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Aversive Stimuli Drive Drug Seeking in a State of Low Dopamine Tone

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ABSTRACT

BACKGROUND: Stressors negatively impact emotional state and drive drug seeking, in part, by modulating the activity of the mesolimbic dopamine system. Unfortunately, the rapid regulation of dopamine signaling by the aversive stimuli that cause drug seeking is not well characterized. In a series of experiments, we scrutinized the subsecond regulation of dopamine signaling by the aversive stimulus, quinine, and tested its ability to cause cocaine seeking. Additionally, we examined the midbrain regulation of both dopamine signaling and cocaine seeking by the stress-sensitive peptide, corticotropin releasing factor (CRF).

METHODS: Combining fast-scan cyclic voltammetry with behavioral pharmacology, we examined the effect of intraoral quinine administration on nucleus accumbens dopamine signaling and hedonic expression in 21 male Sprague-Dawley rats. We tested the role of CRF in modulating aversion-induced changes in dopamine concentration and cocaine seeking by bilaterally infusing the CRF antagonist, CP-376395, into the ventral tegmental area (VTA).

RESULTS: We found that quinine rapidly reduced dopamine signaling on two distinct time scales. We determined that CRF acted in the VTA to mediate this reduction on only one of these time scales. Further, we found that the reduction of dopamine tone and quinine-induced cocaine seeking were eliminated by blocking the actions of CRF in the VTA during the experience of the aversive stimulus.

CONCLUSIONS: These data demonstrate that stress-induced drug seeking can occur in a terminal environment of low dopamine tone that is dependent on a CRF-induced decrease in midbrain dopamine activity.

Keywords: Addiction, Cocaine, Dopamine, Relapse, Stress, Voltammetry

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Stressful life events are potent modulators of mood and can trigger a variety of destructive behaviors, including drug abuse (1). While addiction is a multifaceted disorder, it has been suggested that aversive life events can promote relapse in addicts by inducing negative affect and craving (2–5). Likewise, drug-associated stimuli evoke a negative affective state in abstinent cocaine users that is predictive of relapse (2,4,6). Ultimately these stimuli are thought to promote a spiral of maladaptive behaviors in which substance abusers, attempting to remain abstinent, are prompted to correct an environmentally induced negative affective state through the resumption of drug use (7–11).

Aversive events and their attendant emotional states most likely drive drug seeking by impinging upon the mesolimbic dopamine system, but the manner by which they do this is poorly understood. In fact, while the evidence is mounting that negative affect is a critical determinant of the resumption of drug taking following periods of abstinence, the literature is conflicted on the basic question of the directionality of the dopamine response to aversive stimuli (12,13). Electrophysiological and electrochemical studies that measure dopamine neuron activity and terminal dopamine release, respectively, commensurate with the immediate sensation and perception of aversive stimuli routinely characterize rapid reductions in dopamine signaling in response to aversive stimuli and their predictors (14–19). This reduction in dopaminergic activity is reportedly induced, in part, by stress-sensitive neuromodulators such as corticotropin-releasing factor (CRF) (20,21). Unfortunately, electrophysiological recordings of dopamine neurons indicate that neither the aversion-induced decrease in dopamine neuron activity nor the CRF regulation of that response is uniform (22–25), necessitating an approach that examines rapid terminal signaling in dopamine neuronal projection targets.

Little is known about the nature of rapid, aversion-induced dopamine release patterns in relevant terminal regions. It is unclear how such stimuli could cause reductions in dopamine signaling and how decreased dopamine may promote stress-mediated maladaptive behaviors, like drug seeking. In the nucleus accumbens (NAc), a critical locus of the reward circuit, increases and decreases in dopamine concentration selectively activate D1- and D2-receptor–expressing medium spiny neurons (MSNs), respectively, which have opposing effects on motivated behavior (26,27). Activation of these distinct circuits has long been known to differentially regulate a diverse array of motivated behaviors, including responses to drugs of abuse (28–33). Therefore, characterizing whether aversive stimuli increase or decrease NAc dopamine concentration is likely

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essential to determining how stressful life events activate specific striatal circuitry to cause relapse to drug use. Previously, we observed that cocaine-predictive stimuli can induce a negative affective state, while simultaneously reducing dopamine signaling in the NAc (19). However, the behavioral impact of either of these observations remains to be tested. Critical questions of how aversive stimuli negatively regulate dopamine signaling and whether this mechanism is one that can lead to drug-seeking-like behaviors in rodents must be addressed. In these studies, we scrutinized the precise temporal dynamics of aversion-induced reductions in dopamine signaling, the regulation by stress-induced CRF release into the ventral tegmental area (VTA), and the behavioral impact on hedonic processing and drug seeking. Overall, our findings reveal temporal complexity in dopamine signaling and the ability of CRF to regulate dopamine tone and promote drug seeking.

METHODS AND MATERIALS

Subjects

Twenty-one male Sprague-Dawley rats (275–300 g; Harlan Laboratories, St. Louis, Missouri) were individually housed in a temperature- and humidity-controlled, Association for Assessment and Accreditation of Laboratory Animal Care accredited vivarium. Rats were maintained on a 12/12-hour reversed cycle (lights off at 7 AM) and had ad libitum access (unless otherwise noted) to water and food (Teklad; Harlan Laboratories). All experimental protocols were approved by the Institutional Animal Care and Use Committee at Marquette University in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Surgery

All surgical procedures were conducted under ketamine/xylazine (100 mg/kg/20 mg/kg, intraperitoneal) anesthesia. Intraoral and intrajugular catheter implantations were conducted as previously described (11). Guide cannulas for microinjections (26-gauge; Plastics One, Roanoke, Virginia) were implanted bilaterally immediately above the VTA (anterior-posterior: −5.6; medial-lateral: ±2.2 at 11° angle; dorsal-ventral: −7.0). To prepare for voltammetric recordings, electrode guide cannulas were implanted above the NAc shell unilaterally (anterior-posterior: +1.3; medial-lateral: ±1.3), and a silver/silver chloride reference electrode was placed contralateral to the guide cannula. Additionally, a combined bipolar stimulating electrode/microinjection guide cannula (Plastics One) was placed immediately above the ipsilateral VTA, and a guide cannula was placed above the contralateral VTA. For all surgical procedures, rats were treated with the anti-inflammatory med-cam (1% oral suspension) the day of and for 2 days following the surgery to reduce inflammation and postoperative pain. To maintain patency, the intraoral and intrajugular catheters were flushed daily with distilled water (intraoral) or heparinized saline and the antibiotic cephazolin (intravenous [IV]), respectively.

Microinjections

Microinjectors extended .5 mm from the end of the guide cannula. Artificial cerebrospinal fluid (aCSF) (.3 μL/min) or the selective CRF receptor antagonist CP-376395 (.3 μg/.3 μL/min) was bilaterally injected into the VTA (n = 6 aCSF, n = 6 CP-376395). CP-376395 is a selective CRF-R1 antagonist, but interactions with R2 are likely at this dose. Microinjectors were left in place for 2 minutes after the injection to allow for diffusion. In both procedures, quinine delivery was (re)initiated immediately after the injection.

Voltammetric Recordings

After recovering from surgery, rats were habituated for 2 hours in the voltammetric recording environment, consisting of a clear Plexiglas chamber (Med Associates, St. Albans, Vermont) housed in a custom-designed Faraday cage. The VTA stimulating electrode was harnessed to a rotating commutator (Crist Instrument Co., Hagerstown, Maryland), and one intraoral cannula was harnessed to a fluid swivel (Instech Laboratory, Plymouth Meeting, Pennsylvania) that could receive fluid from a syringe pump (Razel, St. Albans, Vermont). On the following day, voltammetric recordings were conducted as previously described (16). Details of the recording procedure and analysis are described in Supplement 1. Briefly, a carbon fiber electrode was lowered into the NAc shell, a fluid line was attached to the intraoral cannula, and the behavioral session was initiated. The experiment consisted of a 30-minute baseline dopamine monitoring phase (phase 1); a 30-minute quinine delivery period (phase 2); bilateral VTA microinjections; and a 50-minute postinjection quinine delivery period (phase 3). Throughout the quinine delivery phases, a 6-second infusion of .2 mL quinine (.001 mmol/L) was delivered approximately every minute.

Voltammetry Data Analysis

Analyte identification details are described in Supplement 1. Data from each trial (−20 sec before and 30 sec postinfusion onset) were background subtracted using a 1-second block at the local minima in the 20 seconds before infusion onset. For each rat, data were averaged across the quinine infusion trials in the 10 seconds following the initiation of the quinine infusion period (quinine) compared with the previous 10-second period (prequinine) and the next 10-second period (postquinine). The resultant current changes over time were analyzed for dopamine changes using principle component regression. For all rats (n = 12), reductions in naturally occurring (non-time-locked) dopamine tone were quantified and analyzed by comparing the first 5 trials (early) with trials 11 to 15 (middle) and the last 5 trials (late) in the prequinine period, 10 seconds before quinine infusion, using a repeated measures analysis of variance (ANOVA). Significant changes in dopamine concentration over time, time-locked to the quinine infusion, were evaluated using two within-subjects repeated measures ANOVAs varying phase (baseline, quinine, and quinine + drug [aCSF or CP-376395]) × period (prequinine, quinine, postquinine). When significant main or interactive effects were detected, all pairwise comparisons were made with Tukey's post hoc tests for multiple comparisons with alpha set at .05.

Dopamine release events occurred independent of any applied stimuli or experimenter controlled behavioral action in the baseline period. To determine how aversive stimuli affected the likelihood of high concentration dopamine release