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RENEWAL OF EXTINGUISHED COCAINE-SEEKING

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Abstract—Rats were trained to self-administer cocaine in a distinctive context (context A). They were then extinguished in a second context (context B) prior to test for cocaine-seeking in the original training context, context A (group ABA), context B (group ABB) or no test (group AB0). Group ABA showed renewal of extinguished cocaine-seeking associated with c-Fos induction in basolateral amygdala, lateral hypothalamus, and infralimbic prefrontal cortex. Groups ABA and ABB showed test-associated c-Fos induction in prelimbic prefrontal cortex, nucleus accumbens (core, shell, rostral pole), striatum, lateral amygdala, perifornical hypothalamus, and ventral tegmental area. Double immunofluorescence revealed that renewal-associated c-Fos was expressed in orexin-negative lateral hypothalamic neurons whereas test-associated c-Fos was expressed in orexin-positive perifornical hypothalamic neurons. Retrograde tracing from lateral hypothalamus with cholera toxin revealed only sparse dual-labeled neurons in basolateral amygdala and infralimbic prefrontal cortex, suggesting that these regions contribute to renewal of cocaine-seeking independently of their projections to lateral hypothalamus. Retrograde tracing from the ventral tegmental area suggested that hypothalamic contributions to cocaine-seeking are likewise independent of projections to the midbrain. These results suggest that renewal of cocaine-seeking depends critically on basolateral amygdala, lateral hypothalamus, and infralimbic prefrontal cortex. Whereas basolateral amygdala and lateral hypothalamus contributions may be common to renewal of extinguished cocaine-, alcohol-, and sucrose-seeking, infralimbic prefrontal cortex contributions appear unique to renewal of cocaine-seeking and may reflect the habitual nature of relapse to cocaine.

Key words: relapse, orexin, reinstatement, cocaine, rat, tracing.

The contexts where drugs are self-administered play an important role in regulating persistent drug-taking and in relapse to such taking after periods of abstinence. The role of contexts is best exemplified by the phenomenon of "renewal." Renewal is the term used to describe recovery of extinguished behavior with a change in context (Bouton and Bolles, 1979). Typically, subjects are trained to seek a reward in one context, context A. This is then extinguished in a second context, context B. When tested in context B, reward-seeking is low (i.e. it has been extinguished). However, when tested in context A reward-seeking returns (i.e. it has been renewed). Renewal has been demonstrated for instrumental responding based on numerous reinforcers, including natural rewards such as food pellets (Nakajima et al., 2000) or liquid sucrose (Hamlin et al., 2006) as well as drug rewards such as alcohol (Hamlin et al., 2007; Zironi et al., 2006), cocaine (Crombag et al., 2002; Fuchs et al., 2005), heroin (Bossert et al., 2004), and a heroin–cocaine mixture (speedball) (Crombag and Shaham, 2002). Renewal shows that extinction is context-specific and that it is lost with a change in context between extinction and test. It is evidence that extinction is not simply erasure of drug-seeking, but additionally involves imposition of a mask on drug-seeking which reduces drug-seeking in the extinction context but not elsewhere (Bouton, 2002, 2004). Removal of this mask by context change is a source of relapse after behavior change (Bouton, 2002; Conklin and Tiffany, 2002).

The neural mechanisms mediating renewal of extinguished drug-seeking remain only poorly understood. Dorsomedial prefrontal cortex, basolateral amygdala (BLA), and dorsal hippocampus have all been implicated in renewal of extinguished cocaine-seeking because infusions of tetrodotoxin into each of these structures prior to testing for ABA renewal prevents the recovery of extinguished cocaine-seeking (Fuchs et al., 2005). Recent data suggest that serial interactions between BLA and dorsal hippocampus may be important for renewal of extinguished cocaine-seeking (Fuchs et al., 2007). The actions of dopamine are also critical to renewal of extinguished cocaine-seeking (Crombag et al., 2002). Ventral tegmental area (VTA) has been implicated in renewal of extinguished heroin-seeking (Bossert et al., 2004) but its involvement in renewal of cocaine-seeking is unknown. Likewise glutamate actions, including in nucleus accumbens, are important for renewal of extinguished heroin- (Bossert et al., 2006a) and sucrose-seeking (Bossert et al., 2006b) but their involvement in renewal of cocaine-seeking is less well understood. More generally, it is unclear from the available evidence whether common neural mechanisms mediate renewal to seeking different rewards (e.g. cocaine, alcohol, heroin, sucrose), or whether there are distinct neural mechanisms for renewal depending on the specific reward.

The experiments reported here had three aims. The first was to use c-Fos immunohistochemistry to identify the neural correlates of renewal of extinguished cocaine-seeking. We have used this approach to identify the neural correlates of renewal of extinguished alcohol- (Hamlin

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Abbreviations: BLA, basolateral amygdala; CTb, cholera toxin, B subunit; DMH, dorsomedial hypothalamus; f, fornix; ilPFC, infralimbic region, prefrontal cortex; IR, immunoreactive/immunoreactivity; LH, lateral hypothalamus; mt, mammillothalamic tract; NHS, normal horse serum; PB, phosphate buffer; PBS, phosphate buffer saline; PBT-X, phosphate buffer with Triton-X 10; PeF, perifornical hypothalamus; TH, tyrosine hydroxylase; VTA, ventral tegmental area; 3V, third ventricle.

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et al., 2007) and sucrose-seeking (Hamlin et al., 2006). Identification of the neural correlates of renewal of extinguished cocaine-seeking would permit comparison with our recent results and provide important insights into whether there are common or unique correlates for renewal of seeking different rewards. In those studies, ABA renewal of extinguished alcohol- and sucrose-seeking was associated with significant induction of c-Fos protein in BLA, nucleus accumbens shell, and lateral hypothalamus (LH). c-Fos induction was elevated in several other structures including medial prefrontal cortex and nucleus accumbens core, but it was also elevated in these structures among rats tested in the extinction context (group ABB), suggesting that these structures may be necessary but not sufficient for renewal of extinguished reward-seeking. Neisewander et al. (2000) showed that re-exposure to a cocaine-associated context after forced abstinence in a second, different context, was associated with significant c-Fos protein induction in the BLA, hippocampal CA1 region, dentate gyrus, nucleus accumbens shell and core, and anterior cingulate. However, because this study employed forced abstinence rather than extinction of drug-seeking, it is unclear which, if any, of these correlates would also be associated with renewal of extinguished cocaine-seeking.

The second aim of the experiments reported here was to study the possible role of hypothalamic orexin neurons in renewal of extinguished cocaine-seeking by studying their recruitment during renewal. Recent evidence implicates orexin in multiple aspects of reward processing and seeking. For example, exposure to food, opiate, psychostimulant, or ethanol-related contexts or stimuli induces c-Fos in hypothalamic orexin neurons (Harris et al., 2005; Dayas et al., 2007; Hamlin et al., 2007). The midbrain VTA is one efferent of hypothalamic orexin neurons and orexin actions there contribute to synaptic and behavioral responsiveness to psychostimulants and opiates (Borgland et al., 2006; Narita et al., 2006). Importantly, orexin has been implicated in other forms of recovery of extinguished drug-seeking. Systemic injections of orexin receptor antagonist SB-334867 prevent cue-induced reinstatement of extinguished alcohol-seeking (Lawrence et al., 2006) and stress-induced reinstatement of extinguished cocaine-seeking (Boutrel et al., 2005). However, to date only a single study has assessed c-Fos induction in hypothalamic orexin neurons during renewal of extinguished drug-seeking and this was based on an alcohol reinforcer (Hamlin et al., 2007). There are no available data on the recruitment of orexin neurons during renewal of extinguished cocaine-seeking.

The final aim of these experiments was to combine c-Fos immunohistochemistry with retrograde tracing to begin to identify possible neuroanatomical pathways recruited during renewal of extinguished cocaine-seeking. There has been little attention to date on identifying the pathways, as opposed to structures, implicated in renewal of extinguished reward-seeking (but see Fuchs et al., 2007), and to the best of our knowledge, there have been no studies combining tract tracing with activity marker expression during recovery of extinguished drug-seeking. The focus here was on determining which, if any, forebrain afferents to LH or VTA were recruited, as indexed by c-Fos induction, during renewal of extinguished cocaine-seeking. We focused on afferents to VTA and LH because recent research has suggested an important role for LH in mediating the behavioral effects of drug-associated contexts (Harris et al., 2006) and the VTA has been implicated in renewal of extinguished opiate-seeking (Bossert et al., 2004).

To achieve these aims we trained rats to self-administer i.v. cocaine in one context, context A. Cocaine-seeking was then extinguished in a second context, context B. On test in the first experiment, rats were placed in either context B (group ABB) or context A (group ABA). An additional control group was included: group AB0. Group AB0 received identical training and extinction as groups ABA and ABB but they were never tested. Group AB0 therefore controls for several variables (e.g. cocaine history; training history; transport; handling etc) which may confound interpretation of patterns of c-Fos induction. In the second experiment we employed only group ABA and applied the tracer cholera toxin B subunit (CTb) to either the LH or the VTA prior to cocaine self-administration training in context A. Rats were subsequently extinguished in context B, and tested for renewal of cocaine-seeking in context A.

EXPERIMENTAL PROCEDURES

Subjects

Subjects were experimentally naive male Long-Evans rats (250–350 g) obtained from a commercial supplier (Monash Animal Services, Gippsland, Victoria, Australia). After arrival and prior to surgery, rats were housed in groups of eight in plastic cages maintained on a reverse 12-h light/dark cycle (lights on at 19:00 h). After surgery, rats were housed individually in plastic cages maintained on a reverse 12-h reverse light/dark cycle (lights on at 19:00 h). Food and water were freely available prior to surgery and during recovery. During sucrose training rats were allowed 1 h access to food and water following daily training sessions. At the conclusion of the first day of cocaine training, water was freely available and food was restricted to 1 h per day. The experiments were designed to minimize the number of animals used and their suffering. The procedures were approved by the Animal Care and Ethics Committee at the University of New South Wales and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

Surgery

Rats were injected intraperitoneally with 1.3 ml/kg of the anesthetic ketamine (Ketapex; Apex Laboratories, Sydney, Australia) at a concentration of 100 mg/ml and with 0.3 ml/kg of the muscle relaxant xylazine (Rompun; Bayer, Sydney, NSW, Australia) at a concentration of 20 mg/ml followed by 0.1 ml of 50 mg/ml carprofen administered s.c. as a preoperative analgesic. Surgery was conducted to implant a chronic indwelling catheter into the right jugular vein, terminating proximal to the right atrium of the heart and secured with sutures. Catheters consisted of 100 mm of Tygon Micro Bore tubing (ID 0.02 in., OD 0.06 in., Small Parts, FL, USA) attached to a back-mount cannula connector pedestal (Plastics One, VA, USA) secured in place with sutures. Catheters were