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A molecular correlate of memory and amnesia in the hippocampus

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Memory consolidation in humans and other species is profoundly disrupted by lesions of either the medial temporal lobes or regions of the thalamus[1–3]. It has been proposed that these structures regulate the neuronal gene expression necessary for long-term memory[4]. Evidence suggests that long-term memory formation requires the activity of members of the cAMP response element (CRE) binding protein (CREB) transcription factor family[5,6], and that CRE-regulated genes are expressed in the hippocampus in response to inhibitory avoidance training[7,8]. Here we show that lesions of the fornix, a massive fiber bundle connecting the hippocampus with the septum and hypothalamus, specifically disrupt both consolidation of inhibitory avoidance memory and CREB-mediated responses in the hippocampus. We propose that inputs passing through the fornix regulate this memory consolidation by regulating CREB-mediated gene expression in hippocampal neurons.

Rats were given electrolytic lesions of the fornix and were trained on an inhibitory avoidance task one week after surgery (Fig. 1). Lesioned rats did not differ from controls in initial latency to enter the dark chamber ($p > 0.05$). The two groups of animals did, however, differ in their overall retention profiles. ANOVA revealed a significant group × delay interaction ($F_{3,42} = 2.988$, $p < 0.04$) and a significant main effect of group ($F_{1,14} = 20.559$, $p < 0.0005$). At 0 and 6 hours, control and fornix-lesioned rats showed similar retention, whereas at 24 and 48 hours, lesioned rats were severely impaired. This time course suggests that deficits in fornix-lesioned animals reflect impaired memory consolidation.

CREB has a fundamental role in memory consolidation[5,9,10], and CRE-mediated gene expression is induced in the hippocampus following inhibitory avoidance training[8]. Because phosphorylation of CREB at Ser-133 is a necessary step for CREB-dependent tran-

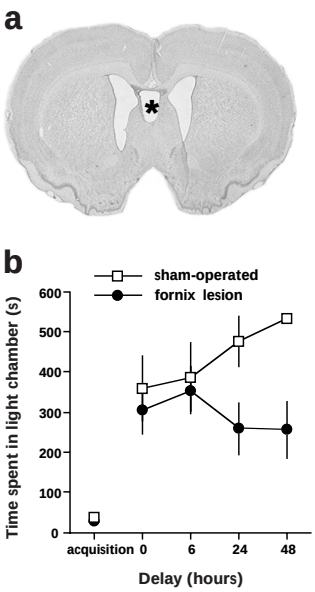

Fig. 1. Fornix lesions produce impairment on the inhibitory avoidance task. **(a)** Representative lesion (asterisk). Surgery was done on male Long-Evans rats (200–250 g) as described[15]. In 25 of 32 lesions, the dorsal fornix was severed and the fimbria extensively damaged. In addition, the anterior aspects of the lateral and triangular septal nuclei and the septofimbrial nucleus were at least partially damaged in all subjects. The remaining seven animals had partial damage to the fornix and fimbria, and only minor damage to the septal nuclei. No damage to the underlying thalamic structures or to the hippocampal formation was observed in any case. **(b)** Inhibitory avoidance training involved placing the rat in a lighted chamber connected with a dark chamber. Ten seconds later, the door separating the chambers was opened, allowing the rat to enter the dark chamber, where it received a footshock (2 s, 1.5 mA). Retention was assessed 0, 6, 24 and 48 hours later by returning the rat to the lighted chamber and measuring latency to enter the dark chamber. Fornix-lesioned rats performed similarly to controls in initial training and in retention tests zero and six hours later. By 24 h, fornix-lesioned rats were severely impaired.

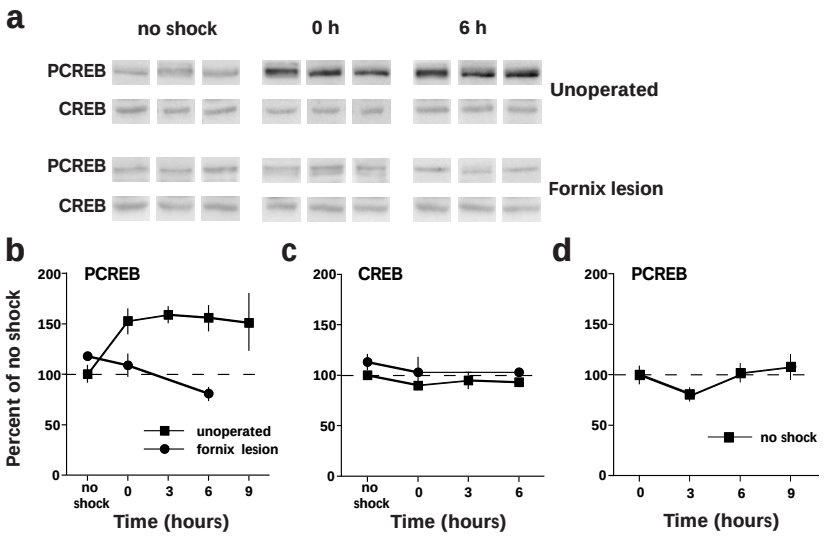

Fig. 2. Inhibitory avoidance training increases hippocampal CREB phosphorylation in normal rats but not in rats with fornix lesions. Hippocampi from unoperated or lesioned rats were homogenized in lysis buffer (0.2 M NaCl, 0.1 M HEPES, 10% glycerol, 2 mm NaF, 2 mM $Na_4P_2O_7$, 5 mM EDTA, 1mM EGTA, 2 mM DTT, 0.5 mM PMSF, 1 mM benzamidine, 10 µg per ml leupeptin, 400 U per ml aprotinin, 1 µM microcystin) and resolved by SDS-PAGE before electroblotting. Membranes were incubated with anti-Ser133-PCREB (1:2000) or anti-CREB (1:1000) antisera (Upstate Biotechnology, Lake Placid, New York) and visualized with ECL (Amersham, Arlington Heights, Illinois) before densitometric analysis (NIH Image). **(a)** PCREB and CREB western blots of hippocampal extracts from unoperated and fornix-lesioned animals. Examples are shown for three conditions: killed without receiving shock on entering chamber, killed immediately after training shock in chamber (0 h) and killed six hours after training shock (6 h). **(b–d)** Densitometric analysis of western blots of hippocampi taken from unoperated and fornix-lesioned animals at various timepoints after training and compared to 'no-shock' controls. **(b)** Unoperated animals showed significant increases ($p < 0.05$) in PCREB over 'no-shock' controls ($n = 8$) at 0 h ($n = 8$), 3 h ($n = 4$) and 6 h ($n = 8$) after training. In contrast, lesioned animals showed no increase in PCREB after training ($n = 4$ per timepoint). **(c)** Total CREB is unchanged in both lesioned and unoperated rats 0, 3 or 6 h after training ($n = 4$ per timepoint). **(d)** PCREB levels in unshocked rats were unchanged at 0, 3, 6 or 9 h ($n = 8, 4, 8, 4$) after exposure to the apparatus.

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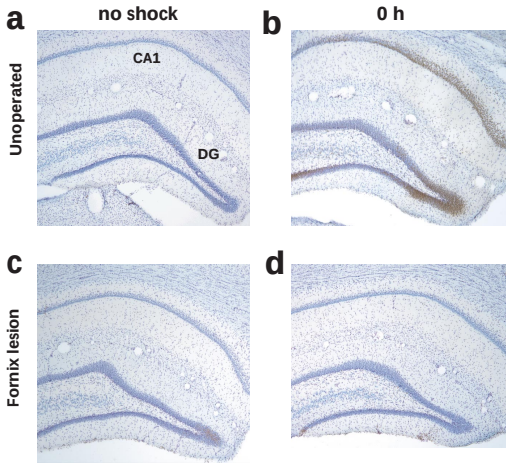

Fig. 3. CREB phosphorylation after inhibitory avoidance learning is induced mainly in CA1 and dentate gyrus (DG). Examples of anti-PCREB (1:1000) staining in 40-µm coronal brain sections from unoperated (**a** and **b**) and lesioned (**c** and **d**) rats without (no shock) and immediately after footshock training (0 h; $n = 4$ per group).

scriptional activation[11], we analyzed western blots of phosphorylated CREB (PCREB) in the hippocampus following inhibitory avoidance training in normal and fornix-lesioned rats (Fig. 2).

In unoperated rats immediately after training, hippocampal PCREB increased to 152.3 ± 12.4% of levels in control animals that entered the dark chamber but received no shock and were immediately killed (Fig. 2a and b). PCREB was increased three, six and nine hours after training (158.6 ± 8.3%, 155.1 ± 12.4%, 150.7 ± 28.6%, respectively). One-way ANOVA revealed a significant main effect of time ($F_{4,26} = 3.346$, $p < 0.02$), and Dunnet post-hoc comparisons confirmed that PCREB was elevated over control 'no-shock' levels at 0, 3 and 6 hours after training ($p < 0.05$). Western blots showed no change in CREB expression after training (Fig. 2a and c). Thus, training increased phosphorylation of hippocampal CREB protein.

To determine whether increased PCREB in unoperated animals was related to the consolidation of the inhibitory avoidance task or to other stimuli evoked by exposure to the apparatus, we measured PCREB in hippocampi of unoperated animals that entered the inhibitory avoidance chamber but received no shock (Fig. 2d). CREB phosphorylation was unchanged from immediately killed control levels 3, 6 or 9 hours after exposure to the apparatus (80.3 ± 6.3%, 101.6 ± 8.9%, 107.5 ± 12.2%; $p > 0.05$). Similarly, no change in PCREB was observed in animals that received the shock only (86.5 ± 7.1%; $n = 4$). These data suggest that persistent elevation in PCREB was specifically associated with consolidation of inhibitory avoidance memory.

Immunohistochemical staining in hippocampi of untrained animals revealed low levels of PCREB (for example, Fig. 3a). PCREB staining was variable in neurons of the dentate gyrus and CA3 and generally negative in CA1. Inhibitory avoidance training, however, produced a strong, regionally specific increase in PCREB immunostained neurons in CA1 and dentate gyrus (Fig. 3b) and, to some extent, in CA3 (data not shown). PCREB immunoreactivity increased immediately after training (Fig. 3a and b) and persisted three and six hours after training (data not shown).

We next investigated whether the PCREB increase following inhibitory avoidance training is affected by fornix lesions. Western blots of hippocampal extracts from untrained, lesioned rats showed basal levels of PCREB (117.4 ± 4.6%) comparable to those found in unoperated no-shock controls. However, unlike levels in controls, the levels of PCREB in hippocampi of rats with fornix lesions did not increase either zero or six hours after training (108.7 ± 11.2%, 80.1 ± 6.7%; Fig. 2a and b). Two-way ANOVA ($F_{5,28} = 6.713$, $p < 0.0003$) and Student Newman Keuls post-hoc analysis revealed significantly lower CREB phosphorylation 0 and 6 hours after training in lesioned rats than in unoperated rats ($p < 0.05$). Total hippocampal CREB was unchanged after training in both lesioned and control groups (Fig. 2c). Immunohistochemistry confirmed that fornix lesions prevented PCREB induction by training in CA1 and dentate gyrus (Fig. 3c and d); no induction was found in any hippocampal subregions in lesioned rats. Thus, hippocampal CREB phosphorylation induced by inhibitory avoidance training is prevented by fornix lesions.

It is interesting that, when tested at early time points, fornix-lesioned animals show normal learning and memory but not hippocampal PCREB increases. Moreover, lesioned rats display significant (although clearly impaired) memory 24 h after training. Initial learning and memory could reflect synaptic modifications independent of new protein synthesis[12]. Residual memory at 24 h might be explained by changes outside the hippocampus, or by mechanisms that do not involve increased CREB phosphorylation.

How might fornix lesions disrupt both PCREB increases and consolidation of long-term inhibitory avoidance memory? The hippocampus receives projections from the septum, hypothalamus and brain stem via the fornix. One hypothesis is that these axons convey signals regulating CREB-dependent gene expression in hippocampus. Indeed, if activity in the fornix is temporarily arrested immediately before inhibitory avoidance training, memory deficits are comparable to those produced by lesioning, whereas inactivation 48 h after training and before testing has no effect[13]. Thus, activity in the fornix is necessary for consolidation, but not expression, of memory. In contrast, inactivation of the dorsal hippocampus prevents both memory encoding and retrieval[14].

Initial learning is likely to result from changes in the transmission of synapses conveying information about where the animal is in space. Whether or not these changes are made permanent depends on the timely occurrence of new gene expression. We propose that signals to hippocampal neurons via the fornix contribute to memory consolidation by modulating CREB-dependent gene expression required for establishment of long-term memory. Identification of the critical chemical signals, their transduction pathways and the genes regulated may suggest treatments for amnesia associated with damage to the temporal lobe memory system.

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