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Bidirectional behavioral plasticity of memory reconsolidation depends on amygdalar protein kinase A

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Reconsolidation—the stabilization of a memory after retrieval—is hypothesized to be a critical and distinct component of memory processing, the disruption of which results in memory impairment. In the rat, we found that activation of amygdalar protein kinase A (PKA) was sufficient to enhance memory only when it was retrieved; in contrast, PKA inhibition impaired reconsolidation. This study demonstrates both a selective enhancement and an impairment of memory reconsolidation dependent on amygdalar PKA.

The retrieval of previously consolidated memories has been hypothesized to induce additional activity-dependent labile periods during which the memory can be modified. Reconsolidation may thus serve to maintain or strengthen memories[1]. The characterization of reconsolidation as an independent mnemonic process requires the demonstration of memory modification in a retrieval-dependent and time-limited fashion[2]. Studies of reconsolidation, like those of consolidation, have focused on molecular mechanisms that, when disrupted, result in a reduction of memory strength or retrieval at a future test[3]. Enhancements in memory reconsolidation, unlike those in consolidation, have not been demonstrated using brain-specific manipulations[4].

We examined the involvement of PKA in the basolateral amygdala (BLA) in the reconsolidation of a previously established auditory fear memory. PKA is a critical intracellular regulator of neuroplasticity. Inhibition of PKA within the BLA markedly reduces[5] memory consolidation, whereas the activation of PKA enhances it[6]; however, the role of PKA in the reconsolidation of fear memories has not been reported. We investigated the effects of both activating and inhibiting PKA after memory retrieval. Rats were initially trained with a single tone-shock pairing in context A. After 24 h, rats were re-exposed to the tone in a new context (context B); immediately after this, they received an infusion of either phosphate-buffered saline (PBS); the specific PKA activator N^6-benzoyladenosine-3′,5′-cyclic monophosphate (6-BNZ-cAMP; Biolog), which has a low affinity toward exchange protein directly activated by cAMP (EPAC; ref. 7); or the PKA inhibitor Rp-adenosine 3′,5′-cyclic monophosphorothioate (Rp-cAMPS, Sigma-Aldrich) into the BLA (see **Supplementary Methods** online; placements of infusion cannulae were according to ref. 8 and are shown in **Supplementary Fig. 1** online). To examine the effect of PKA

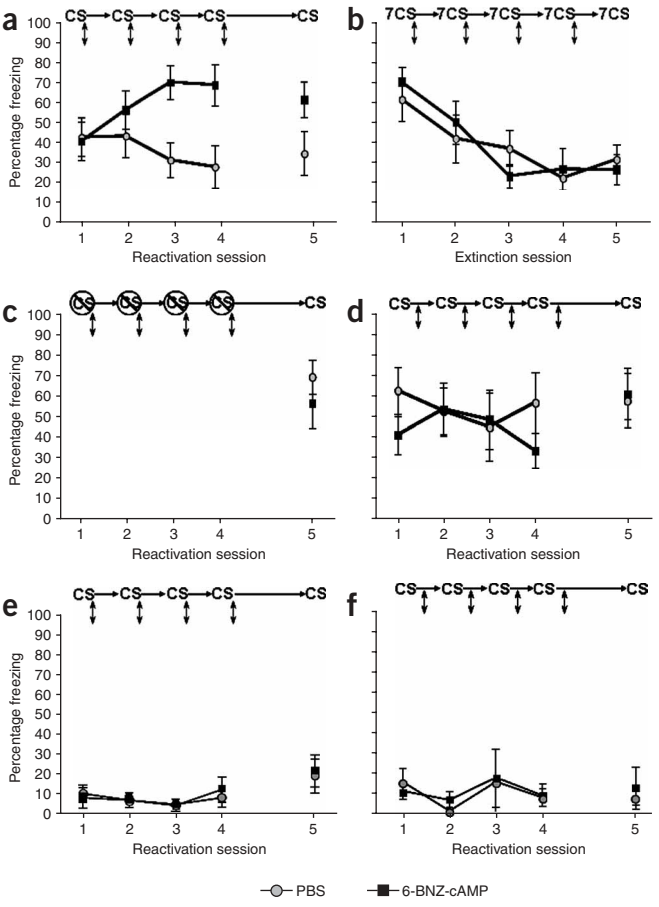

Figure 1 PKA activation in the BLA after retrieval enhanced reconsolidation of a fear memory but did not disrupt extinction. Locomotor activity did not differ between groups. (**a**) The infusion of 6-BNZ-cAMP after memory retrieval increased freezing during the tone over subsequent days (PBS $n = 10$, 6-BNZ-cAMP $n = 11$). (**b**) Postsession infusions of 6-BNZ-cAMP did not disrupt extinction of a fear memory ($n = 8$, $n = 8$). (**c**) No reactivation controls. Infusions of 6-BNZ-cAMP in the absence of memory retrieval did not increase freezing ($n = 9$, $n = 9$). (**d**) Delayed (6 h) infusion controls. The infusion of 6-BNZ-cAMP 6 h after memory retrieval did not increase freezing ($n = 8$, $n = 8$). (**e**,**f**) Reactivation-like controls. Infusions of 6-BNZ-cAMP (**e**) immediately ($n = 5$, $n = 6$) or (**f**) 6 h after ($n = 6$, $n = 6$) an untrained CS did not increase freezing in response to the tone. Error bars represent s.e.m.

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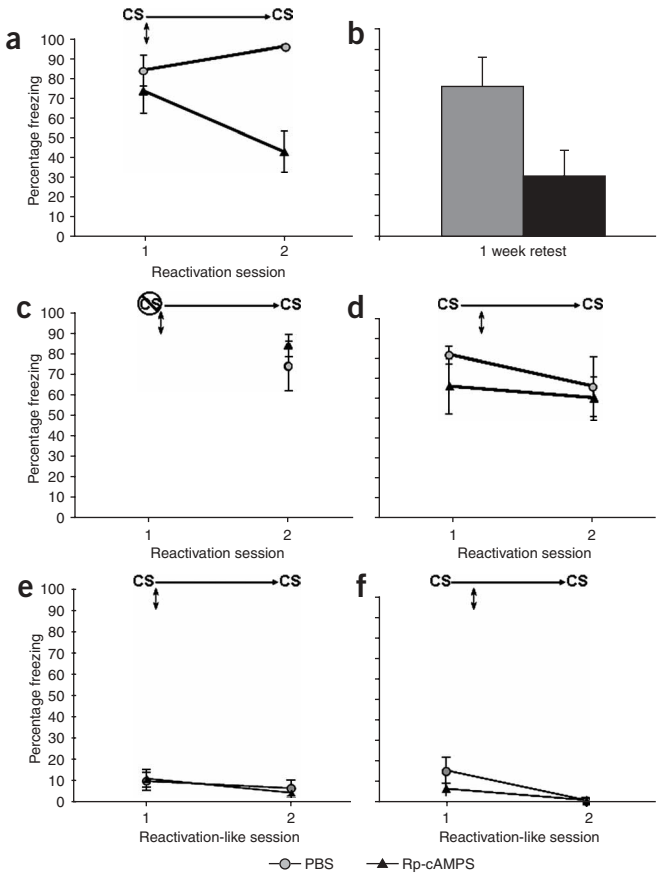

Figure 2 Inhibition of PKA in the BLA after retrieval disrupted reconsolidation of a fear memory. Locomotor activity did not differ between groups. (**a**) Rp-cAMPS infused after memory retrieval attenuates freezing during the tone at a subsequent test (PBS $n = 10$, Rp-cAMPS $n = 10$). (**b**) Disruption of memory was stable at least 1 week after test ($n = 6$, $n = 6$). (**c**) No reactivation controls. Rp-cAMPS in the absence of fear memory retrieval did not decrease freezing at a subsequent test ($n = 8$, $n = 7$). (**d**) Delayed (6 h) infusion controls. Rp-cAMPS infused 6 h after memory retrieval did not disrupt freezing at a subsequent test ($n = 8$, $n = 8$). (**e**,**f**) Reactivation-like controls. Infusions of Rp-cAMPS (**e**) immediately (all $n = 6$) or (**f**) 6 h after (all $n = 6$) an untrained CS did not alter freezing in response to the tone. Error bars represent s.e.m.

activation on reconsolidation, the reactivation trial and postsession infusion were repeated on 4 consecutive days, and a fifth trial was presented 72 h after the fourth day (**Fig. 1**). A separate experiment tested the effect of PKA inhibition on reconsolidation and examined freezing 24 h after a single reactivation and postsession infusion (**Fig. 2**). In all groups and experiments, we observed low levels of freezing in response to the new context on the first reactivation day, suggesting limited context generalization between training and testing contexts (contexts A and B, respectively). All experiments used postsession infusions to avoid nonspecific locomotor effects. Experimental procedures were approved by the Yale University Animal Care and Use Committee.

We found that amygdalar PKA activation enhanced reconsolidation. There were significant differences between rats receiving postretrieval infusions of PBS or 6-BNZ-cAMP across days (**Fig. 1a**; two-way repeated-measures analysis of variance (ANOVA), day × treatment interaction: $F_{4,76} = 3.868$, $P < 0.05$; main effect of treatment: $F_{1,19} = 5.194$; $P < 0.05$). Further analysis showed that rats infused with 6-BNZ-cAMP immediately after successive fear memory reactivations showed significantly more freezing that increased across days (main effect of day: $F_{4,40} = 2.977$, $P < 0.05$). Freezing in PBS-infused rats did not change across the 5 reactivation days (main effect of day: $F_{4,36} = 1.337$, $P > 0.05$). These data suggested that PKA activation enhances reconsolidation.

In contrast, PKA inhibition impaired reconsolidation of memory. We found a significant difference between postretrieval infusions of PBS and Rp-cAMPS (**Fig. 2a**; day × treatment interaction: $F_{1,18} = 7.18$, $P < 0.05$,): the Rp-cAMPS group showed a marked attenuation of freezing at the subsequent test. It is unknown whether disruptions of reconsolidation, like those of consolidation, are permanent or whether the original memory can subsequently be recovered. The disruption of freezing after a postretrieval infusion of Rp-cAMPS into the BLA persisted for at least 7 d after the initial test (**Fig. 2b**; $t_{10} = 2.59$, $P < 0.05$), suggesting that the disruption of reconsolidation resulted in stable changes in the original memory trace. Together, these data supported the idea that PKA in the amygdala has a critical role in the stabilization of fear memories after retrieval.

In several additional control experiments, the specificity of enhanced or disrupted reconsolidation by postretrieval manipulations to mnemonic processes was confirmed. We found that 6-BNZ-cAMP or Rp-cAMPS infusions did not alter freezing in response to a previously trained fear-associated conditioned stimulus (CS) without retrieval of the fear memory (no reactivation controls; 6-BNZ-cAMP: $F_{1,17} < 1$, **Fig. 1c**; Rp-cAMPS: $F_{1,13} < 1$, **Fig. 2c**) and that manipulations to PKA influenced memory reconsolidation only within a limited time window after retrieval ('delayed' (6 h) infusion controls; 6-BNZ-cAMP: $F_{4,56} < 1$, **Fig. 1d**; Rp-cAMPS: $F_{1,14} < 1$, **Fig. 2d**). These important control groups demonstrated the integrity of the reconsolidation process and the specificity of the present experimental manipulations. We therefore showed that the enhancement and attenuation of previously established fear memories by manipulations of PKA activity required both the retrieval of the memory and intervention within a limited time window after retrieval; this confirmed that any changes in behavior were likely due to a modification of the initial memory.

In addition, we ruled out several other unconventional interpretations of reconsolidation. First, the possibility that postreactivation manipulation of PKA activity may alter freezing by influencing second-order conditioning between the CS and the reactivation context (that is, context B) was excluded because freezing in response to the reactivation context before the CS onset on reactivation days was consistently low and unchanged by either treatment (all $F < 1$; see **Supplementary Figs. 2** and **3** online). Second, using a new, untrained CS in reactivation-like trials, we examined the possibility that PKA activators and inhibitors act as aversive unconditioned stimuli. Here, a new auditory CS was presented in the reactivation context; it was followed by infusions of 6-BNZ-cAMP or Rp-cAMPS either immediately or 6 h after CS exposure. The incidence of freezing remained low and was not altered by either treatment (6-BNZ-cAMP: $F_{4,76} < 1$, **Fig. 1e**,**f**; Rp-cAMPS: $F_{1,20} = 2.71$, $P > 0.1$, **Fig. 2e**,**f**). Thus the enhancement and disruption of freezing during a CS presentation by postretrieval manipulations of PKA was dependent on a previous association between the CS and the aversive foot shock.

Although the reactivation procedure used here should primarily initiate reconsolidation[9,10], nonreinforced CS presentations can result in either reconsolidation or extinction. We therefore examined the effects of PKA manipulations in the BLA on extinction using a multiple re-exposure procedure predicted to result in extinction. Here the CS was presented seven times in each of five consecutive extinction

sessions, beginning 24 h after training. PBS or 6-BNZ-cAMP were injected into the BLA immediately after each extinction session. Both groups showed significant extinction across the five experimental sessions (main effect of day: $F_{4,56} = 13.716$; $P < 0.01$). Infusions of 6-BNZ-cAMP did not influence freezing in this procedure (**Fig. 1b**; main effect of treatment: $F_{1,14} < 1$; day × treatment interaction: $F_{4,56} = 2.63$, $P > 0.05$), suggesting that the activation of PKA within the BLA does not affect fear memory extinction under the experimental conditions used here. These data indicate separable effects of post-retrieval PKA activation on reconsolidation and extinction of fear and are consistent with previous studies demonstrating a dissociation between neurobiological mechanisms of reconsolidation and extinction within the amygdala[10,11].

In this study, we showed that PKA in the BLA is required for memory reconsolidation and that activation or inhibition of PKA in the BLA is sufficient to enhance or impair a fear memory, consistent with bidirectional behavioral plasticity after retrieval. We further showed that the PKA-regulated effects of reconsolidation were specific to retrieved memories that occurred within a time window consistent with retrieval-induced lability. Postsession manipulations of PKA activity in the BLA, and the lack of altered extinction, provided additional evidence for our interpretations. PKA exerts its effects on consolidation and neural plasticity by means of a wide range of cellular processes, including the activation of transcription factors such as cAMP response element–binding protein (CREB) that regulate the *de novo* protein synthesis required for long-term memory formation, modulation of intracellular signaling cascades and receptor trafficking. It is as yet unknown which of these pathways are critical for the regulation of fear memory reconsolidation by PKA.

Our findings have important implications for dynamic memory processes. First, the PKA-induced enhancement of reconsolidation, independent of its effects on extinction, provides further evidence for the existence of a distinct reconsolidation process and adds to the growing literature on the molecular substrates of reconsolidation. Second, the enhancement of reconsolidation by PKA activation demonstrates a new mechanism by which consolidated memories can be strengthened after retrieval. Finally, aberrant reconsolidation processes may result in the exceptionally strong and salient emotional memories associated with certain psychiatric disorders. Maladaptive PKA-regulated reconsolidation mechanisms in the amygdala, resulting from physiological states or drug-induced adaptations, are thus hypothesized to self-perpetuate memories in the etiology of chronic relapsing disorders such as post-traumatic stress disorder (PTSD), phobias and addiction.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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1. Sara, S.J. *Nat. Rev. Neurosci.* **1**, 212–213 (2000).
2. Nader, K., Schafe, G.E. & LeDoux, J.E. *Nature* **406**, 722–726 (2000).
3. Duvarci, S. & Nader, K. *J. Neurosci.* **24**, 9269–9275 (2004).
4. Gordon, W.C. *Physiol. Behav.* **19**, 95–99 (1977).
5. Schafe, G.E. & LeDoux, J.E. *J. Neurosci.* **20**, RC96 (2000).
6. Jentsch, J.D., Olausson, P., Nestler, E.J. & Taylor, J.R. *Biol. Psychiatry* **52**, 111–118 (2002).
7. Christensen, A.E. *et al. J. Biol. Chem.* **278**, 35394–35402 (2003).
8. Paxinos, G. & Watson, C. *The Rat Brain Atlas in Stereotaxic Co-ordinates* 4th edn. (Academic, Sydney, 1996).
9. Pedreira, M.E. & Maldonado, H. *Neuron* **38**, 863–869 (2003).
10. Suzuki, A. *et al. J. Neurosci.* **24**, 4787–4795 (2004).
11. Bahar, A., Dorfman, N. & Dudai, Y. *Eur. J. Neurosci.* **19**, 1115–1118 (2004).

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