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Memory traces compete under regimes of limited Arc protein synthesis: Implications for memory interference

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ABSTRACT

Recently encoded information can be lost in the presence of new information, a process called '*retrograde interference*'. Retrograde interference has been extensively described for more than a century; however, little is known about its underlying mechanisms. Different approaches agree on the need of the synthesis of plasticity related proteins (PRPs) to consolidate a long-term memory (LTM). Our hypothesis is that when PRPs are limited, interference of a task over LTM formation of another may be due to the utilization of protein resources common to both tasks. Here, by combining the tasks of inhibitory avoidance (IA) and open field (OF) exploration in rats, we show that memory traces compete for their stabilization if PRPs are limited. As a result, LTM is formed for only one of the tasks with a consequent decrease in the memory for the other. Furthermore, infusing Arc antisense oligonucleotide into the dorsal hippocampus, we found that Arc is necessary for LTM formation of these two types of learning tasks and is one of the PRPs that can be shared between them when animals are trained in both OF and IA. In sum, these findings suggest that under conditions of reduced protein availability, a learning task interferes with LTM formation of another by using the available PRPs.

1. Introduction

Amnesia or poor long-term memory (LTM) for events can occur by impaired encoding, consolidation and/or retrieval processes. Despite their common outcome, the underlying mechanisms can differ among them. Around a century ago, it was postulated that interference by the interpolation of certain materials or tasks could be one of the causes of everyday forgetting (Müller & Pilzecker, 1900).

During our everyday life we experience several events with multiple characteristics. However, not many of them are stored in our LTM. For example, if while rehearsing a phone number you suddenly witness a car crash, the number will probably be forgotten, and instead, the car crash will be remembered. The amnesic effect of a new learning on previously encoded material is known as retroactive interference (RI). This selective memory storage could be related to limitations in the brain structure, the number of synaptic connections and/or the amount of plasticity-related proteins. Wixted (2004) suggests that the interference is the new learning itself which utilizes the resources available to consolidate the original trace. In consequence, the original memory trace is affected. Although this hypothesis has prevailed in the field, little experimental data on the molecular basis of natural memory interference (i.e. what actually happens during the storage of different sets of information) is available.

Studies in hippocampal long-term potentiation (LTP), a cellular model of memory (Martin, Grimwood, & Morris, 2000), introduced the concept of "competitive maintenance" (Fonseca, Nägerl, Morris, & Bonhoeffer, 2004). Under regimes of reduced protein availability, different synapses compete for the available resources, resulting in a depotentiation of activated pathways by the influence of an independently activated pathway. Furthermore, very recent findings provide supporting evidence for the existence of competition for PRPs by activated synapses (Govindarajan, Israely, Huang, & Tonegawa, 2011). In view of these models and considering the requirement of PRPs synthesis for making a long lasting memory, we hypothesized that if different tasks are being consolidated into a LTM under conditions of limited protein resources, intracellular competition for PRPs will define which of the memory traces becomes stabilized. Based on synaptic tagging and capture hypothesis (Frey & Morris, 1997, 1998), we have recently

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postulated that PRPs could be captured by the tags set by learning experiences (learning tags) in order to form its LTM (Ballarini, Moncada, Martinez, Alen, & Viola, 2009; Moncada, Ballarini, Martinez, Frey, & Viola, 2011; Moncada & Viola, 2007).

In this work, we particularly analyzed if activity-regulated cytoskeletal-associated protein (Arc) was a PRP necessary for LTM formation of both tasks. Because of its importance for synaptic plasticity (Barco, Lopez de Armentia, & Alarcon, 2008; Bramham & Wells, 2007) and also for the formation of numerous types of explicit and implicit memories (for review, Bramham et al., 2010), Arc is an attractive candidate to be required for the consolidation of both tasks and, therefore, could also to be one of the PRPs by which learning tags compete.

Hence, we aimed to study if amnesia derived from the interference between two different tasks was due to the competence for the resources required for their LTM formation. Our findings show that under regimes of reduced protein resources, but not when resources are vastly available, a certain learning task can hinder the LTM formation of another because of their common requirement of PRPs.

2. Materials and methods

2.1. Animals

Male adult Wistar rats (180–220 g) were housed in groups of 6 per cage, maintained under a 12-h light/12-h dark cycle (21 °C) with food and water ad libitum. They were handled for three minutes for three consecutive days to avoid emotional stress. All procedures complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publications No. 80-23, revised 1996) and were approved by the Animal Care and Use Committee of the University of Buenos Aires.

2.2. Surgery and drugs

2.2.1. Surgery

For cannulae implantation rats were deeply anesthetized (70 mg/kg ketamine; 8 mg/kg Xylazine) and 22-G cannulae were stereotaxically aimed to the CA1 region of the dorsal hippocampus at coordinates *A* −4.2 mm, *L* ±3.0 mm, *V* 0.3 mm. (Paxinos & Watson, 2007). Cannulae were fixed to the skull with dental acrylic. Animals were allowed to recover from surgery for four days. Drugs were infused using a 30-G needle with its tip protruding 1.0 mm beyond the guide. Cannulae were left in place for 1 additional min to minimize back-flow. Histological examination of cannulae placements was performed. Only data from animals with correct cannula implants (95% of the rats) were included in statistical analyses.

2.2.2. Oligonucleotides

Oligonucleotide pairs (ODNs, Genbiotech, S.R.L) were prepared according to Guzowski et al. (2000). ODNs contained posphorothioate linkages between the three bases on the 5′ and 3′ ends. Arc antisense ODN (Arc ASO) was directed against a 20-mer sequence (bases 209–228, GenBank accession number U19866) covering the Arc start site. Scrambled Arc ODN (Arc SCR) containing the same base composition in randomized order served as control. ODNs (1 nmol/μl saline solution per side) were delivered to the dorsal hippocampus via guide cannulae infusions.

2.3. Behavioral training

2.3.1. Open field (OF, spatial exploration)

The apparatus is a 50 × 50 × 39 cm arena with black plywood walls and wooden floor, divided in 9 squares by black lines. In each session, exploratory activity was measured as the number of crossings between squares and the number of rearings, registered minute by minute. The exploration consists of a 5-min session. When animals were exposed to two different OFs, a second apparatus with similar dimensions but circular shape was used. Habituation percentage for each subject was calculated with the formula: [(OF Tr − OF Ts)/OF Tr] × 100, where "OF Tr" is the total number of events (crossings or rearings) registered during training session and "OF Ts", the total number of events registered during test session. A higher habituation percentage (i.e. a larger decrease in exploratory activity) represents a stronger memory in this task.

2.3.2. Inhibitory avoidance (IA, aversive task)

The apparatus is a 50 × 25 × 25 cm Plexiglas box with a 2.5 cm-high, 8 cm-wide and 25 cm long platform on the left end of a series of bronze bars which constituted the floor of the box. In the training session, rats were placed on the platform facing the left rear corner of the box. When they stepped-down, putting their four paws on the bronze bars, they received a weak (0.24 mA, 2 s) or a strong foot-shock (0.6 mA, 2 s) and were removed from the box immediately after. Animals were returned to their home cage and subjected to a test session to measure LTM 24 h after training. Memory was measured by comparing the step-down latency in the training session (Tr) to that in the test session. In contrast to the sIA training, the wIA training does not induce a LTM. Higher test-latencies represent a stronger memory in this task.

2.4. Western blot analysis

Animals were trained in the different tasks and, 30 min after the end of the last training, they were sacrificed by decapitation. Tissue patches surrounding the infusion area were homogenized. Samples were subjected to SDS–PAGE (10% polyacrilamide, SDS 10%, 20 μg per lane) and transferred onto PVDF membranes for western blot analysis. Membranes were blocked 1 h at room temperature using a 3% BSA-TTBS solution. Anti-Arc primary antibody (1:1000, H-300, sc-15325, Santa Cruz Biotechnology) was dissolved in a 0.5% BSA-TTBS solution and membranes were incubated overnight at 4 °C. For total protein levels, membranes were stripped and incubated with an anti-Actin antibody (1:10,000; C-11, sc-1615 Santa Cruz Biotechnology). Densitometric analysis was performed with Gelpro Analyzer (Media Cybernetics). A value of Arc and Actin was obtained for each experimental animal, relativized to the media of control group and the ratio was calculated as Arc/Actin.

2.5. Data analysis

Statistical analysis of behavioral data was performed with Student's *t* test or Newman–Keuls multiple comparison test after one-way analysis of variance (ANOVA) using GraphPad Prism 5 software (GraphPad Software Inc).

3. Results

3.1. Interference of the wIA training on OF-LTM formation

We and others have demonstrated that a weak inhibitory avoidance (wIA) training session that only induces a short lasting memory can be promoted to a durable memory if rodents explore a novel environment (a novel OF) around the time of training (Lu et al., 2011; Moncada & Viola, 2007; Moncada et al., 2011). As wIA training does not induce PRPs synthesis and only generates short-term memory, the formation of IA-LTM depends on the PRPs synthesis triggered by novel OF exposure (Moncada & Viola, 2007; Moncada et al., 2011). Here we decided to study what happened to

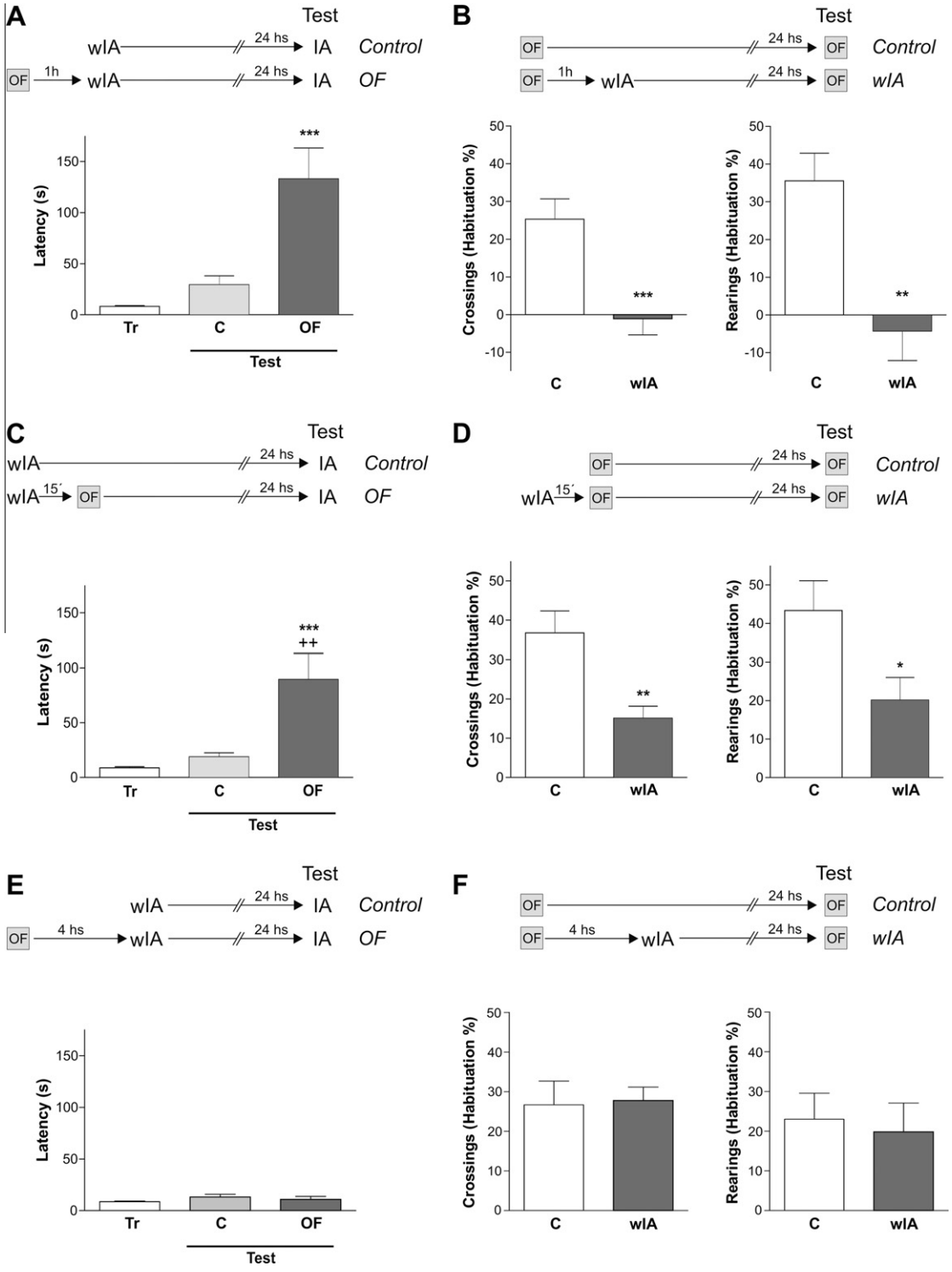

Fig. 1. Open field (OF) exploration promotes IA-LTM formation from a weak IA training (wIA) and this occurs in detriment of the OF's LTM. Schematic representation of the experimental protocol is presented on top of each panel. Control animals (C) received only a wIA training or an OF session. IA-LTM (expressed as the mean ± SEM of the latency to descend from the platform) and OF-LTM (expressed as the mean ± SEM of habituation percentage of test exploratory activity relative to training) were tested 24 h after their training session. (A and B) OF exposure 1 h previous to wIA induces IA-LTM, resulting in an impairment of the OF task's own LTM. Experimental subjects were exposed to OF 1 h before wIA. Half of the subjects were tested for IA-LTM (A, OF group, $^{***}p < 0.001$ vs. both groups; Newman–Keuls test after ANOVA, $n = 14–16$) and the other half was tested for OF-LTM (B, wIA group, $^{***}p < 0.001$, $^{**}p < 0.01$ vs. C; unpaired t-test, $n = 12–14$). (C and D) OF exposure after wIA induces IA-LTM, resulting in an decrease of the OF task's own LTM. Experimental subjects were exposed to OF 15 min after wIA. Half of the subjects were tested for IA-LTM (C, OF group, $^{***}p < 0.001$ vs. Tr, $^{++}p < 0.01$ vs. C; Newman–Keuls test after ANOVA, $n = 13–18$) and the other half was tested for OF-LTM (D, wIA group, $^{**}p < 0.01$, $^{*}p < 0.05$ vs. C; unpaired t-test, $n = 13, 16$). (E and F) OF exposure 4 h before wIA training cannot induce IA-LTM and this does not affect OF-LTM. Experimental subjects were exposed to OF 4 h before wIA. Half of the subjects were tested for IA-LTM (C, OF group, $p > 0.05$, ANOVA, $n = 7–8$) and the other half was tested for OF-LTM (D, $p > 0.05$; unpaired t-test, $n = 7–8$).

the OF-LTM formation when this task promoted the formation of IA-LTM. With this aim we exposed rats to a 5 min session in a novel OF and one hour after that, they were trained with wIA. Twenty-four hours later, we tested the performance of independent groups of animals in the IA paradigm or in the OF arena. Fig. 1A shows the performance of rats when tested in the IA

paradigm. As expected, the control group that had not been exposed to the OF did not show IA-LTM, since the latency to step-down form the platform in the test session is not significantly different to the latency in the training ($p > 0.05$, Fig. 1A). In agreement with our previous findings (Moncada & Viola, 2007), exposure to a novel OF promoted IA-LTM ($p < 0.001$ vs. other groups, Fig. 1A). Figs. 1B show the performance of rats in the OF during test session. The control group of animals trained in OF in absence of wIA showed habituation to the arena, either observed in the crossings or in the rearings, taking this as an index of OF-LTM. Interestingly, rats trained in the OF in presence of wIA, showed a significantly lower OF-LTM with respect to the control group ($p < 0.01$ and $p < 0.001$ vs. C; Fig. 1B). Taken together, these results demonstrate that while IA-LTM was promoted by the exploration of the OF, the OF-LTM was concomitantly impaired. Moreover, when rats were exposed to OF and 1 h later they were delivered a single foot-shock, their OF-LTM showed no significant differences with their corresponding controls (data not shown). This result ruled out the possibility that the mere delivery of the foot shock could account for the impairment in the OF-LTM.

We also followed a similar procedure but placed the OF exploration 15 min after wIA training. Just as done before, memory for both tasks was evaluated in separate groups that had received the same training protocol. Fig. 1 C shows that OF exposure after wIA training can also promote LTM formation for that task ($p < 0.001$ vs. Tr, $p < 0.01$ vs. C). This also had a detrimental effect on the OF-LTM because a significant decrease in this memory can be observed in comparison with its respective control group ($p < 0.01$ and $p < 0.05$; Fig. 1D). In contrast, when tasks were separated by a larger time-lapse, there was no effect on the OF over the wIA and nor of the wIA over the OF-LTM. If animals were exposed to a novel OF session 4 h before the wIA, OF-LTM was preserved ($p > 0.05$, Fig 1D) and IA-LTM was not promoted ($p > 0.05$, Fig 1C). In all, findings shown in Fig 1 indicate that there is a time-window in which the promoting effect of spatial exploration on the IA-LTM formation occurs and it corresponds with the impairment of the OF-LTM.

3.2. Two novel OF training sessions induce a further improvement on IA-LTM

We designed an experimental protocol that would enable an increased availability of PRPs to evaluate if this could exert a beneficial effect on LTM formation. Thus, we sequentially exposed the experimental subjects to two different and novel open field boxes. Just as done in the first experiment, we submitted rats to a wIA one hour after the exposure to a novel square OF for 5 min. This group was compared to a second group of animals exposed to an additional novel circular OF 15 min after wIA had taken place. As expected, exposure to a single novel environment promoted IA-LTM formation ($p < 0.01$ vs. Tr, $p < 0.05$ vs. C; Fig. 2A). Interestingly, the group of rats that had experienced both OF sessions showed greater latencies compared to the other groups ($p < 0.001$ vs. Tr and C; $p < 0.05$ vs. OFx1; Fig. 2A). To evaluate OF-LTM formation, parallel groups of animals receiving the same training protocols as described above, were tested in the corresponding OF arena. Figs. 2B show that whereas OF-LTM for the circular arena was not affected by the multiple training protocol, OF-LTM registered for the first OF arena was decreased only when a wIA was intercalated between the two OF training sessions ($p < 0.001$, $p < 0.01$, vs. their controls). Taken together, Fig. 2 shows that associating a wIA training with two novel exploratory sessions results in a more robust IA-LTM. Hence, it is possible that the greater availability of PRPs derived from both novel OF sessions helped to form a better IA-LTM in detriment of the OF-LTM formation for the first arena.

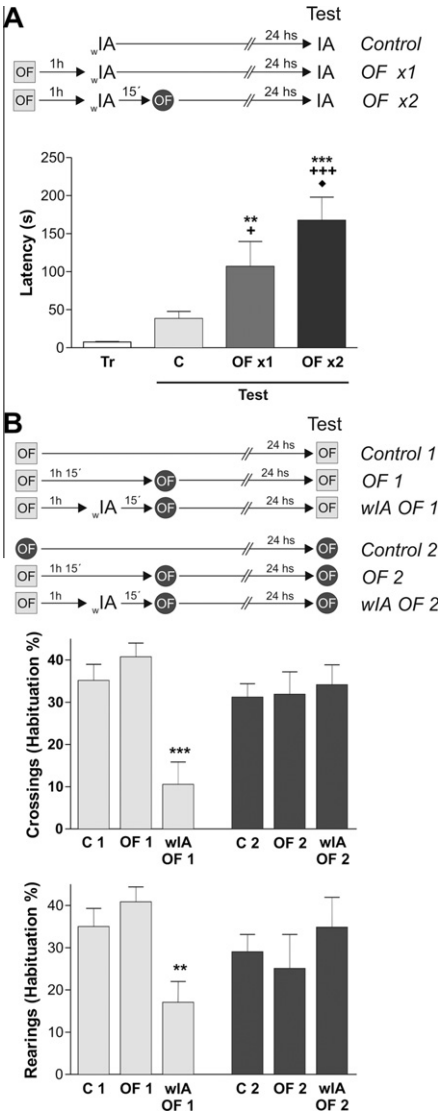

Fig. 2. IA-LTM is further improved if rats are exposed to two novel OF sessions. Schematic representation of the experimental protocol is presented on top of the panels. (A) When subjects are trained with wIA and explore two novel OF arenas, IA-LTM shows a larger increase. Control animals received a weak IA training and latencies were tested 24 h after. Experimental subjects (OF x1) were exposed for 5 min to a novel square OF 1 h before IA training and subjects in the OF x2 group were exposed to and additional novel circular OF, 15 min after IA training. Data are expressed as the mean (±SEM) of training (Tr) or test session latency to descend from the platform. Newman–Keuls test after ANOVA, $^{***}p < 0.001$, $^{**}p < 0.01$ vs. Tr; $^{+++}p < 0.001$, $^{+}p < 0.05$ vs. C; ♦ $p < 0.05$ vs. OF x1; $n = 11–19$. (B) LTM for both arenas is consolidated in absence of wIA training, whereas LTM for the first arena is affected when wIA is intercalated between both exploratory sessions. Control animals were exposed for 5 min to a novel OF and tested 24 h after (square-shaped, Control 1; circular-shaped, Control 2). Subjects in the wIA groups received a weak IA training and were exposed to two different novel OF's 1 h before and 15 min after IA training. These groups were tested 24 h after in one of the two arenas (Square-shaped, wIA OF 1; circular-shaped, wIA OF 2). Subjects in the OF groups were exposed to both OFs separated by 1 h 15' and tested in one of the two arenas, 24 h after training (square-shaped, OF 1; circular-shaped, OF 2). Data are expressed as the mean (±SEM) of habituation percentage of both exploratory activity parameters registered during test, relative to training session. Newman–Keuls test after ANOVA, $^{***}p < 0.001$, $^{**}p < 0.01$ vs. C1 and OF1; $n = 10–16$.

3.3. Arc is one of the proteins required for LTM formation of the different tasks

As introduced before, a likely candidate that could be required for the consolidation of both traces and result in interference is Arc. For this to be true Arc should (1) be required for LTM

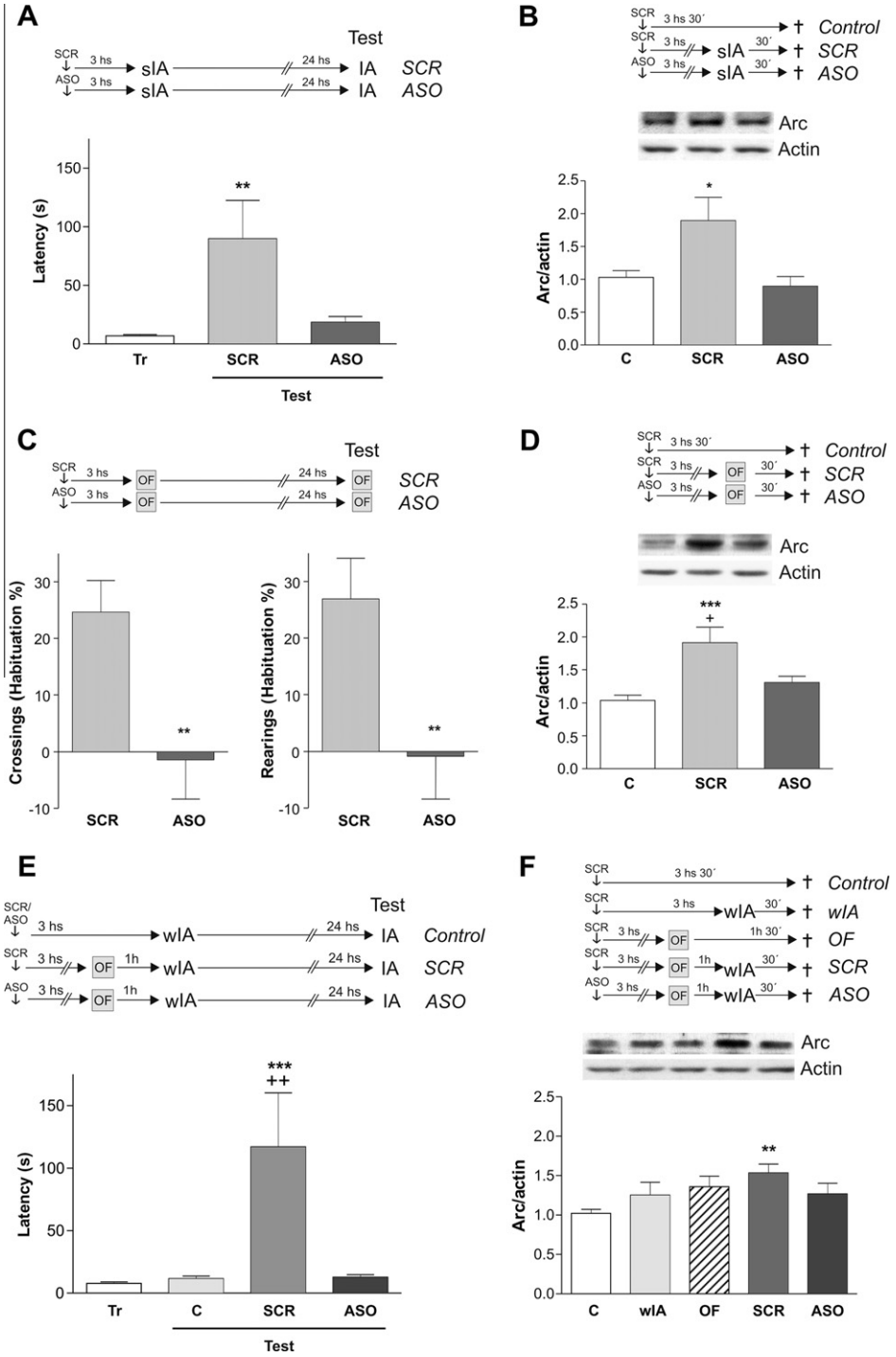

Fig. 3. Arc is needed for the LTM formation of both OF and IA tasks and also for the OF promoted IA-LTM formation. Schematic representation of the experimental protocol is presented on top of the panels. Three hours before training, animals were administered a bilateral intrahippocampal infusion (1 nmol/μl, 1 μl per side) of Arc antisense oligonucleotides (ASO) or Arc scrambled oligonucleotides (SCR) as a control. (A) LTM consolidation for IA is impaired when Arc protein expression is blocked. Subjects received a strong IA (sIA) training and were tested 24 h after. Data are expressed as the mean (±SEM) of training (Tr) or test session latency to descend from the platform. Newman–Keuls test after ANOVA, $^{**}p < 0.01$ vs. the other groups; $n = 11–16$. (B) Arc protein levels increase after sIA training. Representative western blots of the quantification of Arc/Actin protein levels after training are shown on top of the panel. Newman–Keuls test after ANOVA; $^{*}p < 0.05$ vs. the other groups; $n = 6–11$. (C) LTM consolidation for OF is impaired when Arc protein expression is blocked. Subjects were exposed for 5 min to a novel OF and tested 24 h after. Data are expressed as habituation percentage of both exploratory activity parameters registered during test, relative to training session. Unpaired t test $^{**}p < 0.01$ vs. SCR, $n = 12–14$. (D) Arc protein levels increase after OF training. Representative western blots of the quantification of Arc/Actin protein levels after training are shown on top of the panel. Newman–Keuls test after ANOVA; $^{***}p < 0.001$ vs. C; $^{+}p < 0.05$ vs. ASO; $n = 12–17$. (E) The effect of OF exploration on IA-LTM is abolished when Arc protein expression is blocked. Control animals received a SCR or ASO infusion and were submitted to a weak IA training and latencies were tested 24 h after. Experimental subjects received SCR or ASO infusion as indicated and they were exposed for 5 min to a novel OF 1 h before IA training. Data are expressed as the mean (±SEM) of training (Tr) or test session latency to descend from the platform. Newman–Keuls test after ANOVA, $^{***}p < 0.001$ vs. Tr and ASO, $^{++}p < 0.01$ vs. C; $n = 8–14$. (F) Arc protein levels are increased when OF exploration is combined with a wIA training. Representative western blots of the quantification of Arc/Actin protein levels after training are shown on top of the panel. Newman–Keuls after ANOVA; $^{**}p < 0.01$ vs. C; $n = 6–10$.

formation of both the IA and OF tasks separately, and (2) be necessary for the LTM formation of one task and this, in turn, derive in a reduction of the LTM of the other, and (3) if it is highly expressed, it should be sufficient for LTM formation of both tasks. Thus, we first studied if Arc protein was necessary in the formation of these two types of memory. To block Arc expression, we used antisense oligonucleotides against mRNA for Arc protein (ASO, 1 nmol/μl per side) which have been shown to reduce Arc levels by a ≈60% (Guzowski et al., 2000). Rats were bilaterally cannulated in the CA1 region of dorsal hippocampus and, three hours before training, they received an infusion of ASO or its scrambled sequence (SCR) as a control. When rats were trained with a strong IA (sIA) protocol and infused locally with SCR, they showed intact LTM for IA ($p < 0.01$ vs. Tr and ASO; Fig. 3A). In contrast, local ASO infusion impaired IA-LTM formation. In addition, a training-induced increase in Arc protein levels measured 30 min after sIA training was prevented by ASO infusion ($p < 0.05$ vs. C and ASO; Fig. 3B). Moreover, the local infusion of ASO before a novel OF session, impaired the formation of OF-LTM ($p < 0.01$ vs. SCR; Fig. 3C) as well as the increase in Arc protein levels observed 30 min after OF exposure ($p < 0.001$ vs. C; $p < 0.05$ vs. ASO, Fig. 3D). These findings indicate that Arc is needed for LTM formation of both IA and OF tasks, meeting the first of the requirements to be a PRP that could account for the formation of one trace at expense of the other. So we next studied if Arc was necessary for the promotion of IA-LTM after novelty exposure. The infusion of ASO into the dorsal hippocampus before an OF exposure prevented the formation of IA-LTM that was observed in the group of rats infused with SCR ($p < 0.001$ vs. C; $p < 0.01$ vs. ASO; Fig. 3E). Consistent with these results, Arc levels measured 30 min after wIA exhibit a significant increase only when this training is combined with novel OF exploration ($p < 0.01$ vs. C; Fig. 3F). Note that neither Arc levels show an increase when measured 30 min after wIA nor when measured 90 min after OF.

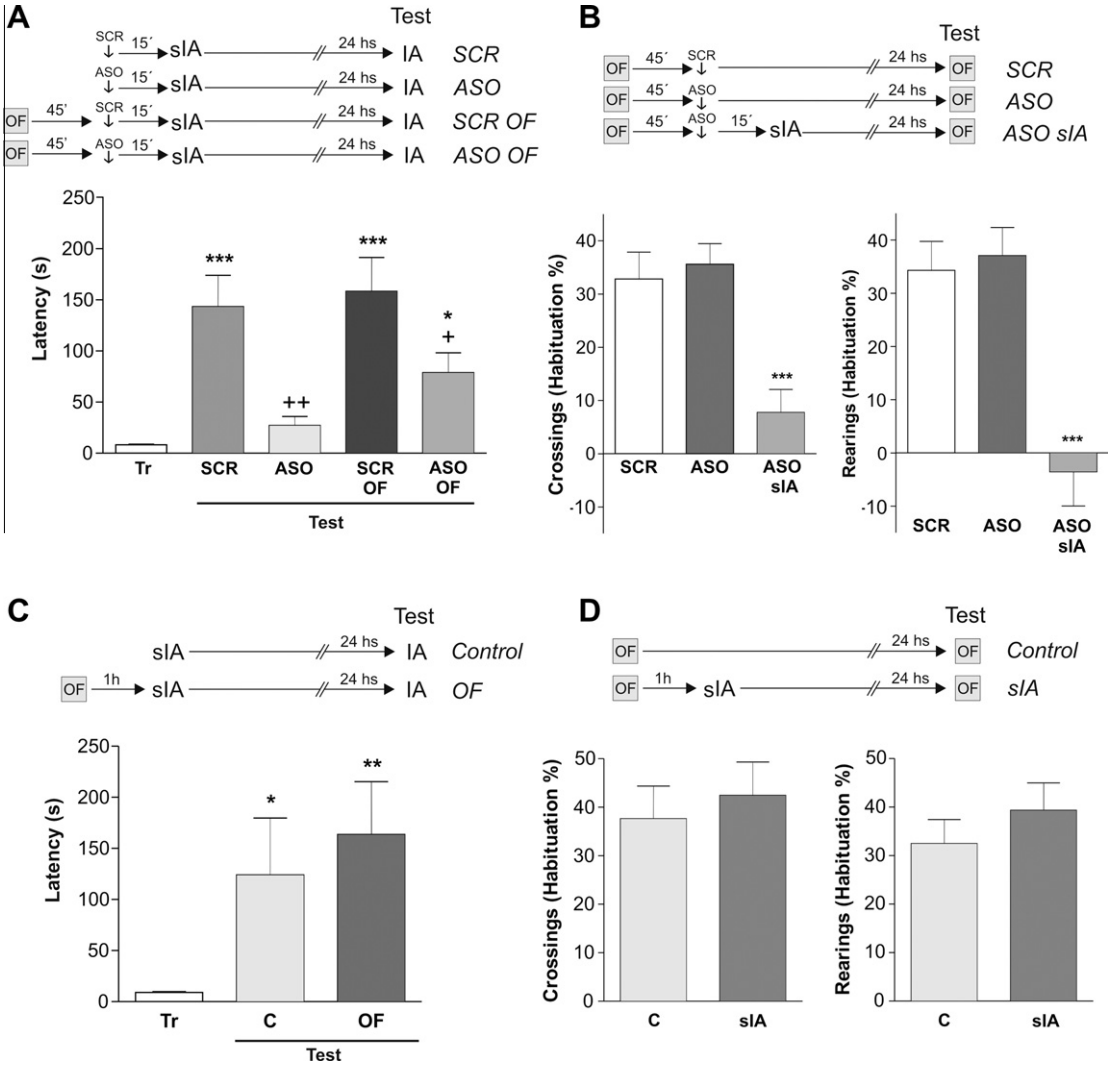

Fig. 4. Limited levels of Arc restricts LTM formation. Schematic representation of the experimental protocol is presented on top of the panels. Control animals received only a strong IA (sIA) training or an OF session. IA-LTM (expressed as the mean ± SEM of the latency to descend from the platform) and OF-LTM (expressed as the mean ± SEM of habituation percentage of test exploratory activity relative to training) were tested 24 h after their training session. (A) Blockage of Arc protein expression previous to training impairs LTM for IA but it can partially be recovered when animals are exposed to a novel OF. Animals were injected with SCR or ASO 15 min before sIA in the absence (SCR, ASO) or in the presence of an OF 1 h before training (SCR OF, ASO OF). Newman–Keuls after ANOVA; $^{***}p < 0.001$, $^{*}p < 0.05$ vs. Tr; $^{++}p < 0.01$, $^{+}p < 0.05$ vs. SCR and SCR OF; $n = 13–20$. (B) OF-LTM is impaired when Arc expression is blocked previous to sIA training. Rats were exposed to an OF and 45 min later were infused with SCR or ASO (SCR, ASO). A third group of animals 15 min after infusion of ASO was also trained in a sIA (ASO sIA). Newman–Keuls after ANOVA; $^{***}p < 0.001$ vs. the other groups, $n = 10–14$. (C and D) LTM for both OF and IA tasks is observed when OF exploration occurs before a sIA. Experimental subjects were exposed to OF 1 h before sIA. Part of the subjects were tested for IA-LTM (C, OF group; Newman–Keuls test after ANOVA $^{**}p < 0.01$, $^{*}p < 0.05$ vs. Tr; $n = 6–12$) and the rest was tested for OF-LTM (D, sIA group; unpaired t-test; $n = 5–8$).

3.4. Limited availability of Arc protein has a detrimental effect on the formation of LTM

In order to fulfil the second requirement mentioned above, in conditions of low Arc availability, traces should be affected by this reduction. To test this, we applied local infusions of ASO 15 min before sIA training. This intrahippocampal application of ASO blocked IA-LTM formation ($p < 0.01$ vs. SCR, Fig. 4A). However, if this procedure was preceded by an exposure to a novel OF, IA-LTM was partially expressed ($p < 0.05$ vs. Tr and SCR OF; Fig. 4A) and this effect was accompanied by the impairment of OF-LTM ($p < 0.001$ vs. all groups; Fig. 4B). Control group of animals revealed that ASO infusion 45 min after OF exposure, in the absence of IA training, did not alter OF-LTM (Fig. 4B). These results suggest that this temporal schedule of ASO infusion selectively affects Arc protein induced by sIA training and that the amount of Arc induced by OF is insufficient for the LTM formation of both tasks.

Having established that when Arc is limited or not available memory traces are affected, we next wanted to address our third requirement for Arc, that is, what would happen in conditions where Arc levels were sufficient to form a LTM of both tasks. Therefore we exposed animals to a novel OF arena for 5 min and 1 h later, they were trained with a sIA which, as shown in Fig. 3A, can generate a LTM by itself. Separate groups of rats were tested in one of the two paradigms. The performance in the IA test shows no significant differences between control and experimental animals as they both exhibit a strong LTM for this task ($p < 0.01$, $p < 0.05$ vs. Tr, Fig. 4C). Interestingly, LTM for OF is preserved in both groups as well ($p > 0.05$ Fig. 4D). Therefore, when OF exploration is combined with a posterior sIA training that elicits a LTM per se, both LTM traces can be formed. Thus, in contrast with what we have shown under limited Arc expression (4A, B), when the system was resourceful and there was a sufficient amount of PRPs both LTM were formed.

4. Discussion

In this work, we demonstrate that when rats are sequentially exposed to two different memory tasks under a regimen of limited protein synthesis, LTM for one of them is formed in detriment of the formation of the other. We postulate that this happens because the amount of PRPs is insufficient to form LTM of the two behavioral tasks. These results suggest the existence of competition between two memory traces – IA vs. OF – for their consolidation when protein resources are limited. We also identified the requirement of Arc as one of the PRPs necessary for the LTM formation of both tasks. Moreover, when training animals sequentially in the OF and in the IA paradigms under a regimen of limited Arc translation, only one of these LTMs was expressed. Therefore, we suggest that interference could be explained by a mechanism of competition for protein resources, being Arc one of the PRPs required for the consolidation of both memory traces.

Here we demonstrated that the promotion of IA-LTM formation by a single novel OF exposure, given 1 h before or 15 min after wIA, is concomitant with the loss or impairment of expression of OF-LTM (Fig. 1A–D). A speculative explanation for the facilitatory effect on IA-LTM formation in detriment of OF-LTM could be the competition for the capture of for newly synthesized proteins derived from OF exposure between the learning tags induced by both behavioral tasks. Thus, when the OF session took place 4 h before wIA, no effects of competition for PRPs were observed probably because the PRPs have already been captured by OF learning tags before the intervention of wIA. As a result only the OF-LTM was formed (Fig. 1E and F). Moreover, when wIA training was accompanied by two novel OF sessions (one hour before and 15 min after training), IA-LTM was more robust than that obtained with the association of a single exposure to spatial novelty (Fig. 2A), suggesting that a larger availability of PRPs could also contribute to an enhancement in the IA-LTM. Besides, with this protocol of a wIA training between two exposures to novelty, OF-LTM was observed for the second novel OF, but not for the first one (Fig. 2B). Interestingly, when there was no wIA training between both exposures to the OF, OF-LTM for both novel OF arenas is formed (Fig. 2B). Thus, competition for protein resources might be taking place by the interposition of this wIA training which, in turn, uses part of the PRPs. Consequently, a reduction in the available PRPs during consolidation of the first mnemonic trace (first OF) resulted in a visible interference in its LTM formation. We think that such competition phenomenon is partly related to the order of the tasks because the impairment of OF-LTM formation was lower when OF training took place after wIA training rather than when it was before wIA. It is a possibility that in the experiments shown in Fig. 2 the requirements for the stabilization of the IA trace have already been covered by supplies derived from the first OF training. In such situation, the support from the second OF could be weaker and, in turn, enough to improve IA-LTM formation this without having any consequence over its own OF-LTM.

As we have previously shown in our behavioral tagging model (Moncada & Viola, 2007), the promoting effect of novelty on a wIA learning depends on the synthesis of PRPs induced by OF exposure. By this behavioral tagging mechanism, PRPs available in time and space will be captured by the tags set by learning experiences (Ballarini et al., 2009; Moncada et al., 2011). i.e. Each training sets a learning tag that can capture the resources available. Hence, we speculate that competition between different learning tasks for the limited amounts of PRPs could be taking place in the activated neuronal population common to both learning experiences.

Regarding the identities of the PRPs intervening in the consolidation of the different tasks, we focused our study on Arc protein because of its relevance in synaptic plasticity and memory processes (Bramham et al., 2010; Tzingounis & Nicoll, 2006). Arc links behavioral experience with the consequent changes in neural plasticity, showing a fast increase in its mRNA levels as rapidly as 5 min after training (Miyashita, Kubik, Haghighi, Steward, & Guzowski, 2009). The infusion of ASO in the dorsal hippocampus impairs memory for a spatial water task (Guzowski et al., 2000) and fear conditioning (Czerniawski et al., 2011). In addition, it has been reported that infusion of ASO in the basolateral amygdala modulates the expression of Arc in the dorsal hippocampus and impairs memory retention of an inhibitory avoidance task (Mc Intyre et al., 2005). Consistent with previous studies using Arc ASO, Arc knockout mice have several memory deficits, as they do not form either spatial, fear, or taste long-term memories (Plath et al., 2006). We found that Arc fulfils the three main requirements to be considered as a PRP for which memories may compete. Arc protein is necessary for both IA- and OF-LTMs formation (Fig. 3A and C). Furthermore, Arc levels were increased 30 min after training procedures that are able to induce LTM for OF or IA (Fig. 3B and D). Moreover, Arc induced by OF exposure was necessary to promote the formation of IA-LTM triggered by a wIA training (Fig. 3E and F), suggesting that Arc is one of the PRPs used by the IA trace. Likewise, when an OF session was followed by a sIA training, LTM for both tasks is observed at 24 h test session (Fig. 4C and D). However, when we selectively blocked the sIA-induced Arc expression, the amount of newly synthesized Arc triggered by OF exposure was not sufficient to consolidate both traces, resulting in the LTM impairment for OF and the partial recovery of the IA-LTM (Fig. 4A and B). These results strongly suggest that under regimes of limited Arc translation one memory trace will become enhanced in detriment of the

other. Therefore, Arc availability can deeply influence the stabilization of the engrams.

Cellular level studies suggest that stimulated synapses would compete for limiting PRPs synthesized at the dendrite compartment. Protein synthesis-dependent L-LTP expression induced at one single spine can facilitate L-LTP and the increase of spine volume at other synapse that received a weak stimulation. Interestingly, stimulation of multiple inputs within a short distance resulted in the growth of one spine, accompanied by the shrinking of the others (Govindarajan et al., 2011). Another example of competition for plasticity factors between synapses has been shown in a model of associative LTP. When a weak and a strong tetanizing stimuli were applied simultaneously, LTP was maintained for hours at both inputs. However, applying a further weak tetanus in the presence of anisomycin resulted in the potentiation of the reactivated pathway at the expense of the persistence of LTP on the other. Moreover, prolonging the anisomycin treatment intensified the competition. These results led to the concept of "competitive maintenance" where the sustained enhancement of the reactivated pathway occurs by the consumption of the PRPs available, impairing the stabilization of LTP at the other input (Fonseca et al., 2004). In this regard, implications of the phenomenon of "competitive maintenance" for memory consolidation have been recently discussed (Redondo & Morris, 2011). In the present work, we propose that competition for protein resources is a mechanism operating in the selection of which memory engrams will consolidate when PRPs are scarce.

In all, sharing, crosstalk and capture in spine neighborhoods (Bramham, 2008) are important mechanisms for the consolidation of plastic changes. It was postulated that during the stabilization of a memory trace, selection of the neuronal network that will be recruited into a given memory representation occurs in a competitive fashion rather than cell-autonomous (Won & Silva, 2008). Here we subjected memory traces to competition by means of two different conditions of reduced protein resources. On the one hand, we trained rats in a task bearing a strong saliency, associated with another different weak training (that did not induce the synthesis of sufficient PRPs, but set a learning tag). On the other, we sequentially exposed rats to two strong training procedures under the effect of a selective blockage of Arc expression in the dorsal hippocampus. These two protocols were based on the hypothesis that protein synthesis induced by a strong training is insufficient for LTM formation for both tasks. Our results demonstrate that under these conditions, only one memory trace survives and is consolidated into LTM.

As discussed before, in the framework of the behavioral tagging model, we propose that the learning tags induced by each of the training sessions could use the same PRPs available in time and place. As a consequence of this, the consolidation of a memory trace will depend on the surrounding context of the training, being information for only one of the events selectively stored within a critical time window. However, when both tasks trigger PRPs synthesis (OF plus sIA) there is no such detrimental effect of one trace on the other, what could be explained in terms of a larger availability of the protein resources.

Centennial observations point that the strength of RI on learning exerted by an interpolated material increases with the proximity between events. The degree of memory forgetting is variable and traces become less vulnerable to empirical forgetting, brain damage or retroactive interference as they consolidate with the passage of time (Jost, 1897; Müller & Pilzecker, 1900; Ribot, 1881). Later, Skaggs (1925) suggested that the interpolated task causing RI could be a mental effort per se or a similar material to be recorded, being the RI effect reduced when tasks are highly similar or, on the contrary, when they are markedly different. These observations could be reinterpreted considering the hypothesis of PRPs-capture by different kinds of learning tags. Thus, we propose that if the interpolated material is identical to the original, it can represent a retraining; probably reinforcing almost the same learning tags set for the original task. In that case, there would be no different kinds of learning tags capturing the PRPs. In contrast, a high dissimilarity of the material could imply its processing in different brain regions; thus, the respective learning tags would not interfere because they do not converge spatially (Ballarini et al., 2009; Redondo & Morris, 2011).

Our findings provide further evidence on the dynamic nature of episodic memories, showing that the consolidation of individual traces may be highly influenced by the surrounding events processed in the same brain structures. The behavioral tagging model proposes a cellular mechanism to explain amnesia by retrograde and also anterograde interference, focusing on the competitive capture of proteins required for the consolidation of those memory traces. This is the first evidence showing the molecular events underlying memory competition that could explain how some pieces of information are stored while others are lost or impaired.

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