

SHORT COMMUNICATION

Amygdalar circuits required for either consolidation or extinction of taste aversion memory are not required for reconsolidation

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Abstract

Recent reports have revitalized the debate on whether, for each item in memory, consolidation occurs just once, or whether, upon their activation in retrieval, items in memory undergo reconsolidation. Further, it has been recently reported that following retrieval in the absence of reinforcer, the activated memory can either reconsolidate or extinguish, depending on the training history. This raises the question whether consolidation, extinction and reconsolidation share neuronal mechanisms, and moreover, whether reconsolidation recapitulates consolidation. In conditioned taste aversion (CTA), consolidation depends on protein synthesis in the central nucleus of the amygdala, whereas extinction depends on protein synthesis in the basolateral nuclei of the amygdala. Here we show that inhibition of protein synthesis in either of these nuclei has no effect on CTA memory under conditions that initiate reconsolidation. This implies that reconsolidation does not recapitulate consolidation, and that consolidation, reconsolidation and extinction are different processes.

Introduction

Considerable evidence indicates that, following their activation in retrieval, items in long-term memory can regain transient sensitivity to agents that block memory consolidation (Nader *et al.*, 2000; Sara, 2000; Debiec *et al.*, 2002). The period of postretrieval sensitivity to consolidation blockers, and the processes involved, are commonly termed 'reconsolidation'. It is not yet clear, however, whether the processes and mechanisms of reconsolidation are similar, let alone identical, to those of the original consolidation, and hence whether the 're' in 'reconsolidation' is actually warranted (Taubenfeld *et al.*, 2001; Anokhin *et al.*, 2002; Thomas *et al.*, 2003). Further, retrieval may activate concomitantly multiple traces that compete for the control of behaviour (Berman *et al.*, 2003), and we have recently demonstrated that a trace becomes susceptible anew to consolidation blockers after retrieval, only if it retains or gains control over behaviour (Eisenberg *et al.*, 2003).

Conditioned taste aversion (CTA) in the rat is a convenient system to investigate the relationship between consolidation and reconsolidation. In CTA, the subject learns to reject a tastant (conditioned stimulus, CS) if this tastant is associated with subsequent malaise (unconditioned stimulus, US) (Bures *et al.*, 1998). CTA can be acquired in a single trial, but the resulting memory can then be extinguished by presentations of the CS in the absence of the US. Application of a consolidation blocker, the protein synthesis inhibitor

anisomycin, into the taste cortex immediately before or after retrieval, blocks extinction without hampering the original, CS–US association (Berman & Dudai, 2001). In other words, under these conditions, the CTA trace does not reconsolidate. In contrast, if the intensity of training is increased, CTA memory becomes much more resistant to extinction. Under these conditions, application of anisomycin impairs the original CS–US association, indicating reconsolidation of the long-term trace (Eisenberg *et al.*, 2003). This ability to obtain and manipulate consolidation, reconsolidation and extinction in the same system is advantageous for understanding of the nature of reconsolidation and its relevance to consolidation on the one hand and extinction on the other.

CTA is subserved not only by the taste cortex (Dunn & Everitt, 1988; Rosenblum *et al.*, 1993; Escobar *et al.*, 1998; Yasoshima & Yamamoto, 1998), but also by other brain areas, including the amygdala (Yamamoto *et al.*, 1994; Lamprecht & Dudai, 2000). We have recently reported (Bahar *et al.*, 2003) that whereas protein synthesis in the central nucleus of the amygdala (CeA) is obligatory for the formation of the long-term CTA trace but not its extinction, the opposite is true for the basolateral amygdala (BLA). This double dissociation provides an opportunity to investigate whether reconsolidation is subserved by the same circuits that subserve either consolidation or extinction.

Here we report that local microinfusion of anisomycin into the CeA or the BLA, immediately after retrieval under conditions that trigger reconsolidation of CTA, does not interfere with the stability of the CTA trace. This indicates that the amygdalar circuits that are obligatory for either consolidation or extinction of CTA are not required for reconsolidation, implying that reconsolidation, consolidation and extinction of CTA are each subserved by different neuronal circuits.

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Materials and methods

Subjects

Male Wistar rats (~60-days-old, 200–250 g) were caged individually at $22 \pm 2^\circ\text{C}$ in a 12 h light: 12 h dark cycle. Water and food were available *ad libitum* unless otherwise indicated. All experiments were approved beforehand by the Weizmann Institute animal care and use committee.

Drugs

The protein synthesis inhibitor anisomycin was from Sigma (St. Louis, MO). The drug was dissolved in physiological saline and adjusted to pH 7. Final anisomycin concentration was $125 \mu\text{g}/1 \mu\text{L}$.

Behavioural procedures

Saccharin (0.1% w/v, sodium salt) was used as the CS, and i.p. injection of LiCl (0.15 M, 2% body weight) as the US. Protocols for the acquisition, extinction and reconsolidation of CTA were as described by Eisenberg *et al.* (2003). In brief, rats were trained over 4 days to get their daily water ration within 10 min from two pipettes, each containing 10 mL. On day 5, the rats were presented with saccharin instead of water. Forty minutes later, they were injected i.p. with the LiCl solution. This is the standard, single-trial CTA training (Rosenblum *et al.*, 1993). To induce conditions under which reconsolidation takes place (Eisenberg *et al.*, 2003), additional training

was performed on day 6 (2-trial, intensive training). Rats that failed to drink at least 0.5 mL of the CS on this conditioning day were excluded from the experiment. On days 7–9, the rats were presented daily for 10 min with two pipettes containing 10 mL of water each, as in training days. On day 10, a multiple-choice test was performed to determine the acquired aversion. In the 10 min choice test, the rats were allowed free access to an array of six pipettes, three with 5 mL saccharin each and three with 5 mL water each. Anisomycin was microinfused into either the CeA or BLA, as indicated in the Results section, immediately after the test. Controls were microinfused with saline only. The aversion index (AI) was defined as a percentage from volumes consumed in the test (Rosenblum *et al.*, 1993):

$$\text{AI} = 100 \times \text{water consumed} / (\text{water} + \text{saccharin consumed})$$

Hence, $\text{AI} = 50$ is equal preference level, and $\text{AI} > 50$ implies higher preference to water over saccharin.

Surgery and microinfusion

Rats were deeply anaesthetized with 4.8 mL/kg Equithesin (2.12% w/v MgSO_4 , 10% w/v ethanol, 39.1% w/v propylene glycol, 0.98% w/v sodium pentobarbitone and 4.2% w/v chloral hydrate), positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and implanted bilaterally with stainless steel guide cannula (23 gauge, thin wall) aimed 1.0 mm above the CeA or BLA [Fig. 1A and D; CeA: AP -2.2 mm, L ± 4.0 mm, V -7.4 mm; BLA: AP -3.0 mm, L ± 5.1 mm, V

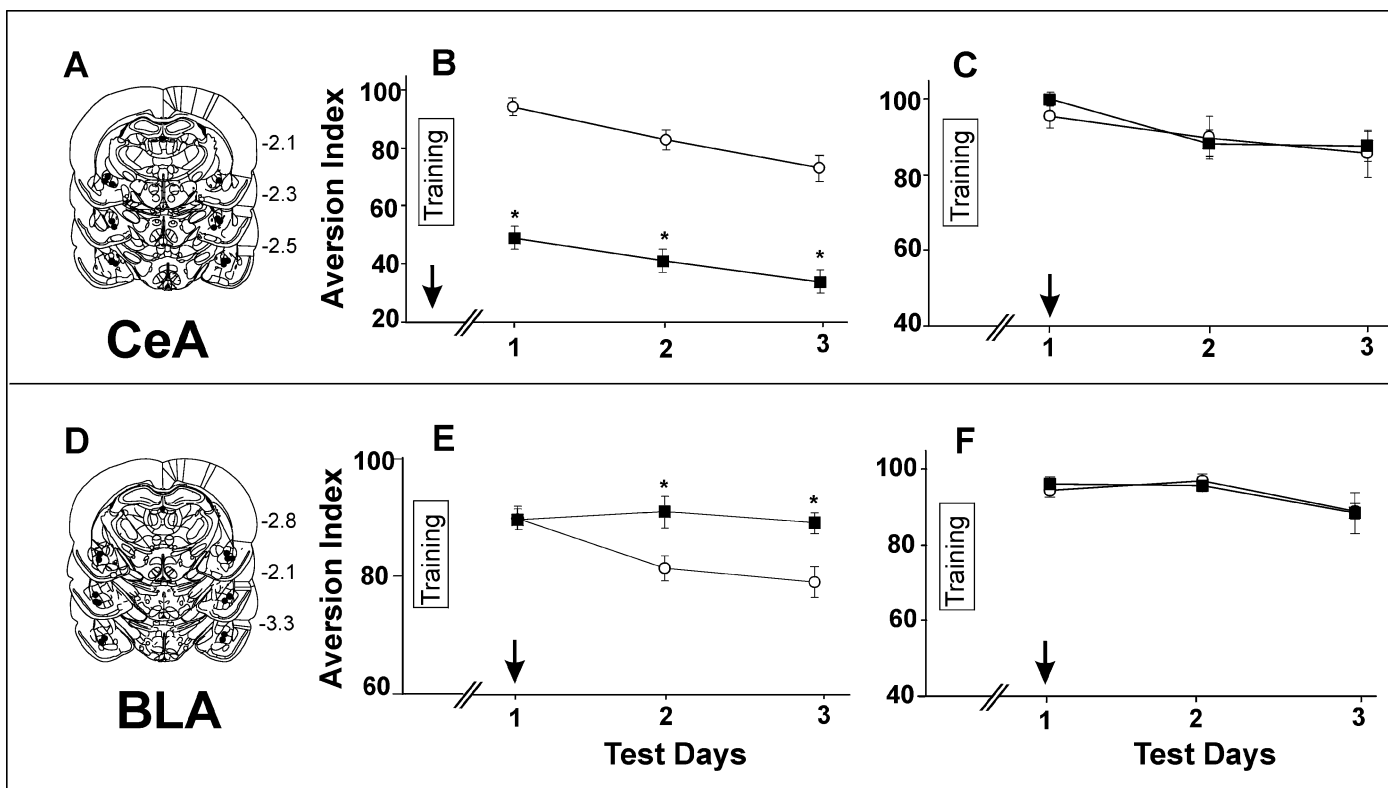


FIG. 1. The effects of anisomycin microinfused into the CeA and the BLA on consolidation, extinction and reconsolidation of CTA memory. Localization of cannula tips in the CeA (A) or the BLA (D). Numbers adjacent to sections denote distance from bregma (sections outline adopted from Paxinos & Watson, 1998). (B) Blockade of consolidation in CeA. Bilateral microinfusion of anisomycin into the CeA during the standard CTA training (see Methods), impaired long-term CTA memory (saline, circles; anisomycin, filled squares; ANOVA with repeated measures, $F_{1,19} = 54$, $P < 0.001$). (C) Lack of effect on reconsolidation in CeA. Bilateral microinfusion of anisomycin into the CeA immediately after retrieval, under the conditions that initiate reconsolidation (intensive training, see Methods), did not affect CTA memory at subsequent test days 2 and 3 (ANOVA with repeated measures, $F_{1,15} = 0.06$, $P = 0.8$). (E) Blockade of extinction in BLA. Bilateral microinfusion of anisomycin into the BLA immediately after retrieval of CTA under the conditions that promote extinction (standard training) impaired extinction of CTA memory (ANOVA with repeated measures, $F_{1,20} = 10.4$, $P < 0.005$). (F) Lack of effect on reconsolidation in the BLA. Bilateral microinfusion of anisomycin into the BLA immediately after retrieval under the conditions that initiate reconsolidation (intensive training), did not affect CTA memory at subsequent test days 2 and 3 (ANOVA with repeated-measures, $F_{1,25} = 0.07$, $P = 0.79$). Data points are mean \pm SEM. Arrows denote time of drug microinfusion.

–7.7 mm; all relative to bregma (Paxinos & Watson, 1998)]. The cannulae were positioned in place with acrylic dental cement and secured by skull screws. A stylus was placed in the guide cannula to prevent clogging. Animals were allowed 1 week to recuperate before being subjected to experimental manipulations. For microinfusion, the stylus was removed from the guide cannula and a 28-gauge infusion cannula, extending 1.0 mm from the tip of the guide cannula, was inserted. The infusion cannulae were connected via PE20 tubing to a Hamilton microsyringe driven by a microinfusion pump (CMA/100; Carnegie Medicin, Stockholm, Sweden). Microinfusion was performed bilaterally, 1 μ L volume per hemisphere, delivered over 1 min. The infusion cannula was left in position before withdrawal for an additional 1 min to minimize dragging of the injected liquid along the injection tract.

Histology

Following completion of the experimental protocol rats were deeply anaesthetized and 1 μ L of India ink was microinfused into each amygdalar structure. Frozen brain slices (30 μ m) were analysed to verify the microinfusion sites.

Results

To determine the potential role of either the CeA or the BLA in reconsolidation, we used the protein synthesis inhibitor anisomycin as the consolidation blocker (Nader *et al.*, 2000; Eisenberg *et al.*, 2003). The inhibitor was microinfused bilaterally into the appropriate amygdalar nuclei, immediately after retrieval of CTA obtained in the intensive training protocol (see Methods). This retrieval does not trigger significant extinction yet initiates reconsolidation of the activated CTA memory (Eisenberg *et al.*, 2003).

Microinfusion of anisomycin into the CeA in standard training blocked long-term CTA memory, as reported previously (Fig. 1B, see also Bahar *et al.*, 2003). However, microinfusion of the consolidation blocker into the CeA immediately after retrieval under the conditions that depress extinction and trigger reconsolidation (intensive training), has no effect on the retrieved trace, suggesting lack of obligatory role of CeA in reconsolidation (Fig. 1C).

Microinfusion of anisomycin into the BLA immediately after retrieval following standard training, i.e. conditions that promote extinction, impaired extinction, as reported previously (Fig. 1E, see also Bahar *et al.*, 2003). However, microinfusion of anisomycin into the BLA under the conditions that depress extinction and trigger reconsolidation (intensive training), has no effect on the retrieved trace, suggesting lack of obligatory role of BLA in reconsolidation (Fig. 1F). The effects of anisomycin infusion into the CeA and BLA on consolidation, extinction and reconsolidation, based on the present data combined with our earlier findings, are summarized in Table 1.

TABLE 1. The effects of the protein synthesis inhibitor anisomycin on consolidation, extinction and reconsolidating of CTA memory

	Long-term CTA memory		
	Consolidation	Extinction	Reconsolidation
Taste cortex	+	+	+
CeA	+	–	–
BLA	–	+	–

Symbols: +, microinfusion of anisomycin disrupted the process; –, no effect of anisomycin on the process; CeA, amygdalar central nucleus; BLA, amygdalar basolateral nuclei. The table is based on the present work as well as on results compiled from Rosenblum *et al.* (1993); Berman & Dudai (2001), Bahar *et al.* (2003) and Eisenberg *et al.* (2003)

The table also depicts, for comparison, the effect of anisomycin on consolidation, extinction and reconsolidation in the taste cortex (Rosneblum *et al.*, 1993; Berman & Dudai, 2001; Eisenberg *et al.*, 2003).

Discussion

Inhibitors of protein synthesis block memory consolidation in all systems and memory paradigms tested to date (Davis & Squire, 1984; Dudai, 2002). In CTA, anisomycin blocks memory consolidation (Rosenblum *et al.*, 1993; Lamprecht *et al.*, 1997) as well as consolidation of experimental extinction (Berman & Dudai, 2001; Bahar *et al.*, 2003). Multiple sites subservise the consolidation of CTA memory and its extinction, including the taste cortex and the amygdala. Inhibition of protein synthesis in the taste cortex or in distinct amygdalar sites immediately before or after the training event, leads to blockade of long-term memory, of either the original CS–US association (in consolidation) or of the inhibitory, CS–noUS association (in extinction). Whether the retrieved CTA memory reconsolidates or extinguishes after its retrieval in the absence of the reinforcer, depends on the training history; standard, 1-trial training results in a trace that is susceptible to extinction but does not reconsolidate, whereas intensive, 2-trial training results in a trace that is resistant to extinction yet reconsolidates (Eisenberg *et al.*, 2003). In the taste cortex, microinfusion of anisomycin immediately after retrieval of the extinction-resistant CTA trace, results in apparent amnesia of this memory (Eisenberg *et al.*, 2003). The taste cortex hence subservises consolidation, extinction and reconsolidation of CTA.

In contrast, different amygdalar nuclei subservise consolidation and extinction. This implies that inhibition of protein synthesis in either the CeA (which is required for consolidation) or the BLA (which is required for extinction), could be used to clarify whether reconsolidation resembles, in terms of the circuits involved, consolidation, extinction, or none of the above. The purpose of the current work was just that. Our data indicate that neither the amygdalar circuits that are obligatory for consolidation of long-term CTA memory, nor those that are obligatory for extinction of long-term CTA memory, are required for the reconsolidation of CTA memory. Hence whatever the mechanisms and function of reconsolidation are, they are not faithful recapitulation of consolidation. It is noteworthy that Nader *et al.* (2000), infusing the same concentration yet half the amount of anisomycin into the lateral and basal nuclei of the amygdala immediately after retrieval, did observe blockade of reconsolidation of fear conditioning memory in the rat. This indicates, on the one hand, that the inhibitor dose used by us can indeed block reconsolidation in the amygdala (as it does in the taste cortex in CTA, Eisenberg *et al.*, 2003), but on the other hand, that the role of amygdala in reconsolidation differs in different aversive conditioning paradigms.

Our present findings on the differential role of amygdalar circuits in consolidation and reconsolidation are in line with molecular studies that indicate that consolidation and reconsolidation might differ (Taubenfeld *et al.*, 2001; Thomas *et al.*, 2003). Furthermore, our data suggest that in CTA, memory consolidation, memory extinction and memory reconsolidation are all different processes.

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Abbreviations

BLA, basolateral nuclei; CeA, central nucleus; CTA, conditioned taste aversion; CS, conditioned stimulus; US, unconditioned stimulus.

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