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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