

# A-kinase anchoring proteins in amygdala are involved in auditory fear memory

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Published online: 12 August 2002, doi:10.1038/nn901

A-kinase anchoring proteins (AKAPs) constitute a family of scaffolding proteins that bind the regulatory subunits of protein kinase A (PKA). AKAP binding to PKA regulates the phosphorylation of various proteins, some of which have been implicated in synaptic plasticity and memory consolidation<sup>1–4</sup>. Here we show that the regulatory subunits of PKA are colocalized with AKAP150 (an AKAP isoform that is expressed in the brain)<sup>5</sup> in the lateral amygdala (LA) and that infusion to the LA of the peptide St-Ht31, which blocks PKA anchoring onto AKAPs, impairs memory consolidation of auditory fear conditioning.

During fear conditioning, rats learn to express fear responses to a previously neutral conditioned stimulus (CS) after it has been paired with an aversive unconditioned stimulus (US). The LA is necessary for the acquisition and consolidation of fear memories<sup>6,7</sup>. PKA, a protein that has been widely implicated in synaptic plasticity and memory, is necessary for memory consolidation of auditory fear conditioning in the LA<sup>8</sup>. It remains unknown, however, whether PKA activity in the LA requires the formation of AKAP-PKA complexes. We therefore examined the role of AKAP-PKA complexes in the LA in fear conditioning.

We first determined if AKAP proteins and the regulatory subunits of PKA—RII $\alpha$  and RII $\beta$ , which bind with highest affinity to AKAP<sup>9</sup>—are expressed specifically in the LA and to what extent they colocalize within the same cells. AKAP150 is the best-characterized brain isoform<sup>5</sup>, so we chose to study its colocalization with PKA. Both RII $\beta$  and AKAP150 are generally expressed in the amygdala<sup>10</sup>; here we examined whether these proteins are expressed specifically in the LA and colocalize within the same neurons.

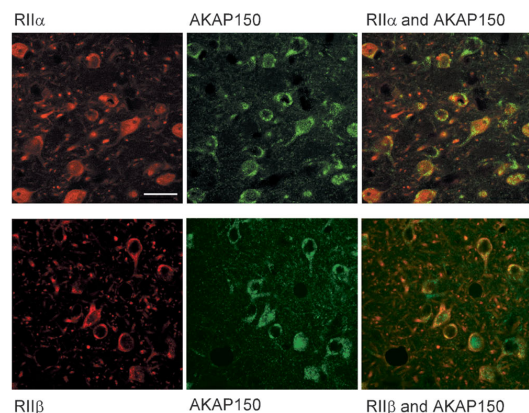
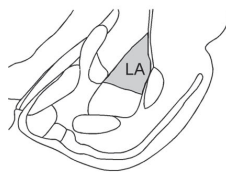
We incubated 40- $\mu$ m coronal sections of rat brain, which included the LA, with

fluorescence-conjugated antibodies against AKAP150 and RII $\alpha$ , or with antibodies against AKAP150 and RII $\beta$  (Supplementary Methods online). We found that RII $\alpha$ , RII $\beta$  and AKAP150 were expressed in the LA (Fig. 1). Both RII $\alpha$  and RII $\beta$  colocalized to a large extent (although not 100%) with AKAP150 within the same neurons.

We next examined whether anchoring proteins are necessary for the formation of memories of fear conditioning. To that end, we used a well-characterized peptide (Ht31)<sup>5</sup> that competes for the binding site of PKA on AKAPs. This peptide dislodges PKA from AKAP<sup>9</sup>, and consequently from its targets, and has been extensively used to study the functional implications of PKA binding to AKAP in several systems<sup>2,5,11,12</sup>. When sterated (St-Ht31), this peptide is cell-permeable<sup>13,14</sup>. Rats received bilateral infusions into the amygdala of St-Ht31 ( $n = 5$ ) or vehicle solution ( $n = 6$ ) one hour before auditory fear conditioning to a tone (see Supplementary Methods and for injection cannula placements, see Fig. 2e). Memory for the tone was assessed 1, 4 and 24 hours after training in the testing chamber by measuring freezing behavior (experimental design in Fig. 2a; freezing data in Fig. 2b). A two-way ANOVA, testing for main effects of group (vehicle or St-Ht31) and session (1, 4 or 24 hour test), showed a significant main effect of group ( $F_{1,9} = 8.6, P < 0.03$ ), a significant main effect of session ( $F_{2,18} = 22.4, P < 0.0001$ ) and a significant interaction effect ( $F_{2,18} = 7.3, P < 0.006$ ). A post-hoc Scheffe test showed that memory 1 hour after training did not differ between the two groups ( $P > 0.4$ ). Both 4 and 24 hours after training, however, the group that received infusions of St-Ht31 showed significantly less freezing than the group that received infusions of the vehicle solution ( $P < 0.03$  and  $P < 0.006$ , respectively). The fact that rats receiving St-Ht31 infusions into LA showed intact freezing 1 hour after training, but impaired freezing 4 and 24 hours after training, suggests that PKA anchoring onto AKAPs is necessary for the consolidation, but not for the acquisition, of conditioned fear to a tone.

To verify that this impairment in freezing was not due to permanent damage to LA resulting from the drug or the injection procedure, we retrained rats in this group ( $n = 5$ ), drug free, one day after the 24-hour test. After retraining, these animals showed high levels of freezing as compared to their first 24-hour test (paired  $t$ -test,  $t_4 = 6.05, P < 0.003$ , see Fig. 2b). Thus, the impairment after St-Ht31 infusions could not have resulted from damage to the LA. Another possibility is that the impairment resulted from a performance deficit, which would not be apparent 1 hour after training if the peptide has

**Fig. 1.** Colocalization of RII $\alpha$  and RII $\beta$  with AKAP150 in the lateral amygdala. Left, diagram of the lateral amygdala (LA, gray), from where the images were taken. Top row, labeling of RII $\alpha$  (left), AKAP150 (middle) and the two images overlaid (yellow when colocalized, right). Bottom row, labeling of RII $\beta$  (left), AKAP150 (middle) and the two images overlaid (right). Scale bar, 25  $\mu$ m. Studies were done using male Sprague-Dawley rats weighing 250–300 g. All procedures were in accordance with US Public Health Service guidelines and were approved by the animal use committee of New York University.



**Fig. 2.** St-Ht31 blocks the consolidation of auditory fear memories. **(a)** Experimental design to test the effects of the infusion on memory consolidation. **(b)** Mean percent freezing (represented as percentage from CS duration, averaged over the trials of each test session) 1, 4 and 24 hours after training. For rats that received drug infusions, a fourth test session was done 24 hours after they were retrained (last time point in the graph). \* $P < 0.05$ , \*\* $P < 0.01$ . **(c)** Experimental design to test effects on performance. **(d)** Effect of the drug on performance (freezing averaged across the three trials of the test session for both groups). **(e)** Example cannula placements (black circles). Numbers in center represent Bregma.

a gradual effect on performance. To control for this possibility, we trained a group of animals and the next day infused either vehicle ( $n = 4$ ) or St-Ht31 ( $n = 4$ ). Five hours after infusion, we gave the test (which corresponds to the time between drug infusion and the post-training 4 hour test). Rats that received St-Ht31 and vehicle showed comparable freezing levels, as revealed by independent  $t$ -tests showing no significant difference between the groups ( $t_6 = 0.16$ ,  $P > 0.8$ ), ruling out the possibility of a performance deficit (Fig. 2c and d).

Taken together, these results indicate that PKA anchoring onto AKAPs in the LA is necessary for the consolidation of auditory fear conditioning. Each isoform of AKAP proteins contains a unique targeting sequence that determines its localization in the cells and a consensus sequence that binds PKA. This property enables AKAPs to direct PKA to a unique subcellular compartment determining which targets will be phosphorylated upon PKA activation<sup>11</sup>. Furthermore, AKAPs function as scaffold proteins that keep various kinases, such as PKA and PKC, and phosphatases, such as calcineurin, close to their targets<sup>15</sup>. Because anchoring proteins are thought to provide specificity to A-kinase activity<sup>11</sup>, manipulating the binding of these proteins to specific targets may provide a new tool to dissect specific cellular and molecular processes downstream of PKA. Our finding constitutes a step toward using AKAP proteins to investigate the cellular mechanisms of memory formation.

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

**Acknowledgments**

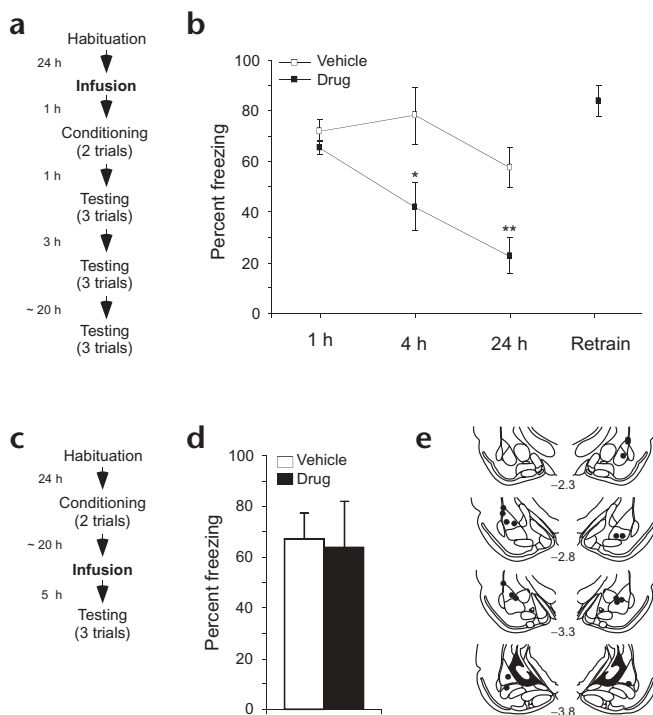
We thank G. Schafe and T. Blair for comments and C. Farb for technical support. M.A.P.M. was supported by the Gulbenkian Foundation and Program PRAXIS XXI/FCT under the Programa Gulbenkian de Doutoramento em Biologia e Medicina. R.L. was supported by a post-doctoral fellowship from the Human Frontier Science Program. National Institute for Mental Health grants MH38774, MH46516 and MH00956 to J.E.L.

**Competing interests statement**

The authors declare that they have no competing financial interests.

RECEIVED 16 MAY; ACCEPTED 21 JUNE 2002

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## Prefrontal cortex in long-term memory: an “interference” approach using magnetic stimulation

Simone Rossi, Stefano F. Cappa, Claudio Babiloni, Patrizio Pasqualetti, Carlo Miniussi, Filippo Carducci, Fabio Babiloni and Paolo M. Rossini

*Nat. Neurosci.* 4, 948–952 (2001)

The title of this article contained a typographical error. It should have read as follows:

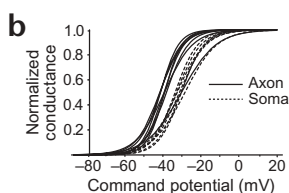
## Prefrontal cortex in long-term memory: an “interference” approach using magnetic stimulation

### Ion channel properties underlying axonal action potential initiation in pyramidal neurons

Costa M. Colbert and Enhui Pan

*Nat. Neurosci.* 5, 533–538 (2002)

A printer’s error introduced an extraneous diagonal line into Fig. 2b on page 534. The correct figure is reproduced below.



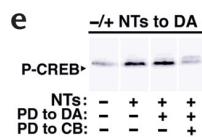
**Fig. 2.** Na<sup>+</sup> channel properties differ between the soma and axon. **(b)** Voltage dependence of activation in somatic and axonal patches. Each curve is the best-fit Boltzmann for an individual patch. Axonal Na<sup>+</sup> channels (solid lines) were activated by less depolarization than somatic channels (dotted lines).

## Neurotrophins use the Erk5 pathway to mediate a retrograde survival response

Fiona L. Watson, Heather M. Heerssen, Anita Bhattacharyya, Laura Klesse, Michael Z. Lin and Rosalind A. Segal

*Nat. Neurosci.* 4, 981–988 (2001)

In Fig. 5e on page 986, the pluses and minuses for lines “PD to DA” and “PD to CB” were incorrect. The conclusions stated in the text and the experimental description in the figure legend were correct. The corrected figure is reproduced below.



**Fig. 5.** Activation of Erk5 promotes survival. **(e)** Neurons in compartmented cultures were treated with PD98059 (PD) to distal axons or cell bodies, as indicated. Distal axons were stimulated with neurotrophins and cell body lysates were immunoblotted for P-CREB. PD treatment of distal axons alone does not prevent CREB phosphorylation. When PD is applied to the cell bodies, CREB phosphorylation is inhibited.

## A-kinase anchoring proteins in amygdala are involved in auditory fear memory

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*Nat. Neurosci.* 5, 837–838 (2002)

The authors wish to correct their supplementary methods online, which gave the wrong sources for three antibodies. The mouse anti-R11 $\alpha$  and anti-R11 $\beta$  antibodies were obtained from Transduction Laboratories (San Diego, California), and the rabbit anti-AKAP150 antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, California).