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Extinction and Reinstatement of Cocaine-seeking in Self-administering Mice is Associated with Bidirectional AMPAR-mediated Plasticity in the Nucleus Accumbens Shell

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Abstract—Experience-dependent synaptic plasticity is an important component of both learning and motivational disturbances found in addicted individuals. Here, we investigated the role of cocaine experience-dependent plasticity at excitatory synapses in the nucleus accumbens shell (NAcSh) in relapse-related behavior in mice with a history of volitional cocaine self-administration. Using an extinction/reinstatement paradigm of cocaine-seeking behavior, we demonstrate that cocaine-experienced mice with extinguished cocaine-seeking behavior show potentiation of synaptic strength at excitatory inputs onto NAcSh medium spiny neurons (MSNs). Conversely, we found that exposure to various distinct types of reinstating stimuli (cocaine, cocaine-associated cues, yohimbine ''stress") after extinction can produce a relative depotentiation of NAcSh synapses that is strongly associated with the magnitude of cocaine-seeking behavior exhibited in response to these challenges. Furthermore, we show that these effects are due to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)-specific mechanisms that differ depending on the nature and context of the reinstatement-inducing stimuli. Together, our findings identify common themes as well as differential mechanisms that are likely important for the ability of diverse environmental stimuli to drive relapse to addictive-like cocaine-seeking behavior. © **2018 Published by Elsevier Ltd on behalf of IBRO.**

Key words: cocaine, self-administration, relapse, plasticity, nucleus accumbens, AMPAR.

INTRODUCTION

Vulnerability to relapse is a hallmark of cocaine addiction in humans, yet current interventions remain largely ineffective (Kampman, 2005, 2010). Animal models of cocaine addiction suggest that the persistent risk of relapse involves experience-dependent synaptic plasticity in mesocorticolimbic circuitry including the nucleus accumbens (NAc), a major limbic-motor interface critical for directing motivated behavior (Lüscher and Malenka, 2011). For example, forced abstinence from either chronic passive or actively self-administered cocaine results in strengthening of excitatory synapses onto gamma-aminobutyric acid (GABAergic) medium spiny neurons (MSNs) in the NAc shell (NAcSh) – a phenomenon that is largely driven by enhancement of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)-mediated neurotransmission (Thomas, 2001; Boudreau and Wolf, 2005; Kourrich et al., 2007; Ma et al., 2014; Pascoli et al., 2014; Dong, 2016). Several studies have indicated that this plasticity is necessary for driving cocaine-related behavior, including the expression cocaine sensitization in mice exposed to passive cocaine injections (Pascoli et al., 2011; Terrier et al., 2016) as well cocaine-seeking or incubation of cocaine craving elicited by exposure to cocaine-associated contexts and/or cocaine-associated cues in animals with a chronic history of volitional, active cocaine self-administration (Ma et al., 2014; Pascoli et al., 2014; Terrier et al., 2016). While these findings strongly implicate potentiation at NAcSh MSNs in driving relapse-related behavior, studies looking at cocaine-seeking behavior in self-administering mice typically do so using forced abstinence/relapse models of self-administration (Venniro et al., 2016). Thus, it is

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Abbreviations: ACSF, artificial cerebral spinal fluid; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; COC, cocaine; CUE, cocaine-associated discrete cue light; D-AP5, D-aminophosphonovaleric acid; FR1, fixed ratio 1 schedule of reinforcement; GABA, gamma-aminobutyric acid; IU, international units; mEPSC, mini excitatory post-synaptic current; MSN, medium spiny neuron; NacSh, nucleus accumbens shell; NMDAR, N-methyl-D-aspartate receptor; NR, non-reinstated mice; PPR, paired pulse ratio; SA, self-administration; SAL, saline; YOH, yohimbine.

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unclear whether findings generalize to mice that have undergone extinction training during a drug-free period, or if maintains after exposure to different types of relapse-promoting stimuli.

Relapse-related behavior during drug-free periods can be induced by exposure to various triggers including exposure to contextual cues (e.g., drug-associated environment), discrete cues (e.g., drug-associated objects in the environment), acute drug re-exposure, or stress. Interestingly, previous work using locomotor sensitization procedures have found that while chronic cocaine exposure followed by a drug-free period potentiates NAcSh synapses, exposure to cocaine or forced swim stress during drug-free periods has the opposite effect and instead reduces excitatory synaptic strength at NAcSh MSNs relative to unchallenged animals (Boudreau et al., 2007; Kourrich et al., 2007; Ferrario et al., 2010; Rothwell et al., 2011; Pascoli et al., 2011; Jedynak et al., 2016). These findings suggest that transient reductions in synaptic strength may also be important for relapse in response to these stimuli. However, it is unclear if such bidirectional plasticity also occurs in animals with a history of chronic volitional cocaine self-administration or how it may relate to relapse-related behavior induced by different stimuli. Therefore, we investigated relationships between NAcSh plasticity and relapse-related cocaine-seeking behavior using an extinction/reinstatement model of cocaine self-administration (de Wit and Stewart, 1981). This model differs from forced abstinence/relapse procedures in that it employs daily exposure to the cocaine-associated context during the drug-free period. Under these conditions, cocaine-seeking behavior in the drug-associated context becomes extinguished, but can then be reinstated by exposure to cocaine-associated cues, cocaine, or stress. We chose this approach as it is a different model than is typically used to examine relapse-related plasticity, and it also allowed us to selectively examine relapse-related plasticity at NAcSh MSNs across multiple independent modalities. These models also have important behavioral and neurological parallels to extinction training approaches used to reduce craving in humans (Bowers et al., 2010; Bossert et al., 2013).

EXPERIMENTAL PROCEDURES

Subjects

A total of 58 adult male C576J/BL6 mice (Jackson Laboratories, 8 weeks old at arrival) were used in this study. Mice were group housed and habituated to the animal vivarium for 1 week prior to surgery. Mice were individually housed after surgery to protect the integrity of the intravenous (i.v.) catheter implant, and were fed *ad lib* at all times to minimize potential stress from food restriction that could impact cocaine-seeking behavior (Campbell and Carroll, 2000). Ethical guidelines outlined in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* were followed, and experimental animal use and procedures were approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

Drugs for surgery and self-administration

Cocaine HCl (Medisca), ketamine, sodium brevital, and yohimbine were obtained through Boynton Pharmacy and ketofen, gentamycin, heparin, and baytril were obtained through Research Animal Resources (all located at the University of Minnesota).

Surgery

Mice were anesthetized with an intraperitoneal injection of a ketamine/xylazine mixture (100 and 10 mg/kg; 10 ml/kg, i.p.) and implanted with a customized chronic, indwelling intravenous (i.v.) catheter into the right jugular vein. The distal end of the catheter was fed subcutaneously and exited approximately 1 cm ventral to the mid-scapular region on the back of the animal. The open end of the catheter port was capped when not in use. Mice were given subcutaneous ketofen (5 mg/kg, s.c.) before surgery and for 3 days after surgery for pain relief. Mice recovered for 5–7 days before beginning self-administration sessions. During self-administration, catheters were flushed before sessions with saline (0.05 ml, i.v.) containing gentamycin antibiotic (0.33 mg/ml) and heparin (20 IU/ml) to prevent blood clotting and catheter occlusion. To further prevent infection, baytril (~8 mg/kg, i.v.) was given immediately after each self-administration session. Catheter patency was checked weekly during self-administration testing and confirmed by the ability of sodium brevital (0.05 ml of 5 mg/ml, i.v.) to induce an immediate loss of righting reflex.

Cocaine self-administration, extinction, and reinstatement

The experimental timeline is illustrated in Fig. 1A. For cocaine self-administration (SA), mice were placed in operant chambers and given access to contingent infusions of cocaine (0.5 mg/kg, i.v.) under a fixed ratio 1 (FR1) schedule of reinforcement for 10 daily sessions (2 h/day). Responses on the active, drug-paired (left) lever resulted in an infusion (25 μl, i.v.) of cocaine over 1.25 s. A discrete cue light located above the active lever illuminated during the 1.25-s infusion, while the house light turned off for 20 s to signal a timeout period where further lever responding was counted, but without consequence. To facilitate acquisition, cocaine self-administration mice received two non-contingent cocaine infusions (one to prime the catheter, one as a sample infusion) and a small amount of 50% vanilla Ensure was put on the active lever for the first 2–3 sessions. Yoked saline control mice (Yoked SAL) were also placed in operant chambers and initially exposed to Ensure, but saline infusions were not contingent of lever responding and were instead yoked to cocaine self-administering mice. Thus, saline control mice received a similar number of infusions (25 μl/inf) under the same temporal pattern as their cocaine self-administering counterparts.

Extinction training began 24 h following the final cocaine session and consisted of 2-h daily sessions in the self-administration context (operant chamber)

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