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Disruption of fear memory consolidation and reconsolidation by actin filament arrest in the basolateral amygdala

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ABSTRACT

The dynamic re-arrangement of actin filaments is an essential process in the plasticity of synaptic connections during memory formation. In this study, we determined in mice effects of actin filament arrest in the basolateral complex of the amygdala (BLA) at different time points after memory acquisition and re-activation, using the fungal cytotoxin phalloidin. Our data show a selective disruption of auditory cued but not contextual fear memory, when phalloidin was injected 6 h after conditioning. In contrast, no effect was observed when phalloidin was applied after 24 h, ruling out an interference with the retrieval or expression of conditioned fear. A comparable result was obtained after memory re-activation, hence suggesting similar actin-dependent mechanisms to be active during consolidation and reconsolidation of auditory fear memory. Biochemical analysis showed that phalloidin-mediated filament arrest leads to a transient increase of highly cross-linked actin filaments in the BLA, evident 2 h after injection. Together, these observations indicate that dynamic re-arrangements of actin filaments in the BLA during a late phase of fear memory consolidation and reconsolidation are critical for fear memory storage.

1. Introduction

Dynamic changes of actin filaments play a critical role in structural plasticity that is believed to underlie information storage in neural networks (Lamprecht & LeDoux, 2004; Matus, 2000). Presumably through trafficking of neurotransmitter receptors and cytoskeletal restructuring of pre- and post-synaptic sites, actin filaments help recently acquired fear memories to be transformed into a more lasting state, a process called memory consolidation. Indeed, changes of synaptic actin filaments and their effect on post-synaptic spines have been demonstrated in cultivated neuronal cells upon induction of synaptic plasticity (Honkura, Matsuzaki, Noguchi, Ellis-Davies, & Kasai, 2008). These match with observations made *in vivo* of post-synaptic actin filament assembly (Fukazawa et al., 2003) and long recognized morphological changes at the synapse (e.g., Fifkova & Van Harreveld, 1977). In fact, interfering with actin filament dynamics disturbs long-term potentiation of neuronal activity in the hippocampus (Kim & Lisman, 1999; Krucker, Siggins, & Halpain, 2000) and local injections of the actin depolymerizing toxins cytochalasin D or lantrunculin A into the amygdala or hippocampus disturb the formation of auditory cued and contextual fear memory (Mantzur, Joels, & Lamprecht, 2009).

Classical fear conditioning is very well suited for the investigation of such memory consolidation processes. Subjects very quickly learn to associate a previously neutral stimulus (conditioned stimulus, CS, such as a tone) or context with a coinciding aversive stimulus (unconditioned stimulus, US, such as electric foot shock). After a single training session, animals form a robust fear memory and to subsequent exposures to the CS respond with species-specific defensive behaviors, such as freezing (Blanchard & Blanchard, 1969). It is now well established that the basolateral complex of the amygdala (BLA) provides a core structure and site of neural plasticity in such classical fear conditioning. The BLA, comprising the lateral and basal amygdala subnuclei, serves as a convergence site for uni- and multimodal sensory signals with ascending nociceptive pathways and affective information processed in limbic brain circuits (Maren, 2003; Maren & Quirk, 2004). Importantly, neural plasticity has been observed in this region following behavioral training as well as after electrical stimulation of thalamic, hippocampal or cortical afferences (Rogan, Staubli, & LeDoux, 1997; Sah, Westbrook, & Luthi, 2008). Molecular and cellular processes that are critical for the acquisition and consolidation of fear memories have been identified and ample evidence has accumulated for an involvement of actin cytoskeleton dynamics (Diana et al., 2007; Lamprecht, Farb, & LeDoux, 2002; Lamprecht, Farb, Rodrigues, & LeDoux, 2006; Mantzur et al., 2009; Stork et al., 2004). Fear conditioning has also been used to investigate the phenomenon

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of memory reconsolidation, i.e., the modification and renewed storage of prior memories upon their retrieval. It was shown that although consolidation and reconsolidation share principle characteristics, neurochemical and molecular details are only partially overlapping (Alberini, 2005; Tronson & Taylor, 2007).

In the current study we tested how an arrest of actin filaments in the mouse BLA through bilateral application of the death cap (*amanita phalloides*) toxin phalloidin affects the consolidation and reconsolidation of Pavlovian fear memory. We focused on the proposed role of actin filaments in the morphological changes in synaptic structures, which typically become evident ca. 0.5 h after stimulation and are thought to provide a long-lasting memory correlate (Bonhoeffer & Yuste, 2002; Korkotian & Segal, 2001). To begin to dissect these processes, phalloidin was applied to the BLA in different animals at time points either 0.5 h, 6 h and 24 h after conditioning or retrieval. Our results not only provide evidence for a similar role of amygdalar actin filament dynamics in consolidation and reconsolidation of auditory fear memory, but also suggest that a temporally distinct actin-dependent cellular process in the BLA is involved in responses to a familiarized background context upon fear memory re-activation.

2. Material and methods

2.1. Subjects

Eight-to-twelve-week old male C57BL/6JBomTac mice (M&B Taconic, Berlin, Germany) were used in all experiments. Mice were purchased at an age of 6 weeks and maintained in our facility in groups of 3–4 under a reversed 12 h day/12 h night cycle (lights on at 19:00) with food and water *ad libitum*. Two days before the experiments, animals were singled out and further-on kept individually in transparent cages, maintaining visual, auditory and olfactory contact to their conspecifics. The time course of experiments is summarized in Fig. 1A. All experiments were performed in accordance with regulation through the German law and were approved by the Landesverwaltungsamt Sachsen-Anhalt (AZ 203.h-42502-2-862).

2.2. Substances

Rhodamine-phalloidin and Alexa 488-coupled phalloidin were purchased from Invitrogen (Karlsruhe, Germany) and anti-actin antibody from Millipore (Schwalbach, Germany). Complete protease inhibitor mixture was from Roche (Mannheim, Germany), and polyvinylidene fluoride (PVDF) membranes as well as ECL plus reagent from GE Healthcare (Munich, Germany). All other substances were obtained from Sigma-Aldrich (Steinheim, Germany).

2.3. Stereotactic implantation and acute injections

For post-training and post-retrieval injections, stainless steel guide cannulae (22 G) were implanted bilaterally under deep pentobarbital anesthesia (50 mg/kg intraperitoneal). Under a 10° angle the cannulae were aimed at the BLA (stereotactic coordinates: AP −1.8 mm, L ±1.7 mm, DV 4.5 mm from midline, see Fig. 1B) and

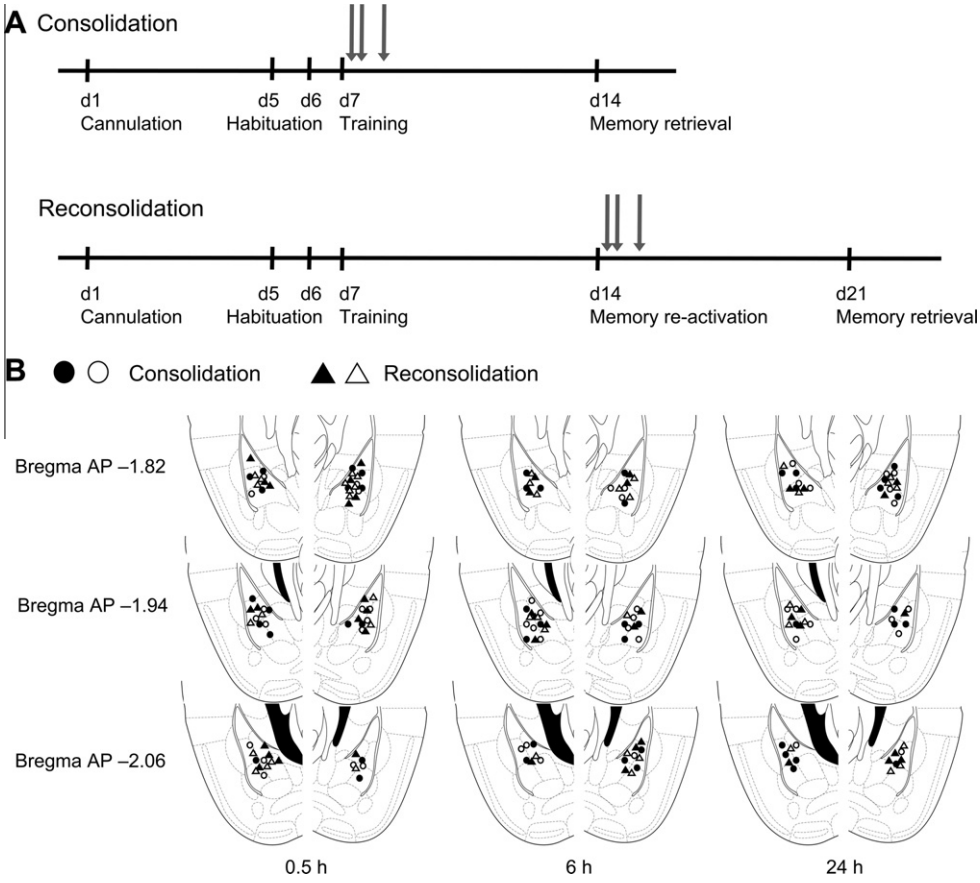

Fig. 1. *Experimental design*. (A) To study effects of phalloidin injection into the BLA on fear memory consolidation, bilateral injections were done either 0.5 h ($n = 8$ vehicle, $n = 8$ phalloidin), 6 h ($n = 8$ vehicle, $n = 8$ phalloidin) or 24 h ($n = 8$ vehicle, $n = 8$ phalloidin) after fear conditioning training and animals were tested one week later (Memory retrieval). To study the potential effects on fear memory reconsolidation, phalloidin was injected either 0.5 h ($n = 9$ vehicle, $n = 9$ phalloidin), 6 h ($n = 7$ vehicle, $n = 7$ phalloidin) or 24 h ($n = 6$ vehicle, $n = 6$ phalloidin) after a retrieval session (Memory re-activation), and tested one week later (Memory retrieval). (B) Schematic illustration of cannula tip placements into the BLA of the left and right hemispheres (phalloidin black, vehicle white).