. Here I studied the competition of two similar memory traces for consolidation non-conditioned recognition memory in adult male C57BL/6JOlaHsd mice using the olfactory cues based social discrimination procedure. My results show that the interference phenomena that cause forgetting are time-dependent, and that retroactive interference can be discriminated from proactive interference. Furthermore, both types of interference can be suppressed by subcutaneous anisomycin treatment immediately after presentation of the interference stimulus. These findings imply that interference phenomena, which result from the competition of two similar memory traces for long-term recognition memory, are related to the progress of memory consolidation and linked to protein synthesis.

1. Introduction

Memory involves not only the ability to acquire and store new information, but also the ability to forget old information. Every day, things that have been learned are confronted with, and sometimes displaced by, newly acquired information. However, most experimental studies of memory consolidation try to omit this dynamic relationship of memory competition for the sake of methodological simplicity, and so the neurobiological basis of memory has largely been investigated using isolated learning events. This approach has helped to form the hypothesis that the consolidation of long-term memory is not a monolithic operation, but consists of several, separate stages (Dudai, 2004; McGaugh, 2000; Wagner, Gais, & Born, 2005). It is of note that most studies designed to investigate these stages and their underlying neuronal mechanisms focused on the learning of an association between an originally neutral and a pronounced emotional (aversive or rewarding) stimulus. However, the addition of an emotional component increases the resistance against forgetting (McGaugh, 2006), and thus if the animals had learned such an association, they would almost never again become naïve of the originally neutral stimulus (Kamprath & Wotjak, 2004). Therefore, a model that is not based on resistant stimulus associations may provide an elegant approach to investigate the interaction of two similar memory traces in long-term memory formation, and thus might also provide new insights into the process of memory consolidation.

Non-conditioned recognition memory, a common paradigm for declarative memory, is well established in primates but has been difficult to apply to laboratory rodents. However, three recent studies show that long-term social recognition memory can be investigated in mice (Kogan, Frankland, & Silva, 2000; Richter, Wolf, & Engelmann, 2005; Wanisch, Wotjak, & Engelmann, 2008). In this task, an adult mouse acquires an 'olfactory memory' of a juvenile conspecific during an initial encounter and is tested 24 h later in a choice session (Fig. 1A). Several aspects render this approach particularly interesting for studies on the dynamic relationship of two memory traces and their competition for consolidation. First, the task is based on the innate drive of an adult animal to investigate unfamiliar conspecifics, so no additional factors are required to encourage the induction of a memory (e.g. punishment). Second, the task allows within-subject comparisons that provide a more powerful statistical analysis. Third, olfactory stimuli are of high ethological relevance, as mice gain information about their environment mainly from such cues (Eibl-Eibesfeldt, 1950, 1958). Finally, studies using the protein synthesis blocker anisomycin (ANI) have shown that this type of memory requires two stages

☆ Interference of recognition memory.

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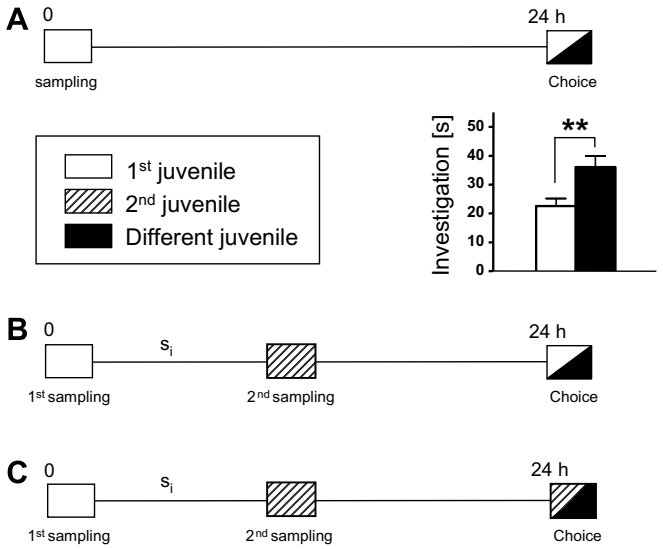

Fig. 1. Experimental protocol for testing the competition to two similar memory traces for long-term juvenile recognition memory in adult male mice. (A) illustrates the standard recognition memory procedure which was modified in (B) for testing the impact of the 2nd sampling (hatched box) for recognizing the conspecific exposed during the 1st sampling. (C) shows the modification allowing the investigation of the impact of the 1st sampling on recognizing a juvenile exposed during the 2nd sampling (hatched box). The two samplings were separated by defined sampling intervals (s_i). Choice took place either 24 h after the1st sampling or, in selected cases, 24 h after the 2nd sampling. The graph in (A) shows a representative result obtained during choice performed 24 h after sampling. The significantly longer investigation of the different juvenile versus the juvenile encountered during sampling (1st juvenile) demonstrates the intact long-term social recognition memory ($^{**}p = 0.01$ paired Students *t*-test; $n = 20$).

of protein synthesis. The first stage is associated with Fos protein synthesis in brain areas associated with the processing of olfactory information and takes place 1–2 h after the encounter (Kogan et al., 2000; Richter et al., 2005). The second stage starts 6–7 h after the encounter, and seems to correspond with the synthesis of proteins other than Fos. Eighteen hours after the encounter, memory consolidation is complete, as ANI treatment at this time does not affect juvenile recognition (Richter et al., 2005).

The present study was designed to investigate the interaction of two similar memory traces for the consolidation of long-term recognition memory. Therefore, I modified the olfactory-cued juvenile discrimination procedure in mice (Fig. 1A) by allowing the adult to acquire the olfactory signature of a second unfamiliar juvenile ("2nd juvenile") within the 24 h retention interval (Fig. 1B and C). I then increased the interval between the presentation of the 1st and the 2nd juvenile (sampling interval; s_i) which correspond to the two stages of protein biosynthesis of recognition memory, with the aim of testing for time-dependent changes in the competition of the two memory traces. Finally, to gain insight into the dependence of the memory traces competition on protein synthesis, I studied the effects of ANI treatment immediately after the presentation of the sampled juveniles.

2. Material and methods

2.1. Animals

Adult male C57BL/6JOlaHsd mice (9–16 weeks old; Harlan–Winkelmann, Borchern, Germany) were used as experimental subjects. They were housed in groups of five per cage (size: 20 × 37 × 15 cm) under standard laboratory conditions with a 12:12 h light–dark cycle (light on: 07:00) for at least one week before starting the experiments. Juvenile C57BL/6JOlaHsd mice of both sexes (25–38 days old) were used as olfactory stimuli. All experimental manipulations were approved by the Committee on Animal Health and Care of the local governmental body and performed in strict compliance with the EEC recommendations for the care and use of laboratory animals (86/609/CEE).

2.2. Olfactory recognition procedure

Olfactory recognition was tested using the social discrimination procedure adapted from rats (Engelmann, Wotjak, & Landgraf, 1995) essentially as described in detail elsewhere (Richter et al., 2005). Briefly, experimental subjects were separated by transferring them to small cages with fresh bedding (size: 14 × 20 × 15 cm) 2 h before starting the session. A social discrimination session consisted of two 4-min exposures of juveniles to the adult in the adult's cage performed under dimmed lighting conditions (approx. 200 lx). During the first exposure ("original encounter"; during the light phase, i.e. between 8:00 and 15:00 a.m.), a juvenile was exposed to the adult animal. The juvenile was then removed and kept individually in a fresh cage with food and water *ad libitum*. After retention interval of 24 h the juvenile was re-exposed to the adult (second exposure; "choice" session) together with an additional, previously not presented juvenile of the same mouse strain. The duration of investigatory behaviour of the adult towards each juvenile was measured separately by a trained observer blind to the animals treatment. A significantly longer investigation duration of the new juvenile compared to the already encountered juvenile during choice is taken as an evidence for an intact recognition memory (Engelmann et al., 1995). After the end of each choice session, the experimental mice were housed in their original groups of five. Fig. 1A shows schematically the olfactory recognition procedure and the typical difference in the investigation durations towards the already encountered juvenile versus the new juvenile during choice after a retention interval of 24 h. It should be noted that during some sessions behaviour was monitored between 19.00–21.00 h (i.e. during the first 2 h of the dark phase of the cycle). During that period the light was switched on in the testing room. Preliminary experiments failed to reveal a significant impact of the circadian rhythm and/or lighting conditions on juvenile recognition using the social discrimination procedure (see also (Reijmers, Leus, Burbach, Spruijt, & van Ree, 2001)).

2.3. Competition of two memory traces I: Choice session with the 1st sampling juvenile

To measure impact of a second memory trace on the consolidation of the recognition memory initiated by the 1st sampling, a second, previously not encountered juvenile was presented for 4 min to the adult mouse during a 2nd sampling session after a defined sampling interval (s_i; either 5 min or 3, 6, 9, 12, 15, 18 or 22 h). During choice, the juvenile presented during the 1st sampling together with a new, previously not exposed juvenile was presented and the investigation durations were measured as indicated above (see Fig. 1B).

2.4. Competition of two memory traces II: Choice session with the 2nd sampling juvenile

To measure the impact of the ongoing consolidation initiated by the 1st sampling on the implementation of a second memory trace, a second, previously not encountered juvenile was presented for 4 min to the adult mouse during a 2nd sampling session after a defined s_i for 4 min, 5 min, or 3, 6, 9, 12, 15, 18 or 22 h after sampling. During the choice session, the 2nd sampling juvenile was presented to the adult together with a new, previously not encoun-