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NMDA AND DOPAMINE D_1 RECEPTORS WITHIN NAc-SHELL REGULATE IEG PROTEINS EXPRESSION IN REWARD CIRCUIT DURING COCAINE MEMORY RECONSOLIDATION

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Abstract—Reactivation of consolidated memory initiates a memory reconsolidation process, during which the reactivated memory is susceptible to strengthening, weakening or updating. Therefore, effective interference with the memory reconsolidation process is expected to be an important treatment for drug addiction. The nucleus accumbens (NAc) has been well recognized as a pathway component that can prevent drug relapse, although the mechanism underlying this function is poorly understood. We aimed to clarify the regulatory role of the NAc in the cocaine memory reconsolidation process, by examining the effect of applying different pharmacological interventions to the NAc on Zif 268 and Fos B expression in the entire reward circuit after cocaine memory reactivation. Through the cocaine-induced conditioned place preference (CPP) model, immunohistochemical and immunofluorescence staining for Zif 268 and Fos B were used to explore the functional activated brain nuclei after cocaine memory reactivation. Our results showed that the expression of Zif 268 and Fos B was commonly increased in the medial prefrontal cortex (mPFC), the infralimbic cortex (IL), the NAc-core, the NAc-shell, the hippocampus (CA1, CA2, and CA3 subregions), the amygdala, the ventral tegmental area (VTA), and the supramammillary nucleus (SuM) following memory reconsolidation, and Zif 268/Fos B co-expression was commonly observed (for Zif 268: 51–68%; for Fos B: 52–66%). Further, bilateral NAc-shell infusion of MK 801 and SCH 23390, but not raclopride or propranolol, prior to addictive memory reconsolidation, decreased Zif 268 and Fos B expression in the entire reward circuit, except for the amygdala, and effectively disturbed subsequent CPP-related behavior. In summary, N-methyl-D-aspartate (NMDA) and dopamine D_1 receptors, but not dopamine D_2 or β adrenergic receptors, within the NAc-shell, may regulate Zif 268 and Fos B expression in most brain nuclei of the reward circuit after cocaine memory reactivation. These findings indicated that the NAc played a key role in regulating addictive memory reconsolidation by influencing the function of the entire addictive memory network.

Key words: IEG, MK 801, SCH 23390, the NAc, reward circuit, memory reconsolidation.

INTRODUCTION

Drug addiction is a chronic and recurrent brain disease that is characterized by compulsive drug use despite terrible negative consequences. For addicts, relapse is often triggered by drug-associated stimuli, which display powerful conditioned reinforcing properties. It was commonly recognized that even after several years of abstinence, drug-associated stimuli could still bring back the experience of initial drug use, thus eliciting intense craving and leading to relapse (Lee et al., 2005). This phenomenon poses a fundamental problem in the treatment of drug addiction, and understanding the possible mechanism underlying this type of dysfunctional memory process is important for helping to prevent drug relapse.

A previous theory suggested that newly acquired memory, which was initially labile, would become permanently stable via a process termed memory consolidation (McGaugh, 2000; Dudai, 2004). Recent evidence suggested that consolidated memory reactivation initiated a memory "reconsolidation" process, during which the memory was susceptible to interference and, thus, was able to be strengthened, weakened or updated (Lee, 2009; Nader and Hardt, 2009). Reconsolidation has been generalized across nearly all types of memory paradigms, and addictive memory reconsolidation critically underlies persistent drug-seeking behavior. Each instance of re-exposure to drug-associated stimuli induces the retrieval of drug-related memory, and the subsequent reconsolidation process strengthens this drug memory; as a consequence, relapse occurs and drug memory is maintained (Tronson and Taylor, 2007; Milton and Everitt, 2010).

Alternatively, the destablilized state of drug-related memory after retrieval also renders it susceptible to disruption. Thus, interventions targeting the drug-related memory reconsolidation process might represent a

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Abbreviations: CPP, conditioned place preference; IEG, immediate early gene; IL, infralimbic cortex; MK 801, dizocilpine; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; NMDA, N-methyl-D-aspartate; PBS, phosphate-buffered saline; PKA, protein kinase A; SuM, supramammillary nucleus; VTA, ventral tegmental area.

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therapeutic strategy for preventing relapse. Behavioral extinction manipulation in the strict passage of time after the retrieval of drug memory could greatly weaken this memory during the reconsolidation stage, thereby preventing drug relapse (Xue et al., 2012). Several studies have shown that systemic administration of the protein synthesis inhibitor anisomycin immediately after the retrieval of drug-related memory effectively disrupted the subsequent memory reconsolidation process (Bernardi et al., 2007; Fan et al., 2010). Additionally, in the conditioned place preference (CPP) paradigm, interventions targeting several neurotransmitters and their receptors, mostly notably the glutamatergic, dopaminergic and β-adrenergic receptors, led to an absence of late CPP memory (Spina et al., 2006; Brown et al., 2008; Otis and Mueller, 2011).

The nucleus accumbens (NAc) plays a pivotal role in the function of the midbrain dopamine cortical limbic circuit, and is well recognized as the pathway component responsible for the addictive memory reconsolidation process. Basic and clinical research evidence suggested that either lesioning (Wang et al., 2002; Gao et al., 2003; Ge et al., 2013) or deep brain stimulation (DBS) of the NAc (Vassoler et al., 2008; Li et al., 2013; Ma et al., 2013; Muller et al., 2013) effectively enabled addicts to maintain permanent abstinence after the intervention. These findings suggest the NAc as the most promising target for treating addiction, although the possible mechanism underlying the effects of such interventions remained unclear. Furthermore, many studies have confirmed that the NAc is a necessary constituent of the memory circuit for several types of memory (Ding et al., 2013; Rasekhi et al., 2014; Wang et al., 2014; Yang and Liang, 2014). Evidence strongly suggested that the NAc acts as a key structure in the process of drug memory reconsolidation (Lv et al., 2015), and this function of the NAc might explain why interventions targeting the NAc function effectively prevented drug relapse among addicts.

Previous data showed that in addition to the NAc, several other brain structures participate in the midbrain dopamine circuit and that most of these structures possess mutual interactions with the NAc. Moreover, these structures are involved in addictive memory reconsolidation (Fuchs et al., 2009; Slaker et al., 2015; Wells et al., 2015). Therefore, we hypothesized that interfering with the NAc function might disrupt drug memory reconsolidation by influencing the function of the entire addictive memory network. Previous studies confirmed that N-methyl-D-aspartate (NMDA) and glutamatergic receptors play important roles in the process of drug memory reconsolidation (Brown et al., 2008; Lee and Everitt, 2008) and found that certain immediate early gene (IEG)-encoded proteins that serve as effective molecular signatures for the functional stimulation of brain regions involved in addictive memory retrieval (Veyrac et al., 2014). In the present study, to test the hypothesis that interventions targeting the NAc might affect drug memory reconsolidation by influencing the function of the entire addictive memory network, we employed the cocaine-induced CPP paradigm to explore the effect of pharmacological manipulations of the NAc function (direct infusion of NMDA, β adrenergic or dopaminergic antagonists) on Zif 268 and Fos B expression in various brain regions after the retrieval of cocaine-related memory. These results might help to elucidate the neuropharmacological mechanism underlying the function of the NAc in drug memory reconsolidation.

EXPERIMENTAL PROCEDURES

Animals

All procedures in the present experiments were conducted in accordance with the guidelines of the Committee for Animal Care and Use at the Fourth Military Medical University (Xi'an, Shaanxi, China). Male Sprague–Dawley rats (initial weight 180–200 g; China SH, Xi'an, China) were individually housed in an animal center at a controlled temperature (21 ± 2 °C) and humidity (40–60%) with a 12-h light–dark cycle (lights on at 07:00) and were provided with food and water *ad libitum*. To avoid interfering with the judgment of the behavior of the animals in the CPP apparatus, all animals were fed separately in customized gray breeding cages.

Surgery

The rats were anesthetized with chloral hydrate (400 mg/kg body weight, i.p.) and fixed in a stereotaxic apparatus (68025, RWD Life Science Co., Ltd, Shenzhen, Guangdong, China) in the prone position. Two stainless steel cannulas (outer diameter 0.41 mm, inner diameter 0.25 mm, length 8.0 mm; 62004, RWD Life Science Co., Ltd., Shenzhen, Guangdong, China) were guided into bilateral NAc-shell (according to the rat stereotaxic atlas: 1.80 mm anterior to the Bregma, 3.00 mm lateral to the midline, and 7.00 mm deep from the brain surface, with a 14-degree angle inward bias) of the rats. The catheters were cemented in place using dental cement to affix them to three stainless steel screws fastened to the skull, and a matched stainless steel probe with a plastic cap (outer diameter 0.20, length 8.5 mm; 62104, RWD Life Science Co., Ltd., Shenzhen, Guangdong, China) was inserted into each cannula to prevent blockage and infection. Following surgery, all of the rats were immediately housed individually in feeding cages, and allowed to recover for at least 5 days. The entire experimental process was conducted during the day.

CPP apparatus

The CPP apparatus (Noldus Information Technology Co., Ltd., Beijing, China) consisted of two large compartments (30 cm × 30 cm × 40 cm) separated by a small compartment (10 cm × 30 cm × 40 cm). The two large compartments were distinguishable by distinct visual and tactile cues: one compartment had a white wall and a floor with many round holes, and the other had a black wall and a floor with many hollow longitudinal lines. The middle section contained a transitional