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# Is spatial memory transformed during the consolidation process? Effect of reminding

**Juan M. J. Ramos**

Department of Experimental Psychology and Behavioural Physiology, University of Granada, Granada, Spain
Email: jmjramos@ugr.es

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Several studies in rats have shown activation of cortical regions and concurrent deactivation of the hippocampus during the retrieval of spatial memory, as the consolidation process progresses. To determine whether during this post-learning period of memory reorganization, spatial memories are transformed from a specific to a more generic representation, in Experiment 1 we compare remote spatial memory measured using a single probe trial *versus* relearning. Results show that spatial memory can be effectively retrieved using a single probe trial during the 18 days following learning; after this time a retraining procedure is necessary. In Experiment 2 and 3 we tested the effect of a reminder treatment on the retrieval of remote memory. Results indicate that when the reminder was applied after experimental day 18, the treatment did not significantly improve the retrieval of spatial memory during a retention test (Exp. 2); however, if the reminder was applied before day 18, a significant improvement during the retention test was observed (Exp. 3). In order to investigate the extent to which memory becomes more difficult to retrieve as time passes due specifically to a retrieval deficit, in experiment 4 no significant differences, but marginal ones, were detected between an overtrained group and a control group when the retention test took place 30 days after the end of learning. Overall, the present results suggest that as the consolidation process progresses, spatial information becomes more difficult to recover, in part because the original detailed trace has gradually been transformed into a more schematic representation.

Key words: memory consolidation, long-term memory, spatial learning, retrieval, amnesia

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Recent studies involving lesions (Kim and Fanselow 1992, Ramos 1998, Riedel et al. 1999, Clark et al. 2002, 2005, Winocur et al. 2005a), genetic (Shimizu et at. 2000), and imaging approaches (Bontempi et al. 1999) conducted on experimental animals, have provided evidence that memories are gradually reorganized over time. Based upon these studies, contemporary memory theories suggest that a consolidation process is necessary during a variable post-learning period, in order for permanent or long-term memory to form (Ramos 2000, Frankland and Bontempi 2005, Squire and Bayley 2007). From a memory systems perspective, the retrieval of recent memory would initially depend on the hippocampus, while remote memory, that is, consolidated memory, would depend on a distributed cortical network (Frankland et al. 2007, Squire and Alvarez 1995). Within this network, specific cortical areas are recruited depending on the type of task. For example, for a task in which olfaction is key (social transmission of food preferences), remote memory retrieval, but not recent memory, was associated with increased activity in piriform, entorhinal and orbitofrontal cortices (Ross and Eichenbaum 2006); however, increased activity in the anterior cingulate cortex (Frankland et al. 2004, Teixeira et al. 2006) or in the medial prefrontal cortex (Takehara et al. 2003) was associated with the retrieval of remote contextual fear or spatial memory and remote trace eyeblink memory, respectively.

How each of these structures contributes to the consolidation process is still not well known. However, according to several theories of consolidation, at the beginning of this period of memory reorganization, the hippocampus encodes and retrieves detailed memories, and then the cortex slowly extracts and stores a more schematic and generic knowledge from original

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*Correspondence should be addressed to J. M. J. Ramos*
*Email: jmjramos@ugr.es*

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specific experiences, as the consolidation process progresses (McClelland et al. 1995, O'Reilly and Rudy 2001, Nadel et al. 2007). Accordingly, recent and remote memories are of a different nature. Some studies have suggested that recent memory (hippocampal-dependent) is detailed and context-dependent (Nadel and Moscovitch 1997, Moscovitch et al. 2006). So, in humans, hippocampal-dependent memories allow for accurate reexperiencing of the content of an episode and the place where it occurred. In contrast, remote or consolidated memories (hippocampal-independent but cortex-dependent) are context-free and more gist-like (Nadel et al. 2007).

The aim of the present series of experiments was to investigate time-dependent changes in the retrieval of a spatial memory as the consolidation process progresses. A central goal was to determine the extent to which these changes in retrieval reflect a transformation of the original memory engram from a detailed to a more generic representation. This study used a radial maze and an experimental procedure that obliged the animals to utilise an allocentric strategy (Ramos 2002). Previous research, using a very similar task and procedure, has shown that the task initially requires the hippocampus, but over time becomes dependent on the neocortex (Maviel et al. 2004). Thus, based on the foregoing data, we hypothesize that immediately after the learning, the memory engram of the task would be specific, highly context-dependent, preserving a detailed representation of the environment. In contrast, as the consolidation process advances and memory becomes dependent on the cortex, the spatial memory engram would become more schematic and contain a coarse representation of the environment.

In the present series four experiments were performed. Experiment 1 was an exploratory study. The goal was to investigate time-dependent differences in spatial memory retrieval. We assume that when animals correctly retrieve the previously-learned spatial task in the first probe trial of the retention test, a detailed memory representation of the environment exists. However, if retraining is necessary to retrieve spatial information, then we assume that the original memory is harder to retrieve, either because a transformation of the memory engram, from a detailed to a more schematic representation, has taken place during the post-training period, or because a retrieval deficit of the original memory has occurred. Experiments 2, 3 and 4 were designed to dissociate between these two possibilities.

In experiment 1 we compare the retrieval of remote spatial memory measured using the first relearning trial (a pure measure of memory) to the total number of errors to criterion during a relearning phase. It is hypothesized that as the spatial memory becomes increasingly remote, a more schematic representation will develop in the cortex and a single probe trial will be less effective in information retrieval. Sixty four naïve male Wistar rats (270–310 g) were used in this first experiment. Rats were individually housed in a vivarium with constant temperature and a 12:12 h light–dark cycle. All experimental procedures were performed in conformity with European (86/609/EEC) legislation and were approved by the Ethics Committee for animal research of the University of Granada. The animals were food-deprived to 85% of their normal body weight during the training of a spatial reference memory task. A radial-arm maze with four arms was used. Three constant arms were used for starting (the east, north and south arms) and the fourth (the west arm) was the goal. Between trials, the maze was rotated 90° in a clockwise direction to prevent the rats from using intramaze signals to reach the goal, although the goal arm always occupied the west position (see Ramos 2002, for specific details on the apparatus and experimental procedure). Rats received eight trials per session and one session per day to reach a learning criterion (14 correct trials on 2 consecutive days). After the end of the training, rats were randomly assigned to a relearning group. A total of 7 independent relearning groups were formed, with training-retraining intervals of 3 ($n$=8), 6 ($n$=6), 12 ($n$=6), 18 ($n$=14), 24 ($n$=15), 48 ($n$=5) and 72 ($n$=10) days. During the training-retraining interval, the subjects remained in their respective cages and were not tested in any way. The procedure used during the retraining phase of testing was identical to that of training (acquisition).

Figure 1A illustrates the percentage of rats in each group that remembered the location of the goal arm during the first trial of the retraining phase (a single probe trial). One-tailed Fisher exact tests showed that for the groups with shorter training-retraining intervals, 3 days ($P$<0.006), 6 days ($P$<0.03), 12 days ($P$<0.03) and 18 days ($P$<0.04), the percentage of animals that remembered the task was significantly greater than would be expected by chance. In contrast, the animals with greater training-retraining intervals did not remember the correct place in the first retraining trial (24 days, $P$=0.23; 48 days, $P$=0.65; 72 days, $P$=0.24; Fischer exact tests).

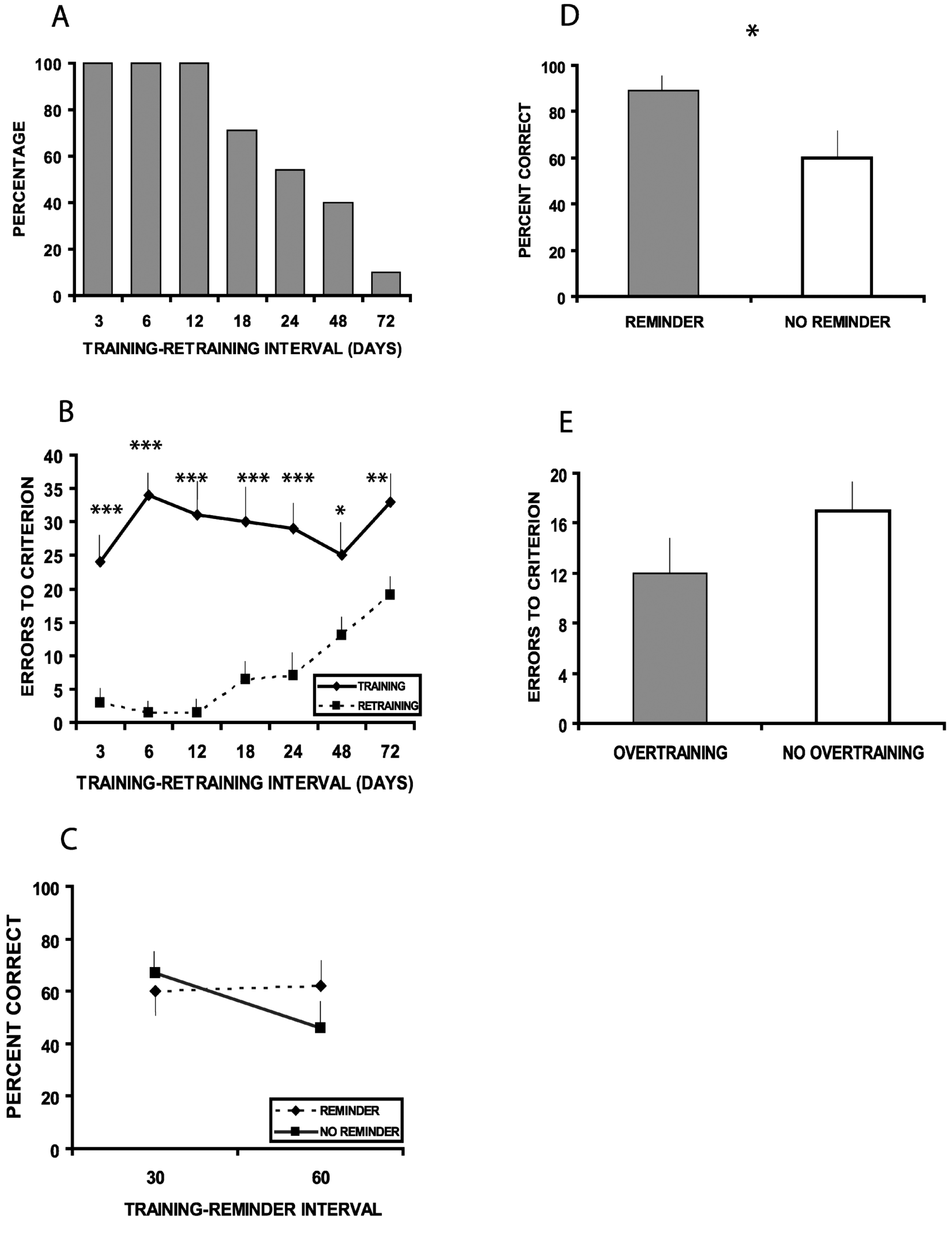

Fig. 1. (A) Percentage of rats of each group that remembered the goal arm during the first trial of the relearning phase. (B) Mean (± SEM) number of errors to criterion for the seven groups during the training and retraining phases of testing. Training versus retraining comparisons with *post-hoc* Newman-Keuls tests: *** $P<0.0001$, ** $P<0.001$, * $P<0.01$. (C) Mean percentage of correct responses (± SEM) during the five trials of relearning 30 or 60 days after the end of learning for the groups with and without reminder treatment. The reminder treatment was applied one day before the relearning session. (D) Mean percentage of correct responses (± SEM) during the five trials of relearning 18 days after the end of learning. The reminder/no-reminder treatment was applied on day 17 post-learning. * $P<0.002$. (E) Mean number of errors to criterion (± SEM) recorded during the retraining phase starting on experimental day 60.

Figure 1B represents the results observed during the retraining period. A two-way ANOVA, with a factor between groups (training-retraining interval, with seven levels) and a factor within groups (with two levels: performance during the training versus retraining phases), revealed a significant effect of groups ($F_{6,57}$=6.68, $P$<0.00002), training-retraining performance within groups ($F_{1,57}$=328.29, $P$<0.00001) and interaction ($F_{6,57}$=4.34, $P$<0.001). *Post-hoc* Newman-Keuls tests indicated that in all the training-retraining intervals, rats performed significantly better during the retraining period as compared with the training phase (Fig. 1B). In an attempt to analyze the interaction, one-way ANOVA comparing the performance of the seven groups during the training did not detect significant differences ($F_{6,57}$=1.73, $P$=0.13). However, a second one-way ANOVA, this time comparing the performance of the seven groups during the retraining, did reveal significant differences ($F_{6,57}$=10.14, $P$<0.0000001). *Post-hoc* Newman-Keuls tests indicated that the number of errors to criterion during the retraining was not significantly different in the groups of 3, 6, 12, 18 and 24 days; however, all these groups differed significantly from the groups with 48 and 72 days of training-retraining interval. Finally the 48 and 72 days groups did not differ significantly from each other ($P$=0.07) in terms of the number of errors before reaching criterion during the retraining period.

Experiment 1 data indicate that spatial memory in the radial maze becomes more difficult to retrieve over time. Two alternative hypotheses may explain these results. One view posits that as memory is stored in the neocortex it loses detail and becomes more schematic in nature (Nadel et al. 2007). A second view, the retrieval deficit hypothesis, suggests that the detailed representation is not lost, but rather becomes more difficult to retrieve with time (Rosas and Alonso 1997). One possible way to test these hypotheses is to apply a reminder treatment before the remote memory test and investigate whether the reminder treatment improves the retrieval of spatial remote memory (Wiltgen and Silva 2007). Thus, in the second experiment, we hypothesize that if, as time passes after learning, the original memory is transformed into a more schematic representation, then a reminder treatment would have little effect in the retrieval of remote memory. In contrast, if the reminder restores remote memory, then the detailed memory was likely not lost (Wiltgen and Silva 2007). Thirty-five naïve male Wistar rats distributed in four groups were trained using the same procedure as in experiment 1. In two groups ($n$=8 no-reminder group, $n$=9 reminder group), the retraining took place 30 days after the end of learning. In the other two groups, each made up of 9 male Wistar rats (reminder vs. no reminder groups), the retraining took place 60 days after the learning. In the reminding groups, one day before the retraining, each animal was allowed to explore the maze freely for 3 minutes. No reward was available in the goal arm. Two hours later, each animal was allowed to explore the maze again, but on this occasion six 45-mg food pellets were placed in the food cup of the goal arm. This second session of reminding ended when the animals consumed the reward. The rats that did not receive the reminder treatment explored an arena in a different room for a period of time similar to the one used by the experimental subjects exploring the maze and received the same amount of food. To determine the effect of the reminding, we recorded the performance of the four groups of rats in five consecutive trials of relearning on day 30 and 60 after the end of learning.

Figure 1C shows the performance of the groups during the five consecutive trials of retraining. A two-way ANOVA showed no significant differences for the reminder/no-reminder factor ($F_{1,31}$=1.16, $P$=0.28), for the training-reminder interval factor ($F_{1,31}$=0.52, $P$=0.47) or for the interaction ($F_{1,31}$=2.53, $P$=0.12). One-tailed Fisher exact tests showed that in none of the four groups used in this experiment was the percentage of animals that remembered the location of the goal arm during the first trial of the retraining phase significantly greater than would be expected by chance (30 days with reminder $P$=0.17, 30 days without reminder $P$=0.23, 60 days with reminder $P$=0.50, 60 days without reminder $P$=0.69).

Results of Experiment 2 show that the reminder was not able to facilitate remote memory. However, it can be argued that the reminder treatment, in itself, could be ineffective in reactivating the memory regardless of the learning-reminder interval. Experiment 3 was designed to investigate this idea. Two groups of 8 naïve male Wistar rats each were trained using the same procedure as in Experiment 2. In this case, however, the reminder treatment was applied on post-learning day 17. To determine the effect of reminding, we recorded the performance of the two groups of animals on five consecutive trials on day 18 after learning.

Results showed that the performance of the reminder group was significantly better than that of the control group ($F_{1,14}$=13.21, $P$<0.002, Fig. 1D).

Results of Experiments 2 and 3 support the view that the spatial memory engram is transformed over time. In Experiment 4, however, we look directly at the retrieval deficit hypothesis. Several studies have shown an inverse relationship between training level and forgetting (Rosas and Alonso 1997). Accordingly, we assume that if the original detailed representation is not lost but simply becomes more difficult to retrieve with time due to a retrieval deficit, then overtraining during the acquisition phase should facilitate the retrieval during the remote memory test. A specific prediction in this experiment is that no significant differences should be found between the overtrained and the control groups when tested at remote points if the memory engram has been transformed to a more schematic format. A total of 19 naïve male Wistar rats were employed ($n$=9 overtraining group, $n$=10 no-overtraining group). The animals were trained following the same experimental procedure used in Experiment 1, except that the overtrained group was trained during 30 consecutive days. In contrast, the control group was trained to criterion, as in Experiments 1, 2 and 3. In total, the control animals in Exp. 4 took an average of 12.3 days to reach criterion. To determine the effect of overtraining on the retrieval of old memory, a relearning procedure was carried out, starting on experimental day 60. This retraining phase ended when the animals reached the performance criterion of 14 correct trials on 2 consecutive days.

Figure 1E shows the mean number of errors to criterion in the experimental and control groups. One-way ANOVA indicated that no significant differences existed between the groups ($F_{1,17}$=3.88, $P$=0.066). In addition, using Fisher exact tests, neither in the overtrained (22%, $P$=0.50) nor in the control (30%, $P$=0.39) animals was the percentage of rats that remembered the location of the goal arm during the first trial of the retraining phase significant.

The main findings of the present series indicate that spatial memory in the radial maze can be retrieved with precision during the 18 days following the learning using a single probe trial. After this time has passed and while the spatial engram is presumibly still in the middle of the consolidation process (Winocur et al. 2005b, Broadbent et al. 2006), a single probe trial is ineffective in retrieving the original memory, and retraining is necessary to significantly improve the retrieval of remote memories. Interestingly, a reminder treatment facilitated the memory if the reminder was applied before post-learning day 18 but not if it was applied later.

Some authors have suggested that one result of the consolidation process is the extraction of general information from specific experiences with the resulting loss of detailed information about the original situation (Winocur et al. 2007). As mere speculation, it may be that in our first Experiment the shift from one single probe trial to retraining as of day 18 following learning, could reflect the loss of detailed information about the original learning as the consolidation process advances. Supporting this idea, the data from Experiment 3 show that the reminder still facilitates the memory when it is applied on Experimental day 17, suggesting that at this point memory is still detailed and a correspondence exists between the engram and the environment. As shown in Experiment 2, however, the reminder is not at all effective in facilitating the memory when applied on day 29 or 59 following learning, which suggests that a transformation in the memory engram has taken place during such period and, accordingly, no correspondence exists between the memory representation and the environment. This would make the retrieval more difficult.

Alternatively, data from Experiment 4 do not support the retrieval deficit hypothesis, since the overtraining, which counteracts the retrieval deficit (Rosas and Alonso 1997), does not significantly improve the retrieval of spatial remote memory. However, the data reveal a marginal difference ($P$=0.066) between the two groups, which suggests a possible partial contribution of the retrieval deficit hypothesis. So, the trend with overtraining in Experiment 4 suggests that a greater number of overtraining trials would have been necessary to have a significant effect. In any case, the two points of view, transformation versus retrieval deficit, are not mutually exclusive, and it is indeed possible that several mechanisms are involved in the retrieval of past memories (Wiltgen and Silva 2007).

In conclusion, the main findings of the present series suggest that with time, spatial memories are transformed from contextually dependent ones to more generic ones (map-like representation). The time-course of this transformation is congruent with a sequential participation of the hippocampus and the cortex during the consolidation of spatial memory (Maviel et al. 2004).

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