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Incubation of cocaine-craving relates to glutamate over-flow within ventromedial prefrontal cortex

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ABSTRACT

Craving elicited by drug-associated cues intensifies across protracted drug abstinence – a phenomenon termed "incubation of craving" – and drug-craving in human addicts correlates with frontal cortical hyperactivity. Herein, we employed a rat model of cue-elicited cocaine-craving to test the hypothesis that the time-dependent incubation of cue-elicited cocaine-craving is associated with adaptations in dopamine and glutamate neurotransmission within the ventromedial prefrontal cortex (vmPFC). Rats were trained to self-administer intravenous cocaine (6 h/day × 10 days) and underwent *in vivo* microdialysis procedures during 2 h-tests for cue-elicited cocaine-craving at either 3 or 30 days withdrawal. Controls rats were trained to either self-administer sucrose pellets or received no primary reinforcer. Cocaine-seeking rats exhibited a withdrawal-dependent increase and decrease, respectively, in cue-elicited glutamate and dopamine release. These patterns of neurochemical change were not observed in either control condition. Thus, cue-hypersensitivity of vmPFC glutamate terminals is a biochemical correlate of incubated cocaine-craving that may stem from dopamine dysregulation in this region.

1. Introduction

Cocaine addiction is a chronic relapsing disorder, characterized by a high propensity for relapse even during protracted abstinence. Re-exposure to drug-associated cues and contexts are known to trigger drug-craving and can even promote relapse in humans (Childress et al., 1999; Volkow et al., 1999). Following a history of drug-taking, the capacity of drug-associated cues to elicit craving in abstinent humans and operant responding (or drug-seeking) in drug-withdrawn laboratory animals intensifies with the passage of time during abstinence/withdrawal (e.g., Gawin and Kleber, 1986; Grimm et al., 2001). This phenomenon, termed the "incubation of craving", is theorized to render addicts highly susceptible to cue-elicited relapse, even following prolonged periods of abstinence (Grimm et al., 2001). In rodent models of incubated drug-craving, the amount of operant-responding for drug-associated cues reinforced by the presentation of those cues progressively increases over the first few months into drug withdrawal and can persist at high levels for up to six months following cessation of drug-taking (e.g., Grimm et al., 2001; Kerstetter et al., 2008). As such, incubation of craving currently serves as a model with which to study the time-dependent, as well as enduring, changes in the brain that underpin persistently high levels of drug craving that is a hallmark feature of addiction.

In rodent models, re-exposure to drug-predictive cues increase the firing rate of prelimbic neurons (e.g., Rebec and Sun, 2005; West et al., 2014) and both neuropharmacological and optogenetic inactivation of the ventromedial aspect of the PFC (vmPFC) attenuates the heightened level of cue-reinforced responding exhibited during protracted withdrawal in cocaine-experienced rats (Koya et al., 2009; Ma et al., 2014). We know from prior work that basal and cocaine-elicited changes in extracellular dopamine and glutamate levels are dysregulated within the medial PFC of rats with a chronic history of cocaine-taking, at least during early (24 h) withdrawal (Ben-Shahar et al., 2012). Yet, to the best of our knowledge, no study to date has investigated the neurochemical anomalies within vmPFC that accompany incubated drug-craving

Abbreviations: Cue test, 2-h test for cue-elicited responding; mPFC, medial prefrontal cortex; NAC, nucleus accumbens; vmPFC, ventromedial prefrontal cortex; WD, withdrawal day.

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in highly cocaine-experienced rats. This being said, incubated cocaine-craving is accompanied by increased levels of the dopamine transporter (DAT) within vmPFC (Grimm et al., 2002; McIntosh et al., 2013), which has been interpreted as reflecting a compensatory response to heightened drug-elicited and/or cue-elicited dopamine release within this region. Furthermore, an intra-medial PFC infusion of dopamine D1 receptor antagonists attenuates cue-induced reinstatement of an extinguished operant response in cocaine-experienced rats, arguing an important role for dopamine-dependent signaling within this region in cue-elicited cocaine-seeking (Ciccocioppo et al., 2001). Interestingly, although re-exposure to cues previously associated with sucrose-taking also elicits cue-reinforced operant responding that can incubate with the passage of time an animal remains sucrose-free (e.g., Grimm et al., 2002), incubated sucrose-seeking is not accompanied by changes in DAT expression within PFC (Grimm et al., 2002). Thus, cocaine appears to elicit changes in dopamine terminals within vmPFC that are distinct from those produced by a non-drug reinforcer. As such, the present study compared the capacity of cocaine- versus sucrose-paired cues to elicit a rise in extracellular dopamine within the vmPFC during early and later withdrawal and their relation to cue-reinforced responding.

Incubated cocaine-craving has also been correlated with changes in the expression of glutamate receptor-related proteins, as well as increased activation of downstream effectors within this subregion (Ben-Shahar et al., 2013; Gould et al., 2015; Koya et al., 2009), suggestive of a hyper-glutamatergic state. While not yet assayed within PFC, the presentation of drug-associated cues after chronic cocaine-taking elicits a rise in extracellular glutamate within both the cell body and terminal regions of the mesolimbic dopamine system, with extracellular glutamate levels varying bi-directionally as a function of cue availability (Suto et al., 2010, 2013; You et al., 2007). Based on the results of neuropharmacological inactivation studies conducted within the confines of the traditional extinction-reinstatement model of drug-seeking, the source of the glutamate regulated within the nucleus accumbens (NAC) by drug-associated cues is likely the PFC (c.f., Kalivas and Volkow, 2005). Given that the activity of corticoaccumbal glutamate projections, and subsequent glutamate release within the NAC, is driven by the activation of somatodendritic glutamate receptors, we hypothesized that the incubation of cue-elicited cocaine-, and perhaps also sucrose-seeking, might reflect heightened glutamate release within the vmPFC.

Using *in vivo* microdialysis procedures, we examined for changes in extracellular levels of glutamate and dopamine within the vmPFC during cue-elicited responding at early versus later withdrawal from a history of excessive cocaine-taking. To determine the reinforcer-specificity of our observed effects, parallel studies were conducted in animals with a history of sucrose-pellet self-administration or in animals allowed to respond for the presentation of neutral cues in the absence of any primary reinforcer.

2. Materials and methods

2.1. Subjects, lever-response training, and surgery

All procedures were approved by the Institutional Animal Care and Use Committee of the University of California Santa Barbara and were consistent with the guidelines of the NIH *Guide for Care and Use of Laboratory Animals*. Male Sprague–Dawley rats (275–325 g; Charles River Laboratories, Hollister, CA) were pair-housed under standard reverse light-cycle conditions (lights off: 0700 h), with *ad libitum* food/water except during lever-response training, during which food was restricted (16 g/day), 24 h prior to 16-h overnight operant sessions (FR1 schedule of reinforcement; 45 mg food pellet; Bio Serv, Frenchtown, NJ; acquisition criterion = 100 responses on the active lever/session). Self-administration training was conducted in standard 2-lever operant chambers (Med Associates Inc., St. Albans, VT). Under ketamine/xylazine anesthesia (respectively 56.25 and 7.5 mg/kg, IM; 2 mg/kg banamine analgesic, SC, for post-operative pain), animals were implanted with a unilateral microdialysis guide cannula (20-gauge; 8 mm long; Synaptech, Marquette, MI) aimed 2 mm above the vmPFC (AP: +3.0; ML ± 0.75; DV: −3.0, in mm from Bregma), with the placement counterbalanced across hemisphere within each group. Animals slated to self-administer cocaine were also implanted with a chronic indwelling jugular catheter as described previously by our group (see Ben-Shahar et al., 2013; Kerstetter et al., 2008). A minimum of 4 days was allowed for recovery, with jugular catheter patency maintained by daily flushing of sterile heparin/timentin/saline (60 IU/ml and 100 mg/ml, respectively; vol = 0.1 ml) and confirmed weekly by intravenous infusion of 5 mg/kg brevital (JHP Pharmaceuticals, Parsippany, NJ).

2.2. Self-administration and in vivo microdialysis during cue-testing procedures

Animals were trained to lever-press under an FI20 schedule of reinforcement for intravenous cocaine (0.25 mg in 0.1 ml saline infusion; NIDA, Bethesda, MD) or a 45 mg sucrose pellet (Bio Serv), with delivery of either reinforcer signaled by a 20-s light-tone compound stimulus. For control rats, active lever-presses resulted in the light-tone stimulus only. Depression of the "inactive lever" had no programmed consequences for any group. During training of the initial cohorts of rats, cocaine animals received an average of 102 reinforcer-stimulus pairings/6-h session. Thus, the total maximum number of reinforcer-stimulus pairings earned by sucrose-trained animals was capped at 102 to equate associative learning across groups. On average, sucrose-trained animals earned 102 reinforcers within 3 h. Thus, control rats were permitted to respond for the neutral cues for 3 h/day. Animals were trained under the above conditions once daily across 10 days, and were then left undisturbed in their home cages for either 3 or 30 days, at which time *in vivo* microdialysis procedures were conducted (e.g., Ben-Shahar et al., 2012) during a 2-h test for cue-elicited responding (Cue Test). For these Cue Tests, active lever-presses resulted in presentation of the light-tone stimulus only. A minimum of 4 h prior to the Cue Test, a microdialysis probe (8 mm long with 2 mm membrane; Synaptech) was inserted into the guide cannula, the animals were placed into their operant chamber with levers retracted and house lights off, and probes were perfused with artificial cerebral spinal fluid (2.0 μl/min; see Ben-Shahar et al., 2012). Dialysate collection occurred, in 20-min intervals, for 1 h prior to the Cue Test and then throughout the duration of the 2-h Cue Test session. 10 μl of preservative (4.76 mM citric acid, 150 mM NaH_2PO_4, 50 μM EDTA, 3 mM sodium dodecyl sulfate, 10% methanol (v/v), 15% acetonitrile (v/v), pH 5.6) was added into each dialysate sample to minimize the oxidation of dopamine. Immediately upon collection, the dialysate sample was stored at −80 °C until assay. Upon completion of the Cue Test, probes were removed, animals were anesthetized with 4% isoflurane, brains extracted and then drop-fixed in 4% paraformaldehyde for later determination of probe placement within the PFC by standard histological methods. Only data from rats exhibiting probe placement within the boundaries of the vmPFC (prelimbic and/or infralimbic subregions) were employed in the statistical analyses of the data. Dialysate content of dopamine (27 μl) and glutamate (20 μl) was determined for each sample using high pressure liquid chromatography with electrochemical detection as described previously (Ben-Shahar et al., 2012).