Neurobiology of Learning and Memory 103 (2013) 72–81

Contents lists available at SciVerse ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

AMPA receptor endocytosis in the amygdala is involved in the disrupted reconsolidation of Methamphetamine-associated contextual memory

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ARTICLE INFO

Article history:
Received 1 October 2012
Revised 27 March 2013
Accepted 9 April 2013
Available online 17 April 2013

Keywords:
Methamphetamine
Drug addiction
Reconsolidation
Anisomycin
Amygdala
AMPA receptor

ABSTRACT

Repetitive drug taking induces neural long-lasting changes and results in compulsive drug-seeking behavior which may arise from enduring drug memory that impairs cognitive control of motivated behavior. Thus, disrupting these memories could reduce drug seeking. Here, we used a conditioned place preference (CPP) procedure in mice to examine the role of AMPA receptor endocytosis in the basolateral amygdala (BLA) in the disrupted reconsolidation of Methamphetamine (MeAM) memory. Conditioning MeAM (2 mg/kg, i.p.) for 3 days in mice markedly increased the time spent in the MeAM-paired compartment tested 24 h after the last injection (CPP test), indicating that MeAM induced a significant rewarding effect. Mice then received anisomycin or vehicle within 1 h after CPP test and CPP was re-assessed 24 h after CPP test. Mice injected with vehicle exhibited CPP for the previously MeAM-paired chamber whereas mice injected with anisomycin did not. Anisomycin had no effect on the CPP when CPP test was omitted. In addition, anisomycin treatment prevented MeAM priming-induced reinstatement of CPP suggesting the disruption of MeAM memory reconsolidation. MeAM CPP increased surface expression of GluR1 and GluR2 subunits of AMPA receptor in the BLA. Bilateral injection of Tat-GluR2$_{3Y}$, a synthetic peptide that blocked AMPA receptor endocytosis, prevented disruption of MeAM memory reconsolidation. These results suggest that AMPA receptor endocytosis in the BLA is critical for the anisomycin-mediated disruption of reconsolidation of MeAM reward memory.

1. Introduction

Drug addiction is a chronically relapsing disorder characterized by compulsive drug-seeking behavior. Repeated drug administration can develop an intense associative memory between drug-paired cues and the rewarding effects of the drug (Milton & Everitt, 2012; Nestler, 2001). As in aversive memories, repeated presentation of drug-paired cues to the subject without pairing with the drug results in a gradual extinction in drug response. This extinction process represents an explicit model of behavioral therapy and is an effective treatment for patients with post-traumatic stress disorder (Yehuda, 2002). However, successful reduction of both aversive and rewarding memories through extinction processes is often followed by a return of conditioned responses (Myers & Davis, 2007; Sotres-Bayon, Bush, & LeDoux, 2004; Torregrossa & Taylor, 2012).

With each drug use, when drug-related memory is reactivated, the memory is thought to become destabilized such that it is susceptible to disruption by amnestic agents (Milton & Everitt, 2010; Sorg, 2012; Torregrossa & Taylor, 2012; Tronson & Taylor, 2007). A growing number of studies have manipulated the reconsolidation process to disrupt the drug reward memory by using pharmacological agents (Lee, Di Ciano, Thomas, & Everitt, 2005; Robinson & Franklin, 2007; Sorg, 2012; Wang, Zhao, Ghitza, Li, & Lu, 2008). It has been demonstrated that an established morphine CPP was persistently disrupted if protein synthesis was blocked by either anisomycin or cycloheximide after the representation of a conditioning session (Milekic, Brown, Castellini, & Alberini, 2006). Although this elegant study has demonstrated persistent disruption of an established memory induced by a drug of abuse, its underlying mechanism remained elusive.

Long-lasting changes in synaptic efficacy are brought about by gene transcription, and protein synthesis and changes in strength of glutamatergic synapses through AMPA receptor trafficking are believed to be the cellular substrates of aversive and rewarding memory. The glutamatergic projection from the prefrontal cortex (PFC) to the nucleus accumbens (NAc) undergoes long-lasting neuroplasticity, including long-term increases in the strength of excitatory synapses on medium spiny neurons (MSNs) (Kourrich, Rothwell, Klug, & Thomas, 2007; Moussawi, Pacchioni, et al., 2009), following chronic cocaine administration (Conrad, Tseng, et al., 2008; Kalivas, 2009; Kauer & Malenka, 2007). Synaptic potentiation is indicated in studies showing increased AMPA

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http://dx.doi.org/10.1016/j.nlm.2013.04.004

relative to NMDA currents in vitro (Kourrich et al., 2007), increased field potentials in PFC to accumbens synapses in vivo (Moussawi et al., 2009), elevated surface expression of GluR1 receptor subunit in the NAc (Conrad et al., 2008), and increased density and diameter of dendritic spines on accumbens MSNs (Robinson & Kolb, 2004; Shen, Toda, et al., 2009). In the present study, we examined the role of AMPA receptor trafficking in BLA in the reconsolidation of MeAM memory. We used a Pavlovian CPP procedure, in which the preference of mice for the drug context cue served as a measure of drug reward (Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982) and the incentive motivational effect of drug (Mueller & Stewart, 2000). We focused on the amygdala because it plays a critical role in the reconsolidation of aversive and drug rewarding memories (Fuchs, Bell, Ramirez, Eaddy, & Su, 2009; Lee et al., 2005; McGaugh, 2004; Nader, Schafe, & LeDoux, 2000; Sanchez, Quinn, Torregrossa, & Taylor, 2010; Tronson & Taylor 2007).

2. Methods

2.1. Animals

All mice in this present study were male C57BL/6 mice purchased from the animal center of National Cheng Kung University (NCKU). All mice were kept on a 12 h/12 h light/dark cycle with a room temperature of 22 ± 2 °C and humidity of 55 ± 5% and were given free access to water and food. All procedures were approved of the Institutional Animal Care and Use Committee of the College of Medicine, NCKU.

2.2. Surgery and Intra-amygdala microinjections

Mice were anesthetized with chloral hydrate (400 mg/kg, i.p.), and subsequently were mounted on a stereotaxic apparatus. A cannula made of 26 gauge stainless steel tubing were implanted bilaterally into the amygdala (anteroposterior, −1.6 mm; mediolateral, ±3.5 mm; dorsoventral, −4.7 mm). The mice were handled daily and were given 7 days to recover. Anisomycin (62.5 μg/side dissolved in saline-containing 10% DMSO, pH value was adjusted to 7.0) was obtained from Sigma (St. Louis, MO). In the experiments of inhibition of AMPA receptor endocytosis, the Tat-conjugated peptide with the sequence 869-YKEGYNVYG-877 ($GluR2_{3Y}$, 15 pmol/side) was designed to block the regulated clathrin-coated endocytosis of AMPARs. The control peptide in which the tyrosine residues were replaced by alanine had the sequence AKEGANVAG ($GluR2_{3A}$, 15 pmol/side). These peptides were purchased from Kelowna International Scientific Inc. (Sanchong Dist., New Taipei City, Taiwan) and dissolved in saline. Drug was administered bilaterally to the amygdala in a volume of 1.0 μl at a rate of 0.1 μl/min. The infusion cannulas were left in place for 2 min before being withdrawn.

2.3. Conditioned place preference paradigm

The CPP apparatus which was made of acrylic plastic box (36 × 15 × 30 cm) consisted of three compartments. Two identically sized compartments (15 × 15 × 30 cm) were constructed at both sides, separated by a narrower compartment (6 × 15 × 30 cm). One of the large compartments was white walls and stainless steel bar grid floor; the other large compartment was black walls and stainless steel wire floor. To give more visual cues, blue and red light bulbs were hung separately above the two large compartments (Lue, Huang, Yang, Wong, & Tao, 2007). The CPP paradigm consisted of three phases: pre-conditioning, conditioning and post-conditioning. In the pre- and post-conditioning phases, animals were placed in the neutral gray center compartment and the sliding doors were removed to allow equal access to the entire apparatus for 15 min. The amount of time each mouse spent in each compartment represented the initial preferred compartment in the pre-conditioning phase. In the conditioning phase, the animals received either saline or MeAM injections in the morning or afternoon sessions, respectively. For the MeAM-paired group, each animal received saline injections in their initially preferred compartment (saline-paired side) in the morning and then MeAM injections in their initially non-preferred compartment (drug-paired side) in the afternoon for 3 days (total six sessions; 30 min/session). For the MeAM-alone control group, the mice received saline and MeAM injections in their home cages. The CPP in the present study was a biased design and thus the relative amount of time spent in each compartment before vs. after conditioning was used to assess the CPP. The CPP data reported here was shown as the "time difference" and was calculated by subtracting the time spent in the initially non-preferred side of the apparatus (drug-paired) and the time spent in the preferred side during the pre- or post-conditioning test. It is important to mention that overall MeAM-treated mice spent significantly more time in the initially non-preferred compartment than the initially preferred compartment following drug conditioning suggesting a true reward measurement.

2.4. Extinction and reinstatement of CPP

Extinction training began the next day after CPP test. In this experiment, extinction was carried out by pairings of saline both in the morning and in the afternoon for 5 consecutive days. The animals did not receive MeAM during this period. CPP was tested 24 h after the last extinction training (Extinction test). Next day, the mice received priming injection of MeAM (1 mg/kg, i.p.) and 5 min later were given CPP test (Reinstatement; Priming test).

2.5. Locomotor activity

Locomotor activity was monitored in a plastic cage with top open. The size of locomotor arena was 15 cm × 15 cm × 30 cm. On the day of experiment, mice were transferred to the behavioral testing room in their home cages and allowed to acclimatize to the testing room for 60 min undisturbed. Then, mice were injected with saline or MeAM in their home cages and moved into the locomotor arena for 30 min. Data were recorded and quantified by a computer-operated video tracking system (Ethovision XT tracking system, Noldus Information Technology, Wageningen, The Netherlands).

2.6. Biotinylation and western blotting analysis of surface AMPA receptor

Brain slices containing amygdala were placed on ice and washed twice with cold artificial cerebrospinal fluid (ACSF; 117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, and 11 mM glucose). The slices were then incubated with cold ACSF containing 0.125 mg/ml EZ-Link Sulfo-NHS-LC-Biotin (Pierce Chemical Co., Rockford, IL) for 1 h on ice. After biotin incubation, the slices were rinsed three times in cold ACSF to quench the biotin reaction, and then frozen at −80 °C until used. The slices were sonicated briefly in homogenizing buffer (1% Triton X-100, 0.1% SDS, 50 mM, Tris–HCl, pH7.5, 0.3 M Sucrose, 5 mM EDTA, 2 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride and 20 μg/ml leupeptin, and 4 μg/ml aprotinin). After sonication, the samples were centrifuged at 14,000 r.p.m. for 30 min at 4 °C and the supernatants were obtained. Protein concentration was then