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Control over stress accelerates extinction of drug seeking via prefrontal cortical activation

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ABSTRACT

Extinction is a form of inhibitory learning viewed as an essential process in suppressing conditioned responses to drug cues, yet there is little information concerning experiential variables that modulate its formation. Coping factors play an instrumental role in determining how adverse life events impact the transition from casual drug use to addiction. Here we provide evidence in rat that prior exposure to controllable stress accelerates the extinction of cocaine-seeking behavior relative to uncontrollable or no stress exposure. Subsequent experimentation using high-speed optogenetic tools determined if the infralimbic region (IL) of the ventral medial prefrontal cortex mediates the impact of controllable stress on cocaine-seeking behavior. Photoinhibition of pyramidal neurons in the IL during coping behavior did not interfere with subject's ability to control the stressor, but prevented the later control-induced facilitation of extinction. These results provide strong evidence that the degree of behavioral control over adverse events, rather than adverse events *per se*, potently modulates the extinction of cocaine-seeking behavior, and that controllable stress engages prefrontal circuitry that primes future extinction learning.

1. Introduction

A core feature of addiction is the ability of drug-associated cues and contexts to evoke intense craving and compulsive drug-seeking behavior (Childress et al., 1987). Consequently, many treatment strategies are directed at reducing the expression of conditioned behaviors produced by repetitive drug use. In pre-clinical models of drug administration, conditioned drug responses can be extinguished following repeated exposure to the drug-paired environment in the absence of the drug. Despite the extensive use of extinction procedures, little is known about experiential variables that modulate the speed and strength of extinction, as well as the neural mechanisms that mediate the effects of these variables. Only a minority of individuals that use substances with addictive potential go on to develop an addictive disorder (Anthony et al., 1994), and so an understanding of circumstances that promote or mitigate the expression of drug seeking is of clinical importance (Potenza, 2014).

Accumulating evidence indicates that the infralimbic region (IL) of the ventral medial prefrontal cortex (mPFCv) is critical for the suppression of both conditioned fear and appetitive behaviors (Quirk et al., 2006; Peters et al., 2009). Following cocaine self-administration and extinction training, inactivation of the IL is sufficient to elicit cocaine-seeking in the absence of any other reinstatement trigger (Peters et al., 2008). Moreover, recent findings support involvement of the IL in learning the extinction of conditioned reward-seeking behavior as well as the retrieval of extinction memory after cocaine-seeking is extinguished (Hsu and Packard, 2008; Peters et al., 2008; LaLumiere et al., 2010, 2012; Van den Oever et al., 2013). This is noted here because recent work suggests that prior exposure to controllable (escapable shock, ES), but not physically identical uncontrollable (inescapable shock, IS), stressors potently reduce conditioned fear responses through an IL-dependent mechanism (Baratta et al., 2007, 2008). Additionally, exposure to ES *after* fear conditioning facilitates the reduction of fear responses during extinction and eliminates the spontaneous recovery of fear. If controllable stress alters the IL in such a way as to

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suppress later conditioned fear responding, then perhaps prior control would also reduce the later expression of other conditioned responses regulated by the IL.

Adverse life events are thought to be important for the transition from recreational to compulsive drug use in humans (Kosten et al., 1986; Sinha, 2008), and exposure to stressors can facilitate the acquisition of drug self-administration (Piazza et al., 1990; Goeders and Guerin, 1994; Miczek and Mutschler, 1996) and reinstate extinguished drug-seeking (Shaham and Stewart, 1995; Highfield et al., 2000; Bossert et al., 2013). Along these lines, several studies have investigated the impact of stressor controllability on reactions to drugs of abuse. Exposure to a single session of IS, but not ES, enhances the later development of morphine conditioned place preference (CPP) (Will et al., 1998). Furthermore, a prior experience of ES can block later IS-induced potentiation of morphine CPP, even when the ES-to-IS interval is as long as 8 weeks (Rozeske et al., 2012). This stress-buffering effect of ES depends on IL activity during ES.

Here we evaluate the selective impact of stress on the acquisition of extinction and whether or not the dimension of control can modulate its impact. Specifically, the present experiments determine if stressor controllability modulates the extinction of drug-seeking behavior following cocaine self-administration, and use rapid and reversible optogenetic silencing to investigate the role of the IL in mediating the effects of behavioral control.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (300–325 g at the time of testing, Institute for Behavioral Genetics, Boulder, Colorado) were kept on a 12-h reverse light/dark cycle; all experiments were conducted during the dark phase. Animals were pair-housed until catheter implantation, after which they were housed individually. Standard lab chow and water were available *ad libitum*. Procedures were performed in accordance with standard ethical guidelines (National Institutes of Health *Guide for the Care and Use of Laboratory Animals*) and were approved by the Institutional Animal Care and Use Committee at the University of Colorado Boulder.

2.2. Cocaine self-administration

Rats were anesthetized with a ketamine/xylazine mixture (100 mg/kg and 8 mg/kg, respectively, i.p.) and implanted with intrajugular catheters. All incisions were sutured, stapled, and sealed with Vetbond (3M). After surgery, catheters were flushed daily with a sterile solution of heparin (2.5 units/mL) to maintain catheter patency. Five to seven days following catheterization, animals were trained to nose poke for intravenous cocaine HCl (0.5 mg/kg/infusion; National Institute on Drug Abuse) during daily 3-h sessions (65 infusion max) over 7 days. The 65-infusion limit was chosen to prevent adverse effects of the drug. Prior to the start of each session, rats were placed into self-administration chambers ($20 \times 20 \times 26$ cm) that were equipped with two nose-poke portals located 5 cm above the floor on opposite walls. A protective cap was removed from the catheter and its external end was connected via tubing (0.02 inch inner diameter; Saint-Gobain Performance Plastics) to a single-channel liquid swivel mounted in the ceiling of the self-administration chamber. Nose pokes into one of the ports (designated active) resulted in a cocaine infusion over 5 s on a fixed-ratio 1 (FR1) schedule of reinforcement. Each reinforced response resulted in a 20-s timeout period, during which time the active port was illuminated with a white LED positioned at the back of the port. Nose pokes made in the other port (designated inactive) were recorded but had no programmed consequence. At the end of the 7 days of self-administration training, the criteria for achieving stable responding were a) minimum of 30 reinforcers earned; b) preference for the active port greater than 70%; and c) no more than 20% variation (both number of reinforcers and active port preference) for at least two of the final three self-administration sessions.

2.3. Stressor controllability

For manipulation of controllability, subjects were run in a triad design. One subject of each triad received ES, a second received yoked IS, and third received no tailshock (home cage control, HC). Each rat was placed in a Plexiglas box ($14 \times 11 \times 17$ cm) with a wheel mounted in the front of the box. The tail was secured to a Plexiglas rod extending from the back of the box, and affixed with two copper electrodes and electrode paste (Parker Laboratories). Each tailshock session consisted of 80 trials of tailshock (27×1.0 mA, 27×1.3 mA, 26×1.6 mA) on a variable interval 60-s schedule (range = 45–90 s). As in prior studies (Baratta et al., 2007), the following procedure was used to insure that the ES rat learned the operant response to terminate the tail shock. Initially, the shock was terminated by a one-quarter turn of the wheel. The response requirements were increased by a one-quarter turn when three consecutive trials were completed in less than 5 s. Subsequent latencies under 5 s increased the requirement by 50% up to a maximum of four full turns. The requirement was reduced if the trial was not completed in less than 5 s. If the requirement was not reached in less than 30 s, the shock was terminated and the requirement was reduced to one-quarter turn of the wheel. An additional rationale for this procedure is that it maintains shocks at durations sufficient to produce the usual behavioral effects of IS in the yoked IS subject. Rats in the IS group received an *identical* amount of shock but had no control over its termination.

2.4. Extinction of cocaine-seeking behavior

Extinction was conducted in 3-h daily sessions, during which a nose poke in the active port resulted in a 20 s presentation of the white LED, but no cocaine delivery. Nose pokes made in the inactive port had no programmed consequences. Extinction sessions continued for each subject until the number of previously reinforced responses (active port) in a given session was <20% of extinction day 1 levels.

2.5. Viral vectors

Adeno-associated virus (AAV) vector was used to genetically target third-generation halorhodopsin (NpHR), a light-activated chloride pump, to IL pyramidal neurons. CaMKIIα::NpHR-eYFP and CaMKIIα::eYFP cassettes were packaged in AAV vectors serotyped with AAV5 coat proteins (titers: $3.0 - 6.0 \times 10^{12}$ genome copies/mL) by the Vector Core at the University of North Carolina at Chapel Hill.

2.6. Viral delivery and optical fiber implantation

For optogenetic experimentation, rats were anesthetized using a ketamine/xylazine mixture and a stainless steel needle with beveled tip (31 gauge; Hamilton Company) was directed to the IL (+2.5 mm anterior to bregma, ±0.5 mm lateral to the midline, −3.2 mm ventral to the cortical surface), and virus (1.0 μL/hemisphere) was infused over 10 min (0.1 μL/min) followed by an additional 10 min to allow for diffusion. After 2–3 weeks, animals were anesthetized and implanted with an intravenous catheter (as described above) into the right jugular and optical fibers (200 μm diameter core, 0.39 NA, approximately 1.0 mm center-to-center distance) into the bilateral IL