Neurobiology of Learning and Memory 91 (2009) 58–65

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

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

Mario Engelmann *

Otto-von-Guericke-Universität, Medizinische Neurobiologie, Leipziger Street 44, D-39120 Magdeburg, Germany

ARTICLE INFO

Article history:
Received 24 July 2008
Revised 28 August 2008
Accepted 30 August 2008
Available online 10 October 2008

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

Keywords:
Learning
Memory
Olfaction
Mice
Proactive interference
Retroactive interference
Protein synthesis
Amnesia
Memory consolidation

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.

* Fax: +49 391 67 14365.
E-mail address: mario.engelmann@med.ovgu.de

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doi:10.1016/j.nlm.2008.08.009

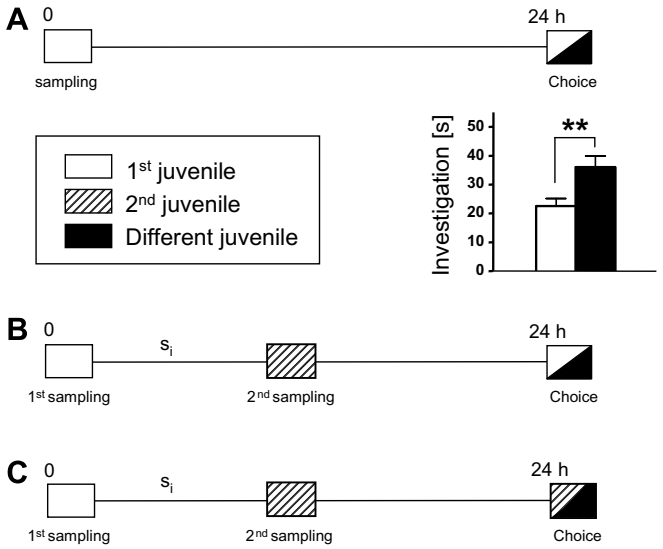

Fig. 1. Experimental protocol for testing the competition to two similar memory traces for long-term juvenile recognition memory in adult male mice. (A) illustrates the standard recognition memory procedure which was modified in (B) for testing the impact of the 2nd sampling (hatched box) for recognizing 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-

tered juvenile (see Fig. 1C). Since this procedure results in shorter retention intervals with increasing s_i (see Fig. 1C), I expanded the retention interval for the s_i = 9, 18 and 22 h, so that the interval between 2nd sampling and choice was 24 h.

2.5. Fos immunohistochemistry

For analysing Fos immunoreactivity, mice (n = 23) were separated 12 h before the experiment. The mice underwent an initial encounter as mentioned above: juvenile presentation lasted 4 min. During presentation, a trained observer measured the duration of investigatory behaviour of the adult mice towards the presented juvenile(s).

Either 7 h after exposure to a "sampling" juvenile or 70 min after exposure to a sampling "interference" juvenile that took place 6 h after sampling, or 70 min after sampling adults were deeply anaesthetised using a mixture of 0.1 ml/0.06 ml Ketavet and Dormitor (Pharmacia GmbH, Erlangen; Bayer Vital GmbH, Leverkusen, respectively, Germany) and transcardially perfused with physiological saline for 2 min, followed by 4% formaldehyde, diluted in 0.1 M phosphate buffer pH 7.4 (PB) for 12 min. Brains were removed, postfixed for 20 h in 4% formaldehyde diluted in PB, transferred to 0.5 M sucrose in PB for 6 h and finally to 1 M sucrose in PB for ca. 40 h. The brains were shock-frozen in isopentanol at −50 °C and stored at −80 °C until cryo-sectioning (Leica Frigocut 2800E, Wetzlar, Germany). Floating frontal sections (25 μm) were incubated with an antibody raised against a peptide mapping at the amino terminus of human c-Fos p62 (identical to the corresponding mouse sequence; c-Fos sc-42; Santa Cruz Biotechnology Inc., Santa Cruz, California, USA). Immunoreactive cells were visualised using the ABC-method as previously described (Laube et al., 2002). The number of Fos-immunoreactive cells was determined using a Zeiss microscope (Axiophot, Jena, Germany) and a computer software program (Image C, Berlin, Germany). The following brain areas were identified using the stereotaxic atlas of (Franklin & Paxinos, 1997): AOB (granular cell subdivision), MOB, medial nucleus of the amygdala, piriform cortex and medial preoptic area. A trained observer unaware of the animal's treatment counted all cells that were immunoreactive in a given brain area on one to three representative slices per mouse. For the MOB, a representative area of 0.7 mm^2 (including both mitral and granular cells) was analysed.

2.6. Blockade of protein synthesis

Protein synthesis was blocked by ANI (Sigma-Aldrich, Steinheim, Germany) diluted in saline by adding 0.1 M HCl. The pH 7.2 was adjusted by 0.1 M NaOH. Mice received ANI (150 mg/kg subcutaneous) under light isofluran (Forene®, Abbott GmbH, Wiesbaden, Germany) anaesthesia either immediately after presentation of the interference juvenile or after the given time interval has elapsed (see Fig. 1). The dose used here has been previously shown to significantly reduce protein synthesis for the first 3–4 h after administration (Flood, Rosenzweig, Bennett, & Orme, 1973; Wanisch et al., 2008). Previous experiments revealed that juvenile recognition with a retention interval of 24 h is insensitive to anisomycin treatment if the drug is applied either 3 or 18 h after sampling (Richter et al., 2005). Each group of mice was tested in multiple sessions. During control sessions, mice received the same volume of sterile saline (Fresenius-Kabi, Bad Homburg, Germany). Experiments were performed in a balanced cross-over design with 1 week between treatments.

2.7. Statistics

Data are presented as means + SEM. Statistical analysis was performed using GB-STAT 6.0 (Dynamic Microsystems, Silver Springs, MD, USA) or GraphPad Prism 4.01 (GraphPad Software, San Diego, CA, USA). Data obtained from the social discrimination were analysed either using the paired Student's t-test or two-way ANOVA with repeated measures. The number of Fos-immunoreactive cells was analysed using one-factor ANOVA. ANOVAs were followed by Neuman–Keuls procedure if appropriate. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Time-dependent interaction of two memory traces

Investigation durations measured during the 1st and 2nd sampling are presented in Table 1. Adult mice 'remember' a juvenile mouse that they first encountered 24 h earlier. However, if the adult mouse was exposed to a 2nd juvenile for 4 min during the 2nd sampling at a s_i of 5 min or 3, 6, 9, 12 and 15 h, its ability to recognize the 1st sampled juvenile was impaired (Fig. 2A; statistics: 5 min: $t(df = 18) = 0.03$, $p = 0.974$; **3 h**: $t(df = 19) = 0.189$, $p = 0.852$; **6 h**: $t(df = 19) = 0.631$, $p = 0.536$; **9 h**: $t(df = 18) = 0.186$, $p = 0.855$; **12 h**: $t(df = 15) = 1.01$, $p = 0.328$; **15 h**: $t(df = 14) = 1.34$, $p = 0.208$). In contrast, if the 2nd sampling took place 18 h or 22 h after the 1st sampling, recognition of the 1st sampled juvenile was unaffected (Fig. 2A, **18 h**: $t(df = 18) = 3.472$, $p = 0.003$; **22 h**: $t(df = 18) = 3.88$, $p = 0.001$).

Table 1
Investigation durations (means + SEM) during sampling and interference, respectively, of the animals presented in Figs. 2, 3, 4 and 5; n indicates the number of animals per group

		1st sampling	2nd sampling	n
Fig. 2				
A				
s_i = 5 min		25.8 + 4.2	14.9 + 2.1	19
s_i = 3 h		42.9 + 6.7	25.1 + 5.6	20
s_i = 6 h		26.3 + 4.0	20.8 + 5.4	20
s_i = 9 h		21.5 + 3.1	13.1 + 1.5	19
s_i = 12 h		37.9 + 3.6	31.6 + 3.5	16
s_i = 15 h		20.8 + 4.8	27.1 + 6.9	15
s_i = 18 h		22.8 + 2.9	20.4 + 2.4	19
s_i = 22 h		37.1 + 3.7	48.2 + 6.9	19
B				
s_i = 5 min		29.4 + 3.9	18.8 + 2.5	18
s_i = 3 h		45.6 + 5.3	25.0 + 3.8	19
s_i = 6 h		27.6 + 3.6	24.5 + 4.8	19
s_i = 9 h		23.1 + 1.9	14.4 + 2.4	19
s_i = 12 h		36.3 + 3.5	31.1 + 5.2	16
s_i = 15 h		27.3 + 5.0	22.5 + 4.9	15
s_i = 18 h		16.8 + 2.7	25.1 + 6.0	10
s_i = 22 h		25.0 + 4.2	32.8 + 4.7	19
C				
s_i = 9 h		33.6 + 7.1	22.3 + 5.1	10
s_i = 18 h		100.2 + 9.5	95.7 + 11.6	10
s_i = 22 h		29.3 + 6.7	22.7 + 5.2	21
	Treatment	1st sampling	2nd sampling	n
Fig. 3				
A	NaCl	24.5 + 3.9	15.2 + 3.1	20
	ANI	21.2 + 3.5	33.3 + 14.1	20
B	NaCl	25.3 + 3.1	24.1 + 3.5	19
	ANI	32.6 + 4.1	32.1 + 3.6	19
Fig. 4				
A	NaCl	29.3 + 3.3	33.4 + 4.8	20
	ANI	25.6 + 3.7	41.5 + 5.9	20
B	NaCl	23.2 + 4.6	37.7 + 9.0	19
	ANI	28.9 + 4.6	37.9 + 7.1	19
Fig. 5				
	NaCl	78.3 + 9.9	84.4 + 11.3	20
	ANI	39.7 + 6.0	48.3 + 5.2	20

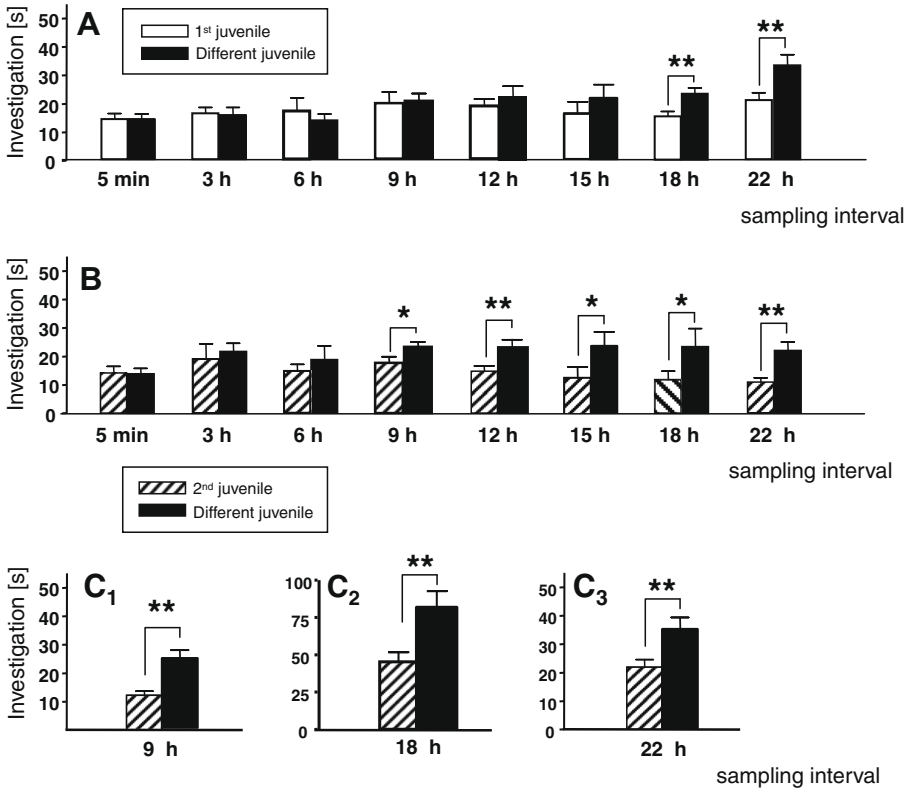

Fig. 2. The competition of two similar memory traces for long-term memory depends upon the sampling interval. Recognition memory of the experimental subjects was tested during choice by exposure to juveniles presented either during the 1st (A) or 2nd sampling (B, C) together with a different unfamiliar juvenile mouse. According to the protocol provided in Fig. 1 B and C the sampling intervals ranged from 5 min up to 22 h. For A and B the 1st sampling and choice were separated by 24 h, for C the 2nd sampling and choice were separated by 24 h. $^{*}p < 0.05$ and $^{**}p < 0.01$ paired Students *t*-test.

Adults were unable to recognize the juvenile exposed during the 2nd sampling if they were tested during the choice session at a s_i of 5 min, 3 h or 6 h (Fig. 2B, 5 min: $t = 0.512$, $df = 17$, $p = 0.615$; 3 h: $t = 0.107$, $df = 18$, $p = 0.916$; 6 h: $t = 0.759$, $df = 18$, $p = 0.456$). Recognition was seen at a s_i of 9, 12, 15, 18 and 22 h (Fig. 2B, **9 h**: $t(df = 18) = 2.443$, $p = 0.025$; **12 h**: $t(df = 9) = 4.04$, $p = 0.003$; **15 h**: $t(df = 14) = 2.86$, $p = 0.012$; **18 h**: $t(df = 9) = 3.170$, $p = 0.014$; **22 h**: $t(df = 18) = 4.083$, $p < 0.001$). Exemplarily investigation of longer RIs confirmed that both at a s_i of 9, 18 and 22 h, the recognition of the 2nd juvenile was still detectable 24 h after the 2nd sampling (Fig. 2C; **9 h**: $t(df = 9) = 4.386$, $p = 0.002$; **18 h**: $t(df = 9) = 3.280$, $p = 0.009$; **22 h:** $(df = 20) = 4.004$, $p < 0.001$).

3.2. Impact of protein synthesis blockade on interaction of two memory traces

Mice exposed to the 2nd juvenile 3 h after the 1st sampling could remember the 1st sampled juvenile if they were given a subcutaneous injection of ANI immediately after the 2nd sampling, but not if they were given a subcutaneous injection of 0.9% NaCl (vehicle) instead (Fig. 3A; ANOVA, interaction $F_{1,36} = 10.41$; $p < 0.001$).

Similarly, mice tested at a s_i of 3 h which received a subcutaneous injection of ANI immediately after the 1st sampling could recognize the 2nd juvenile, but could not recognize the 2nd juvenile if given a subcutaneous injection of 0.9% NaCl instead (Fig. 3B; ANOVA interaction $F_{1,79} = 19.08$; $p < 0.001$).

If the 2nd juvenile was presented 18 h after the original encounter, neither NaCl-solution nor ANI affected recognition of the juvenile presented during the 1st sampling (Fig. 4A; ANOVA juvenile $F_{1,38} = 14.43$; $p < 0.001$; interaction $F_{1,38} = 0.04$; $p = 0.04$). Exposure of the 2nd juvenile at a s_i of 18 h followed by ANI impaired recognition of this juvenile during the choice session (Fig. 4B; ANOVA interaction $F_{1,36} = 5.736$; $p = 0.022$), showing that this type of intermediate-term memory is sensitive to protein synthesis blockade.

Exposure to the 2nd juvenile 9 h after the 1st sampling with subsequent ANI treatment abolished the ability of the mice to recognize the 2nd sampled juvenile during choice (Fig. 5; ANOVA interaction $F_{1,38} = 6.46$; $p = 0.015$).

3.3. Fos protein synthesis

Fig. 6 shows the number of Fos-immunoreactive cells in mice exposed to the interference juvenile 6 h after the original encounter and killed 1 h later. In these mice, there were significantly more Fos-immunoreactive cells in distinct parts of the accessory (Fig. 6A; ANOVA statistics: accessory olfactory bulb, granular cells: $F_{3,18} = 18.42$, $p < 0.01$; medial preoptic area: $F_{3,19} = 13.40$, $p < 0.01$; medial nucleus of the amygdala: $F_{3,19} = 27.04$, $p < 0.01$) and main olfactory systems (Fig. 6B; ANOVA statistics: main olfactory bulb, granular and mitral cells: $F_{3,19} = 7.48$, $p < 0.01$; piriform cortex: $F_{3,19} = 23.76$, $p < 0.01$) than in untreated controls or mice exposed to the sampling juvenile 7 h before without interference. This result is similar to that seen in animals killed 70 min after the initial encounter (Fig. 6, group **JT**).

4. Discussion

Amnesia induced by the competition of two memory traces plays an outstanding role in daily life, but is technically difficult to perform in laboratory rodents. Here I used a non-conditioned olfactory recognition task as a tool to investigate memory forma-

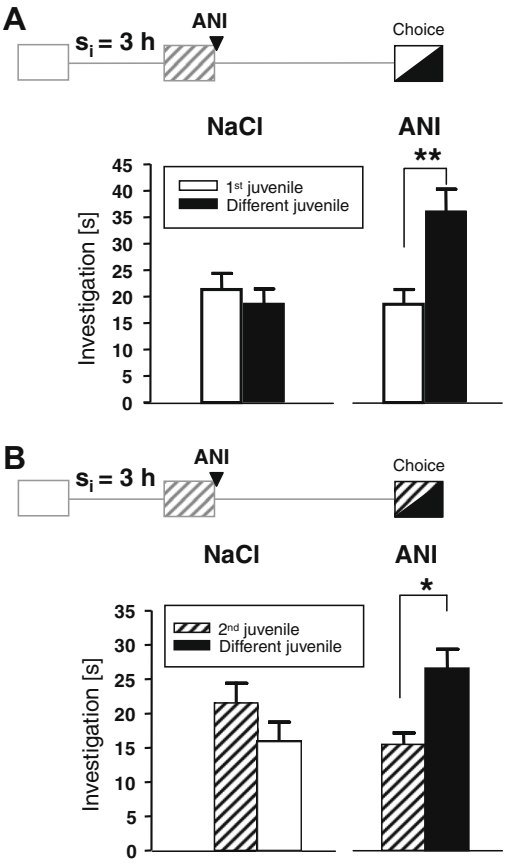

Fig. 3. At a sampling interval (s_i) of 3 h ANI treatment blocks the amnesic effects observed after repeated sampling. (A) Administration of ANI immediately after the 2nd sampling blocked the amnesic effect for the 1st sampled juvenile observed after saline treatment under otherwise similar conditions. (B) Similarly, ANI treatment immediately after the 1st sampling blocked the amnesic effect observed for the 2nd sampled juvenile observed after saline treatment under otherwise similar conditions. $^{*}p < 0.05$ and $^{**}p < 0.01$, ANOVA followed by Newman–Keuls test. The symbols of the timeline diagrams correspond with those provided in Fig. 1B and C.

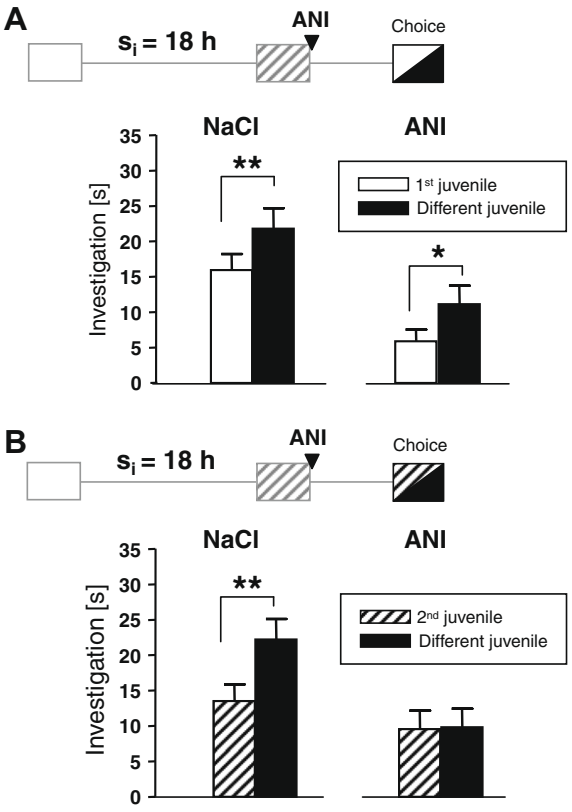

Fig. 4. At a sampling interval (s_i) of 18 h ANI failed to affect the recognition of the 1st sampled juvenile, but impaired that of the 2nd juvenile. (A) Administration of neither saline nor ANI immediately after exposure to the 2nd juvenile affected recognition memory. (B) Administration of saline immediately after exposure of the 2nd juvenile failed to affect recognition of this juvenile during the choice session. In contrast, ANI treatment blocked this recognition under otherwise similar conditions. $^{*}p < 0.05$ and $^{**}p < 0.01$, ANOVA followed by Newman–Keuls test. The symbols of the timeline diagrams correspond with those provided in Fig. 1B and C.

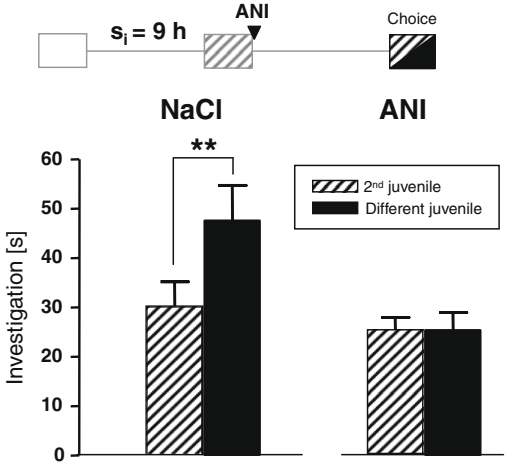

Fig. 5. ANI blocks recognition of the 2nd juvenile presented at a sampling interval (s_i) of 9 h. Administration of ANI immediately after the presentation of the 2nd juvenile blocks the recognition of this juvenile. $^{**}p < 0.01$, ANOVA followed by Newman–Keuls test. The symbols of the timeline diagram correspond with those provided in Fig. 1C.

tion and extinction in laboratory mice. Most attempts to investigate the competition of two memory traces for consolidation are hampered by the fact that these studies are based on conditioning, which makes memories become more resistant against extinction (McGaugh, 2006). The model of olfactory discrimination learning provides insight into memory formation that is not based on such resistant stimulus associations, and thus may result in memory traces that are – at least for a defined period after learning – more sensitive to stimuli that produce amnesia. Müller and Pilzecker (1900) described how a dramatic loss in memory can occur as a result of interference by subsequently acquired similar information. This process, known as *retroactive interference* (Müller & Pilzecker, 1900), has been the subject of many psychological studies, but little is known of its neurobiological basis (Baddeley, 1979; Wixted, 2004). Retroactive interference is known to be closely linked to the amnesic effect called *proactive interference*, whereby the acquisition and consolidation of new memories is impaired by prior learning. The approach of first exposing the experimental subjects to two different juveniles, allowing them to acquire their olfactory signatures, and finally testing their ability to recognize these signatures, fulfils the criteria of retroactive (Fig. 1B) and proactive interference (Fig. 1C).

Evidence has accumulated in the literature which suggests that the power of interference is closely related to the interval between two learning events, and that retroactive and proactive interference are closely linked (Baddeley, 1979; Wixted, 2004). My data show that retroactive interference of an acquired olfactory memory occurs only for s_i's shorter than 18 h (Fig. 2A). This corresponds with the observation that the synthesis of proteins that are required for the consolidation of long-term olfactory recognition

memory is finished at 18 h (Richter et al., 2005; Wanisch et al., 2008). The fact that the exposure of the 2nd juvenile, from as little as 5 min to as much as 9 h after the 1st sampling, produced a retroactive interference implies that there is an impact on mechanisms that are involved in the consolidation of information acquired during the original encounter. Importantly, while social recognition at about 3 h after the original encounter is insensitive to ANI (Richter et al., 2005), exposure to the 2nd juvenile at the same time point corrupted juvenile recognition (Fig. 2A). This finding demonstrates that amnesia induced by retroactive interference differs qualitatively from that induced by ANI administration (Gold, 2006), and seems to indicate that these effects involve different mechanisms. However, as I will later argue, there is good evidence that ANI treatment and exposure of an interference juvenile act at the same neuronal substrate.

To further characterize the cellular mechanisms that underlie retroactive and proactive interference, I used ANI to block translation. Since ANI treatment 3 h after the original encounter does not affect pure recognition memory tested 24 h later (Richter et al., 2005), I chose this time window for my experiments. When ANI was administered immediately after presentation of the 2nd juvenile 3 h after the original encounter, there was no retroactive interference (Fig. 3A). This suggests that exposure to the 2nd juvenile initiates new protein synthesis that impairs the second wave of protein synthesis induced by the original encounter (Richter et al., 2005). As shown by my Fos immunohistochemistry, exposure to an interference juvenile stimulates protein synthesis in the same neuronal populations that are stimulated by the original encounter (Fig. 6; see also (Richter et al., 2005)). This suggestion that retroactive interference is related to confounded protein synthesis processes is supported by the fact that presentation of a 2nd juvenile 18 h after the original encounter did not affect recognition memory (Fig. 4A). At this time, the protein synthesis needed for juvenile recognition is finished (Richter et al., 2005; Wanisch

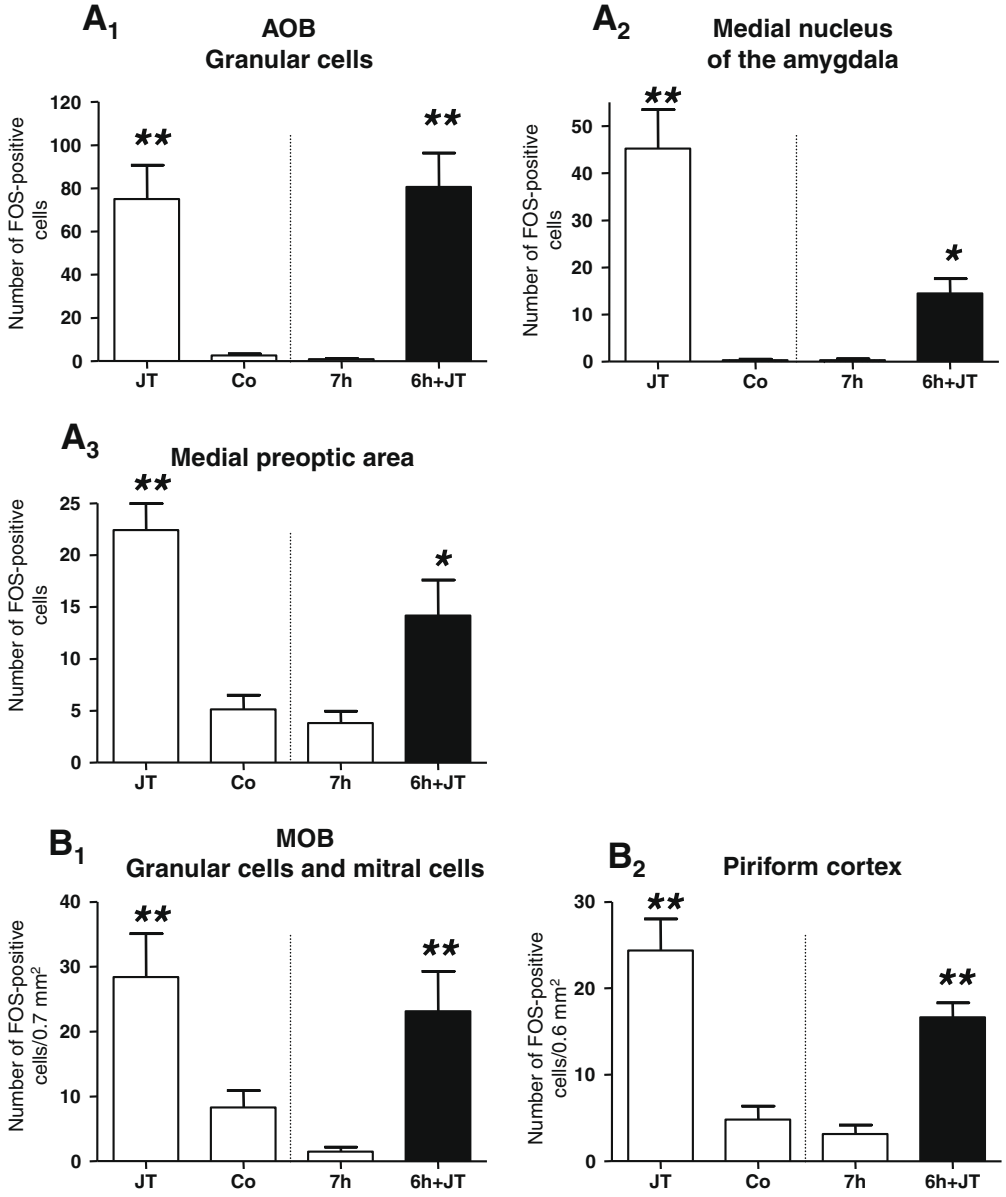

Fig. 6. Exposure to a 2nd juvenile 6 h after the 1st exposure induces c-Fos synthesis selectively in brain areas that are also activated after the initial encounter. Means + SEM are shown. Mice were exposed for 4 min to a conspecific sampling juvenile and killed either 70 min (JT) or 7 h later (7 h) or mice were re-exposed to another juvenile 6 h after the initial encounter and killed 70 min later (6h+JT; black bar). Control mice (Co) remained undisturbed. (A_{1-3}) illustrates the number of Fos-immunoreactive cells in brain areas primarily involved in processing non-volatile olfactory stimuli, (B_{1-2}) illustrates the number of Fos-immunoreactive cells in brain areas mainly involved in processing volatile stimuli. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. Co and 7 h, ANOVA followed by Neuman–Keuls test. AOB: accessory olfactory bulb, MOB: main accessory bulb.

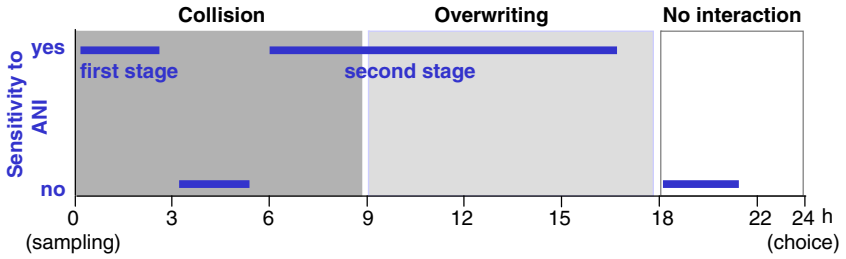

Fig. 7. Hypothetical scenario illustrating the effects of sampling on juvenile long-term recognition memory in mice. The observed effects of the exposure of a 2nd juvenile on recognition memory are plotted against the response to the administration of the protein synthesis inhibitor ANI (black line) induced by sampling with a retention interval of 24 h until choice (based on the results obtained by Richter et al. (2005)). "Collision" refers to the interval (up approx. 9 h after 1st sampling) during which new and earlier acquired information extinguish each other possibly due to two competing and subsequently interfering protein synthesis processes. This period is followed by "overwriting" (from approx. 9–18 h after sampling), during which the memory for the earlier learned stimulus is replaced by the subsequently acquired memory. Finally, new memories can be formed without loosing earlier acquired information during the period where "no (interfering) interaction" between the two memory traces can be measured (i.e. after 18 h after sampling; modified after (Wanisch et al. (2008)).

et al., 2008) and, therefore, cannot be affected by interference juvenile-related protein synthesis. My data fit well with the observation that the amnesic effect of retroactive interference is linked to intracellular processes that depend on the organelles within the neuronal somata (Sangha et al., 2005).

The hypothesis of a time-dependent interaction between the two protein synthesis processes is strengthened by my proactive interference studies. As shown in Fig. 2B, juvenile exposure during the original encounter blocks the memory for a 2nd juvenile presented up to 6 h later. However, if protein synthesis for the recognition of the 1st sampled juvenile is completed (about 18 h after sampling (Richter et al., 2005; Wanisch et al., 2008)) no collision with the protein synthesis of the 2nd sampled juvenile occurs, and there is no proactive interference. If protein synthesis induced by the 1st sampled juvenile is blocked by ANI treatment, then animals will recognize the 2nd sampled juvenile during choice (Fig. 3B). Moreover, the results obtained by presenting the 2nd juveniles 9, 18 and 22 h after sampling, and testing for recognition 24 h later support the observation that mice can generate and recall independent long-term lasting memory traces for similar stimuli (Fig. 2C).

Strikingly, there was no proactive interference if I exposed the 2nd juvenile at 9 h after the original encounter (Fig. 2B and C). This contrast with the results obtained for retroactive interference, and suggests that retroactive and proactive interference can be dissociated. The most plausible explanation is that ongoing protein synthesis underlying consolidation of recognition memory is confounded by a potential interference stimulus in a unidirectional manner. Indeed, ANI treatment revealed that essential protein synthesis processes induced by sampling and required for successful recognition are still ongoing 9–15 h later (Wanisch et al., 2008). Furthermore, ANI given immediately after presentation of the 2nd juvenile 9 h after the original encounter re-installed proactive interference (Fig. 5). This implies that the recognition of the 2nd sampled juvenile also requires protein synthesis; a conclusion confirmed by studying proactive interference using the interference interval of 18 h (Fig. 4B). This suggests that the choice test performed 6 h later refers to intermediate-term memory which has been reported to depend on protein synthesis (Crow, Redell, Tian, Xue-Bian, & Dash, 2003). Indeed, own unpublished data suggest that, in contrast to shorter retention intervals (e.g. 1 h, c.f. (Richter et al., 2005), intermediate-term juvenile recognition memory is both sensitive for ANI and retroactive interference.

On the basis of the data obtained here, I postulate three different time windows during which either (a) the synthesis of proteins related to the consolidation of the memory for the 1st sampled juvenile collides with that of the memory for the 2nd sampled juvenile or (b) the protein synthesis initiated by exposure of the 2nd juvenile overwrites that induced by sampling or (c) no perturbing interaction occurs (Fig. 7). Obviously, the critical parameter is the progress of protein synthesis initiated by the originally sampled olfactory signature.

The present study provides new insight into the interaction between two newly formed memories under animal laboratory conditions. My results support the hypothesis that the processes of retroactive and proactive interference, which are applicable to my model of declarative memory in mice, are time-dependent and related to memory consolidation processes that are sensitive to the translation blocker ANI. I also conclude that forgetting, which is related to both retroactive and proactive interference in my animal model, is linked to effects on the formation of the memory trace rather than to impairments in memory retrieval. Further studies will investigate whether the effects observed in response to ANI administration relate indeed exclusively to the blockade of protein synthesis, or whether specific intracellular cascades and/or neurotransmitter systems affected by the drug may impair memory (and thus may be relevant for memory consolidation) independent of protein synthesis inhibition (Canal, Chang, & Gold, 2007; Routtenberg & Rekart, 2005) in my model of social recognition memory.

Acknowledgments

The author thanks Rita Murau and Andrea Rudloff for expert technical support and Laura Baur for correcting the English.

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