Updating contextual information during consolidation as result of a new memory trace

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ABSTRACT

Reconsolidation studies have led to the hypothesis that memory, when labile, would be modified in order to incorporate new information. This view has reinstated original propositions suggesting that short-term memory provides the organism with an opportunity to evaluate and rearrange information before storing it, since it is concurrent with the labile state of consolidation. The *Chasmagnathus* associative memory model is used here to test whether during consolidation it is possible to change some attribute of recently acquired memories. In addition, it is tested whether these changes in behavioral memory features can be explained as modifications on the consolidating memory trace or as a consequence of a new memory trace. We show that short-term memory is, unlike long-term memory, not context specific. During this short period after learning, behavioral memory can be updated in order to incorporate new contextual information. We found that, during this period, the cycloheximide retrograde amnesic effect can be reverted by a single trial in a new context. Finally, by means of memory sensitivity to cycloheximide during consolidation and reconsolidation, we show that the learning of a new context (CS) during this short-term memory period builds up a new memory trace that sustains the behavioral memory update.

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1. Introduction

Understanding the role of labile periods of memory is an open challenge. Nevertheless, most of the discussion about the functional value of the labile states, strengthening or rearranging memory, is nowadays principally restricted to the reconsolidation process (Alberini, 2007; Dudai, 2009; Sara, 2000). After learning, long-term memory formation must undergo a stabilization period (consolidation) at which memory can be interfered or strengthened by several treatments, including hormonal changes and experiences (McGaugh, 1966; McGaugh, 2000; Sara & Hars, 2006). During this time, behavior relies on short-term memory, a process that is believed to be parallel to long-term memory formation (Izquierdo et al., 2002; Sherff & Carew, 2004; Shirahata, Tsunoda, Santa, Kirino, & Watanabe, 2006). It has been proposed that the transient short-term memory phase, a period that is concurrent with consolidation, when memory is still labile, provides the organism with a better opportunity to evaluate, classify and rearrange information before long-term memory is stored (Dudai, 2002a; Gerber & Menzel, 2000; McGaugh, 2000; Menzel, 1999). For instance, it has been proposed that during this period it is possible to integrate new information into one single experience (Izquierdo & Chaves, 1988; Loftus & Palmer, 1974). Accordingly, there is a lot of evidence across phylum that a weak memory

undergoing consolidation can be strengthened by a weak learning experience or by retrieval (Parvez, Stewart, Sangha, & Lukowiak, 2005; Summers, Crowe, & Ng, 2000). In this sense, a training procedure that induces short- but not long-term memory, may be enhanced by presenting a reminder trial during both consolidation and the period of short-term memory expression (Summers et al., 2000; Smal, Suárez and Delorenzi, unpublished results).

Here, the *Chasmagnathus* associative memory model is used to investigate whether during consolidation it is possible to update some attributes of a recently acquired memory. The associative learning paradigm is based on the escape response elicited by the presentation of a visual danger stimulus (US), an opaque rectangle passing above the animal. Upon the iterative presentation of US, the crab's escape response declines and a strong freezing response is built up (Pereyra, González, & Maldonado, 2000). The response decrement lasts for at least 5 days (Lozada, Romano, & Maldonado, 1990; Pedreira, Dimant, Tomsic, Quesada-Allue, & Maldonado, 1995). The memory formed using this paradigm is based on the association between the environmental features of the training context (CS) and the features of the screen moving above the animal (the signal, US) (Tomsic, Pedreira, Romano, Hermitte, & Maldonado, 1998); such memory was termed Context-Signal Memory (CSM). Studies performed on the mechanisms underlying different memory phases have shown that CSM consolidation, extinction and reconsolidation are blocked by protein synthesis inhibitors (Hermitte, Pedreira, Tomsic, & Maldonado, 1999; Pedreira, Dimant, & Maldonado, 1996; Pedreira, Perez-Cuesta, &

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Maldonado, 2002; Pedreira et al., 1995). Findings from studies done to investigate the mechanisms underlying reconsolidation showed a reliable CSM sensitivity to amnesic agents by re-exposing the animals for 5 min to the learning context, without US presentation, 24 h after training (Maldonado, 2002; Pereyra et al., 2000). This labile memory is sensitive to cycloheximide and other pharmacological agents (Pedreira & Maldonado, 2003; Pedreira et al., 2002). Nevertheless, memory does not become labile again if the reminder (the learning context) is reinforced with a US presentation (Frenkel, Maldonado, & Delorenzi, 2005; Pedreira, Pérez-Cuesta, & Maldonado, 2004).

Here, we explore whether during consolidation it is possible to update some attributes of a recently acquired memory, and whether this change in a memory's feature can be explained as modifications of the consolidating memory trace, or as result of the formation of a new memory trace. Specifically, we tested whether memory can be modified during this period in order to update information (i.e. to incorporate new context information). Firstly, we showed that strong training protocols generate a short-term memory that, in contrast to long-term memory, is not context specific. Secondly, in order to test whether this change in behavioral memory features can be explained as modifications of the consolidating memory trace, we found a reversion phenomenon of the cycloheximide-induced amnesia during consolidation by a single trial presentation in a new context. Finally, we used an experimental approach involving reconsolidation to show that causing the updated memory to become transiently labile again in the new context does not imply that the original memory trace will become transiently labile as well. Using this approach, we present evidence supporting the view that context memory update is due to a new memory trace, generated when memory is still being consolidated, i.e. labile.

2. Materials and methods

2.1. Animals

Intermolt adult male crabs of the species *Chasmagnathus granulatus* between 2.7 and 3.0 cm across carapace were collected from the narrow coastal inlets of San Clemente del Tuyú, Argentina. In the laboratory, crabs were kept on a 12:12 h light–dark cycle, in collective tanks (20 animals each) filled up to 2 cm deep with 12‰ seawater prepared with hw-Marinex (Winex, Germany) salt, pH 7.4–7.6. The holding and experimental rooms were kept at 22–24 °C and 80 ± 10% relative humidity. Experiments were carried out at daytime within the first week after the arrival of animals. Each crab was used in one experiment only. Experimental procedures are in compliance with the policies on the use of Animals and Humans in Neuroscience Research. All efforts were made to minimize the number of animals used and their suffering.

2.2. The experimental device

The experimental device, the actometer (Maldonado, 2002), consists of a container covered to a depth of 0.5 cm with artificial sea water, where the crab remains during each experimental session. The actometer is illuminated from above with a 5 W bulb. During each trial (9 s), an opaque rectangular screen (25–7.5 cm), termed the visual danger stimulus (US), was moved horizontally over the animal, cyclically from left to right and vice versa, at a constant speed (one trial = two passages of the visual danger stimulus). The US provokes in the crab an escape response and consequent container vibrations, which are converted into electrical signals through four piezoelectric transducers (microphones) placed on the external wall of the container. These signals are

amplified, integrated during each 9-s trial, and translated by computer into numerical units (activity scores). In all experiments on this work, a single trial consists in two passages of the visual danger stimulus over the animal. The experimental room has 40 actometers, separated from each other by panels.

2.3. Escape response and freezing

The magnitude of container vibrations during the 9 s of US presentation (a trial) depends on the type and magnitude of the defensive responses the displayed by the crab when faced with an impending threat. Two types of defensive responses are distinguished: namely, escape response and freezing response (Pereyra et al., 2000). The escape response is a directional run of the animal in an attempt to move away from the passing screen (US), while the freezing response consists of a rigid motionless display in which the crab lies flat on the substratum. During repeated US presentations (training), the escape response decreases in intensity and is replaced by the progressive building up of a strong and long-lasting freezing (Pereyra et al., 2000; Romano et al., 2006; Tomsic et al., 1998). During context exposure without US presentation the crabs show no defensive responses, instead they are observed exploring or wandering. Throughout this article, data was only recorded during trials, i.e. during the 9-s US.

2.4. Training protocol

A strong *Training Protocol* consists of fifteen trials (9 s, two visual danger stimulus presentations), with 3 min intertrial intervals (total training duration: 42 min), after a 10 min adaptation period. Animals are kept in training context without receiving any stimulation from the US during 10 min before training (adaptation period). This protocol builds up a long-term memory that persists for at least 5 days (Maldonado, 2002).

2.5. Experimental context

Three kinds of containers were used during these experiments. Context A: the classical actometer: an orange plastic container with a steep concave wall 12 cm high (23 cm top diameter and 9 cm floor diameter). Context B: a cylindrical (15 cm diameter and 15 cm height) plastic container with black and white striped walls. Context C: a brown hexagonal plastic container (16 cm top length, 13 cm floor length and 15.5 cm height) with white spots. All containers were covered to a depth of 0.5 cm with artificial seawater.

As described in Section 2.2, the experimental room has 40 actometers (context A), each one with four microphones firmly attached to its base. Context B an C are arranged to fit inside the actometer, thus vibrations provoked by the motor activity of the animal can not be registered properly. Consequently, Context A is the only one in which the activity of the crab can be measured. Thus, experiments were designed in such a way that the *Testing Session* occurs in Context A. For the other sessions, any of the three contexts may be used. This imposes a limitation in experimental designs, since context presentation can not be counterbalanced. However, these contexts have been used as reactivation controls and as context-dependence controls in a number of works, proving that animals recognize them as different contexts (Frenkel et al., 2005; Pedreira & Maldonado, 2003).

2.6. Experimental procedure and design

Experiments included three or four sessions: a *Training Session* (Day 1), a *Single Trial Session* (Day 1; 0.5, 4 or 8 h after the *Training Session*), and a *Testing Session* (Day 2 or 3). Reconsolidation experiments included an additional *Reactivation Session* (Day 2). Experi-