SHORT COMMUNICATION
The amygdalar circuit that acquires taste aversion memory differs from the circuit that extinguishes it

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Abstract
Experimental extinction is the decline in the frequency or intensity of a conditioned behaviour resulting from repetitive performance of the behaviour in the absence of the unconditioned stimulus or reinforcer (Pavlov, 1927). Ample behavioural evidence indicates that experimental extinction does not reflect unlearning of the original trace, but rather a relearning process, in which the new association of the conditioned stimulus with the absence of the original reinforcer comes to control behaviour (Rescorla, 1996). If experimental extinction is indeed learning rather than forgetting, are the neuronal circuits that subserve learning and extinction identical? We address this question by double dissociation analysis of the role of the central (CeA) and the basolateral (BLA) nuclei of the rat’s amygdala in the acquisition and extinction, respectively, of conditioned taste aversion (CTA). Whereas local blockade of protein synthesis or β-adrenergic receptors in the CeA blocks acquisition but not extinction of CTA, a similar intervention in the BLA blocks extinction but not acquisition. Hence, the amygdalar circuit that acquires taste aversion memory differs functionally from the circuit that extinguishes it.

Introduction
Conditioned taste aversion (CTA) is a form of learning in which the subject learns to associate taste with visceral malaise (Garcia et al, 1955; Bures et al., 1998). Robust CTA can be acquired in single-trial training, yet under the appropriate circumstances the aversion can be readily extinguished (Berman & Dudai, 2001). This implies that CTA can be used to compare the molecular, cellular, circuit and system mechanisms of acquisition and extinction of memory. Parts of the circuits that subserve CTA are known; major stations include the taste area in the insular cortex, the amygdala, the parabrachial nucleus and the nucleus of the solitary tract (Bures et al., 1998; Lamprecht & Dudai, 2000). The amygdala is of particular interest as this collection of nuclei is known to play a key role in multiple types of aversive and emotional learning (Lamprecht & Dudai, 2000; LeDoux, 2000; McGaugh, 2002). The precise role of amygdalar nuclei in CTA is, however, unclear (reviewed by Lamprecht & Dudai, 2000). We have recently demonstrated that the CeA is essential for the formation of the CTA trace; inhibition of protein synthesis and blockade of the expression of the transcription factor cAMP-response element-binding protein (CREB) in this nucleus block consolidation of CTA memory (Lamprecht et al., 1997). Other studies have indicated that the BLA may also be involved in CTA (reviewed by Lamprecht & Dudai, 2000).

As ample behavioural evidence indicates that experimental extinction does not reflect unlearning of the original trace, but rather a relearning process (Rescorla, 1996), the question can be raised whether the neuronal circuits that subserve learning and extinction are identical. The amygdala appears to provide a convenient experimental system to address this question. Here we describe double dissociation analysis of the role of CeA and BLA in the acquisition and extinction of CTA, respectively. Our approach was based on the blockade in each of these nuclei of identified molecular targets that are known to subserve learning and consolidation in a variety of systems. This was carried out by local microinfusion, via chronically implanted cannulae, of anisomycin, an inhibitor of protein synthesis, or propranolol, a blocker of the β-adrenergic receptor, into the target nucleus in the amygdala at the appropriate time-points relative to acquisition or extinction training. Our results suggest that the amygdalar circuit(s) that subserve acquisition are functionally different from those that subserve the extinction of CTA.

Materials and methods
Subjects
Male Wistar rats (≈ 60 days old, 200–250 g) were caged individually at 22° ± 2 °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 and the β-adrenergic receptor antagonist propranolol were from Sigma (St Louis, MO, USA). The drugs were dissolved in physiological saline and adjusted to pH 7. Microinfusion of saline alone served as control. The particular drug dosages used for microinfusion as well as the timings of drug administration relative to training or retrieval, were selected on the basis of previous in vivo studies (Naor & Dudai, 1996; Berman et al., 2000; Nader et al., 2000) or preliminary experiments (A. Bahar, unpublished data). Identical drug concentrations were used for each amygdalar nucleus: 120 μg/μL anisomycin; 20 μg/μL propranolol. All drugs were microinjected in a volume of 0.5 μL per hemisphere. To test the effect of the drugs on CTA acquisition, anisomycin was...
microinfused 20 min before, and propranolol immediately after the presentation of the CS on the conditioning day. To test the effect of the drugs on CTA extinction, anisomycin was microinfused 20 min before, and propranolol immediately after performance of the first multiple-choice test (day 8, see below). No statistical difference was detected between the behaviour of rats that were microinfused with saline in the different application schedules.

**Behavioural procedures**

Different groups were used for the study of acquisition and extinction of CTA. Unless otherwise indicated, saccharin (0.1%, w/v, sodium salt) was used as the unfamiliar taste (conditioned stimulus, CS), and i.p. injection of LiCl (0.15 M, 2% body weight) as the malaise-inducing agent (unconditioned stimulus, US). Protocols for the acquisition and extinction of CTA were essentially as described by Berman et al. (2000). In brief, rats were trained over 4 days to obtain 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. On days 6 and 7, the rats were presented with two pipettes each containing 10 mL of water, daily for 10 min, as in the training days. On day 8, a multiple-choice test was performed to determine the acquired aversion. To measure extinction of CTA, the test was repeated for at least 3 successive days (days 8–10). In each 10 min 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. The aversion index (AI; Rosenblum et al., 1993) was defined as a percentage, with consumption in mL:

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100 \times \frac{\text{water consumed}}{\text{water + saccharin consumed}}
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Hence, 50 is equal-preference level, and >50 implies higher preference to water over saccharin.

**Surgery and microinfusion**

Rats were deeply anaesthetised with 4.8 mL/kg Equithesin [2.12% MgSO4 (w/v), 10% ethanol (w/v), 39.1% propylene glycol (w/v), 0.98% sodium pentobarbitone (w/v), and 4.2% chloral hydrate (w/v)], restrained in a stereotactic apparatus (David Kopf Instruments, Tujunga, CA, USA), and implanted bilaterally with stainless steel guide cannulae (23 gauge, thin wall) aimed 1.0 mm above the central or basolateral nuclei of the amygdala [Fig. 1a and b; CeA: AP ① -2.2 mm, L ±4.0 mm, V -7.4 mm; BLA: AP -3.0 mm, L ±5.1 mm, V -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

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**Fig. 1.** Double dissociation of the effect of anisomycin and propranolol in the CeA and BLA on the acquisition and extinction of CTA. (a and b) Localization of cannula tips aimed to the CeA (a) or BLA (b). Numbers adjacent to sections denote distance from bregma (section outline adopted from Paxinos & Watson, 1998). (c) CTA memory following bilateral microinfusion of propranolol (prop, triangle) or anisomycin (ani, square) into the CeA. Both drugs impaired encoding of CTA memory compared to control (saline, ctl, circle; ani, n = 12, t(21) = 9.7, p < 0.05; prop, n = 7, t(17) = 3.1, p < 0.05). (d) In the BLA, anisomycin did not affect encoding of CTA (n = 11, t(20) = 0.3, p > 0.05). Propranolol displayed a tendency to decrease AI compared to control but the effect was insignificant (n = 12, t(21) = 1.5, p > 0.05). (e) Anisomycin and propranolol [(same drug dosages as in (d)] in the CeA did not affect extinction compared to control (saline, ctl, circles; ani, squares, n = 12; t(22) = 0.3, p > 0.05; prop, triangles, n = 13; t(26) = 0.2, p > 0.05). (f) Anisomycin and propranolol microinfused into the BLA impaired extinction of CTA compared to control. The effect of anisomycin remained significant also on the 3rd test day (ani, n = 23; test day 2, t(43) = -2.3, p < 0.05; test day 3, t(43) = -3.3, p < 0.05, prop, n = 13: test day 2, t(25) = -2.6, p < 0.05). Data points are mean ± SEM. Arrows in (e) and in (f) denote time of drug microinfusion in extinction experiments.
Our study focused on two molecular targets, the protein synthesis machinery and the β-adrenergic receptor. Protein synthesis is known to be universally required for memory consolidation (reviewed by Davis & Squire, 1984), whereas the β-adrenergic system is known to be required for learning in a variety of systems, including the insular cortex in CTA (Berman & Dudai, 2001). As can be seen in Fig. 1c, local microinfusion of the protein synthesis inhibitor anisomycin or the β-adrenergic antagonist propranolol into the CeA in the training session impaired CTA memory. In contrast, local microinfusion of the same drugs and under the same conditions into the BLA did not significantly impair CTA memory (Fig. 1d). Propranolol showed a nonsignificant tendency to lower the acquired aversion, whereas anisomycin had no effect whatsoever, in contrast to the marked effect seen in the CeA (compare Fig. 1c and d). However, the effect of the aforementioned interventions on experimental extinction of CTA was the opposite. Although anisomycin and propranolol in the CeA had no significant effect on extinction (Fig. 1c), they did delay extinction when microinfused into the BLA (Fig. 1f; similar results were obtained when both the concentration and the volume of anisomycin were doubled, data not shown).

It may be possible that the application of anisomycin or propranolol into the BLA attenuates extinction because it induces aversion that becomes associated with the taste. To rule this possibility out, we microinfused either anisomycin or propranolol into the BLA of rats exposed during training to the taste CS in the absence of the US (i.e. LiCl i.p.). No acquired aversion was detected (Fig. 2) indicating that under the conditions of this study, anisomycin or propranolol could not substitute the US in CTA. The question could similarly be raised whether the effects of anisomycin or propranolol in the amygdala, when seen, are due to lingering damage induced by administration of these drugs. This was also ruled out, as evident by the observation that rats retrained on CTA to another taste, glycine (1% w/v), after completing the experimental protocol in which the effect of anisomycin or propranolol on the CTA to saccharin was tested as above, acquired normal CTA to the new CS (Fig. 2).
retrieval (Nader et al., 2000). Whereas local microinfusion of anisomycin into the lateral and basolateral amygdala immediately after retrieval of fear conditioning resulted in a marked decline in fear memory (ibid.), in our CTA training and extinction protocol, the same treatment in either the CeA or the BLA did not lead to diminution of the original trace. Our data, nevertheless, do point out that, theoretically, there might be a potential for intervention so that old memories can be extinguished without affecting the ability to acquire new ones. This is because there are cellular targets that are differentially required for both processes. This could have important practical implications.

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Abbreviations
Ani, anisomycin; BLA, basolateral nuclei; CeA, central nucleus; CREB, cAMP-response element-binding-protein; CS, conditioned stimulus; CTA, conditioned taste aversion; Prop, propranolol; US, unconditioned stimulus.

References


