Selective, Retrieval-Independent Disruption of Methamphetamine-Associated Memory by Actin Depolymerization

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Background: Memories associated with drugs of abuse, such as methamphetamine (METH), increase relapse vulnerability to substance use disorder. There is a growing consensus that memory is supported by structural and functional plasticity driven by F-actin polymerization in postsynaptic dendritic spines at excitatory synapses. However, the mechanisms responsible for the long-term maintenance of memories, after consolidation has occurred, are largely unknown.

Methods: Conditioned place preference ($n = 112$) and context-induced reinstatement of self-administration ($n = 19$) were used to assess the role of F-actin polymerization and myosin II, a molecular motor that drives memory-promoting dendritic spine actin polymerization, in the maintenance of METH-associated memories and related structural plasticity.

Results: Memories formed through association with METH but not associations with foot shock or food reward were disrupted by a highly-specific actin cycling inhibitor when infused into the amygdala during the postconsolidation maintenance phase. This selective effect of depolymerization on METH-associated memory was immediate, persistent, and did not depend upon retrieval or strength of the association. Inhibition of non-muscle myosin II also resulted in a disruption of METH-associated memory.

Conclusions: Thus, drug-associated memories seem to be actively maintained by a unique form of cycling F-actin driven by myosin II. This finding provides a potential therapeutic approach for the selective treatment of unwanted memories associated with psychiatric disorders that is both selective and does not rely on retrieval of the memory. The results further suggest that memory maintenance depends upon the preservation of polymerized actin.

Key Words: Addiction, amygdala, dendritic spine, memory maintenance, myosin, structural plasticity

A hallmark of memory is its longevity and relative stability (1,2). However, rather than being static after the traditional consolidation period, evidence now points to memories being actively maintained (3–10). Our understanding of protein turnover rates has influenced this notion (8,11). Although memories are encoded through functional and structural plasticity as well as through activation of the upstream epigenetic, transcriptional, and signaling events that support plasticity (12–14), the proteins that maintain these changes are themselves transient. This would suggest that the brain and, by extension, long-term memories are surprisingly dynamic in storage. Additional evidence pointing to the active maintenance of memories comes from the relative ease with which they can be manipulated after consolidation, through extinction and reconsolidation (3,5,10). We and others suspect that these phases of memory largely exist to enable the constant updating and integration of memories (15–17), although they also present a potential therapeutic avenue for weakening aberrant memories associated with psychiatric disorders, such as posttraumatic stress disorder and substance use disorder (SUD) (6,18–21).

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There is a growing consensus that memory is supported by structural and functional plasticity at excitatory synapses (22). Most excitatory synapses are located on dendritic spines, which are small structures that protrude from dendrites and enable input-specific biochemical and electrical isolation of synapses that facilitate signal integration and information storage. Spines undergo volumetric and functional changes that are important for long-term memory (23,24). The actin filaments that comprise the cytoskeleton in dendritic spines are surprisingly dynamic, capable of making rapid changes on the order of seconds (25). Actin polymerization, the process of linking actin monomers (G-actin) into complex, branched filaments (F-actin), is a critical regulator of dendritic spine synapse structure and function (26,27). Indeed, the plasticity that occurs during long-term potentiation (LTP) is dependent upon actin polymerization (28–31). Long-term potentiation stimulation initiates a multitude of molecular processes that tightly control actin polymerization and the structural enlargement of dendritic spines (32,33). Importantly, memory formation fails when learning-induced F-actin dynamics are disrupted. Furthermore, current studies report that depolymerizing agents are only effective around time of training, because F-actin rapidly stabilizes after learning (30,34–37).

The invasive, drug-associated memories associated with SUD, including associations formed with the widely abused psychostimulant methamphetamine (METH), elicit a motivational state capable of triggering relapse. This occurs through activation of several brain regions, including the emotional memory center of the brain, the amygdala (38–43). These long-lasting and extinction-resistant memories can trigger a motivation for the drug even after prolonged drug-free periods produced by seemingly successful rehabilitation. Thus, a therapeutic challenge presented by SUD is to disrupt drug-associated memories after

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they have formed. We and others have taken the approach of postconsolidation manipulations focused on the time of retrieval (e.g., blockade of reconsolidation and accelerated extinction) (44–48). In the current study we hypothesized that the actin cytoskeleton supporting structural plasticity might be a target for manipulating the strength of METH-dependent associations after the typical consolidation period. Although very little is known about spine plasticity associated with drug-related memories, there is a precedent for drugs of abuse altering F-actin dynamics in the brain. Withdrawal from the psychostimulant cocaine and development of aversive associations with morphine withdrawal both involve actin polymerization (49–51). These suggest that F-actin cycling might mediate unique aspects of memories formed through associations with drugs of abuse.

Conditioned place preference (CPP) and context-induced reinstatement of self-administration animal models of contextual reward associations and drug seeking use a circuit that includes the basolateral amygdala complex (BLC) (lateral and basolateral) (52–58). Here we investigated the postconsolidation contribution of F-actin dynamics to METH-associated memory and structural plasticity within the BLC through direct disruption of actin polymerization and upstream inhibition of myosin II, a molecular motor that drives memory-promoting dendritic spine actin polymerization (30). We originally hypothesized that interfering with F-actin dynamics in the postconsolidation period would affect the rate at which METH-associated memory extinguished. However, we were surprised to find that actin depolymerization produced an immediate and highly specific loss of METH-associated memory that was independent of retrieval in both rats and mice, indicating a disruption of memory in the maintenance phase. These postconsolidation disruptions to cycling F-actin were ineffective at interfering with the maintenance of other types of associations and reversed dendritic spine density increases produced by METH-associated memory formation. Thus, this unique feature of cycling F-actin renders METH-associated memories susceptible to postconsolidation manipulations and suggests that a general requirement for memory maintenance might be the preservation of polymerization actin.

Methods and Materials

Animals

Adult male Sprague-Dawley rats (300–350 g; Charles River, Wilmington, Massachusetts) and Thy1-GFP(m) mice (10 weeks) were housed under a 12:12 light/dark cycle, with food and water ad libitum. All procedures were performed in accordance with the Scripps Research Institutional Animal Care and Use Committee. Animals were handled for 3–5 days before behavioral conditioning.

Surgery

Rats and mice received implantation with 26G bilateral stainless steel guide cannulae directed at the BLC, and rats received implantation with chronic indwelling jugular catheters for intravenous METH self-administration (for additional details and drug infusion rates, see Supplement 1).

Drugs

Methamphetamine hydrochloride (Sigma-Aldrich, St. Louis, Missouri) was delivered intraperitoneal at the appropriate dose (rats: 1.0 mg/kg, mice: 2.0 mg/kg). Latrunculin A (LatA) (Calbiochem, Darmstadt, Germany), an actin depolymerizing agent, was infused at a concentration of 25 ng/μL in 2% dimethyl sulfoxide. Both enantiomers of Blebbistatin (− indicates active Blebbistatin, + indicates inactive control; Calbiochem), a myosin II inhibitor, were infused at a concentration of 90 ng/μL in 10% dimethyl sulfoxide.

Behavioral Procedures

CPP. Animals were trained with an unbiased procedure in an apparatus with three chambers (Med Associates, St. Albans, Vermont) that differed in terms of visual, tactile, and olfactory cues. During the pretest, rats were given free access to the apparatus for 15 min, and the time spent in each chamber was recorded. During training, METH was given before placement in the conditioned stimulus (CS+) chamber for 30 min (counterbalanced between chambers within groups). On alternating days, rats were injected with an equivalent volume of .9% saline and confined to the opposite chamber (CS−). This was repeated three times for a total of 6 days, with starting treatment (METH vs. saline) also counterbalanced within groups. Testing began 48–72 hours after the final training session, with free access to the apparatus for 15 min. For additional details with regard to modifications for CPP with food reward and in mice, see Supplement 1.

Auditory Fear Conditioning. Fear conditioning was performed in rats as previously described (37). See Supplement 1 for additional details.

Context-Induced Reinstatement of METH Seeking. The behavioral protocol was performed in rats as previously described and consisted of four phases: 1) training with food pellets to establish lever pressing; 2) METH self-administration in Context A; 3) extinction of lever pressing in Context B; and 4) reinstatement in Context A in the absence of METH reward (58,59). For additional details, see Supplement 1.

Spine Density Analysis

To determine spine density, 10 dendrite sections/animal, ranging from 20 to 45 μm in length and <1 μm in width, were selected for analysis from the BLC of Thy1-GFP(m) mice after METH or saline CPP training and intra-BLC vehicle (Veh) or LatA infusion. For additional details, see Supplement 1.

Statistical Analysis

One-way analysis of variance and repeated measures analyses of variance were used to analyze all data, with Fisher's protected least significant difference post hoc tests performed when appropriate.

Results

Maintenance of METH-Associated Memory Is Supported in the Amygdala by a Dynamic Actin Cytoskeleton

For CPP, animals were trained to associate the rewarding effects of METH with the multi-modal environmental context in which it was administered. Two days after training that produced a lasting memory for the METH-paired context (CS+), rats were given a single intra-BLC infusion of LatA, a highly-specific inhibitor of cycling but not stable actin (60). Fifteen minutes after infusion, METH-associated memory was assessed in the absence of the unconditioned stimulus (US), METH reinforcement (Figure 1A). Although Veh-treated control subjects displayed a robust METH-associated memory, preferring the CS+ over a saline-paired context (CS−), LatA-treated animals displayed an immediate memory disruption (Figure 1B). Suspecting that this effect might