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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

events, every 100-msec sample on every trial for each rat was time-stamped if its concentration was 40 nmol/L or higher. This threshold is within the range of affinities for high-affinity D1 receptors and is the approximate average value of spontaneous dopamine release events (34,35). From this characterization, transient frequency and amplitude were quantified and analyzed. A two-way ANOVA was used to identify main effects of period (quinine versus postquinine) and drug (aCSF vs. CP-376395). Tukey's post hoc tests for multiple comparisons were used to identify significant differences within period and drug. In all cases, the alpha level for significance was .05. Statistical comparisons were made using commercially available software (Statistica; StatSoft, Tulsa, Oklahoma).

Taste Reactivity Data Analysis

Taste reactivity was analyzed in a frame-by-frame analysis using digital video recorded on the test day in aCSF- and CP-376395-injected rats ($n = 5$ in each group). Appetitive and aversive taste reactivity was counted in the prequinine and quinine periods using the technique of Grill and Norgren (36). Mouth movements that matched the triangle shape for a duration exceeding 90 msec were counted as aversive. These criteria excluded all neutral and ingestive mouth movements, which were counted separately. Instances in which the tongue protruded and crossed the midline were counted as appetitive. The remaining licking behavior was counted as neutral licking. Statistical analyses of all behavioral data were performed using commercially available software (Statistica).

Self-Administration and Reinstatement

Mildly food-restricted rats (15–18 g/day) were trained to press a lever for sucrose pellets. Upon acquisition of lever pressing (~3–5 days), intraoral and intravenous catheters were implanted as described above. After recovery, rats were food restricted again and trained to self-administer cocaine (.3 mg/.2 mL/infusion, IV) on a fixed-ratio 1 schedule in computer-interfaced operant conditioning chambers enclosed in sound-attenuating cubicles (Med Associates). When the cocaine session began, a house light illuminated the chamber, and a cue light located above the active lever signaled cocaine availability. Each cocaine infusion was accompanied by turning off the house light and cue light, and a time-out period lasting 20 sec, during which the lever remained extended and responses were recorded but yielded no reinforcement. Responding on a second inactive lever was also recorded. After the time-out period, the house light and cue light were turned on and signaled cocaine availability. Self-administration sessions occurred in a series of four experimenter-controlled 6-day cycles consisting of 3 days of cocaine self-administration and 3 days without cocaine in the home cage. After the third cycle, all rats received VTA cannulation surgery and began their fourth cycle after 2 weeks of recovery. Each daily cocaine session ended when rats achieved a fixed maximum number of cocaine infusions (25 infusions for the first 9 days of access before VTA cannulation and 30 infusions for the last three cocaine sessions following VTA cannulation). Extinction consisted of daily 2-hour sessions during which each lever press resulted in a saline infusion but no cue light signaling or cocaine delivery. Once the extinction criterion was met (<15 active lever responses for the terminal 2-day average; Table S1 in Supplement 1), each rat was tested for quinine-induced reinstatement. To prevent the potential confound of spontaneous recovery, reinstatement testing was conducted for each animal the day after extinction criteria were met. Before each reinstatement session, rats received intra-VTA microinjections of aCSF ($n = 4$) or CP-376395 ($n = 5$). Reinstatement sessions began with 15 intraoral infusions of quinine delivered in the cocaine self-administration chamber in the same manner as in the previous experiment for 15 minutes. Five minutes after quinine delivery, the levers were extended and responses were recorded for 1 hour.

Reinstatement Data Analysis

Changes in lever pressing behavior in the first hour of each session were analyzed using a two-way ANOVA varying the between-subjects factor of drug (aCSF, CP-376395) × the within-subjects factor of day (extinction, reinstatement, posttest). Extinction responding was defined as the last day of extinction training, and posttest responding was a final session tested under extinction conditions without quinine administration. Significant differences in drug-seeking behavior were identified when appropriate by Tukey's post hoc tests for multiple comparisons with alpha set at .05.

Histology

After the completion of experimental procedures, all subjects were euthanized with carbon dioxide. To verify placement of recording electrodes, small electrolytic lesions were created by running a current (250 μA) through a stainless steel electrode placed at the depth at which the recording took place. Brains were then removed and submerged in 10% formaldehyde for 14 days. They were then sliced into 40-μm sections, mounted, stained with .25% thionin, and coverslipped. Depictions of the cannula and electrode placements from the voltammetry and reinstatement experiments are presented in Figures S1 and S2 in Supplement 1, respectively (37).

RESULTS

To probe the temporal dynamics of dopamine reductions triggered by aversive events, we employed fast-scan cyclic voltammetry in freely moving rats exposed to brief intraoral infusions of the unpalatable, bitter taste of quinine. This design allows for the simultaneous monitoring of an animal's affective response coincident with the assessment of terminal dopamine release in the NAc on a subsecond time frame (16,19). As expected, across the 30-minute test session (1 infusion/min), quinine exposure evoked the expression of aversive taste reactivity time-locked to reductions in terminal dopamine release events (Figure 1B; phase 2, prequinine compared with quinine/postquinine periods, left and right; Figure S3 in Supplement 1). Intriguingly, dopamine reductions displayed two discrete temporal signatures: an immediately apparent, transient drop during each exposure to quinine, as well as a longer-lasting reduction in naturally occurring dopamine tone that emerged only after repeated exposure to quinine. This

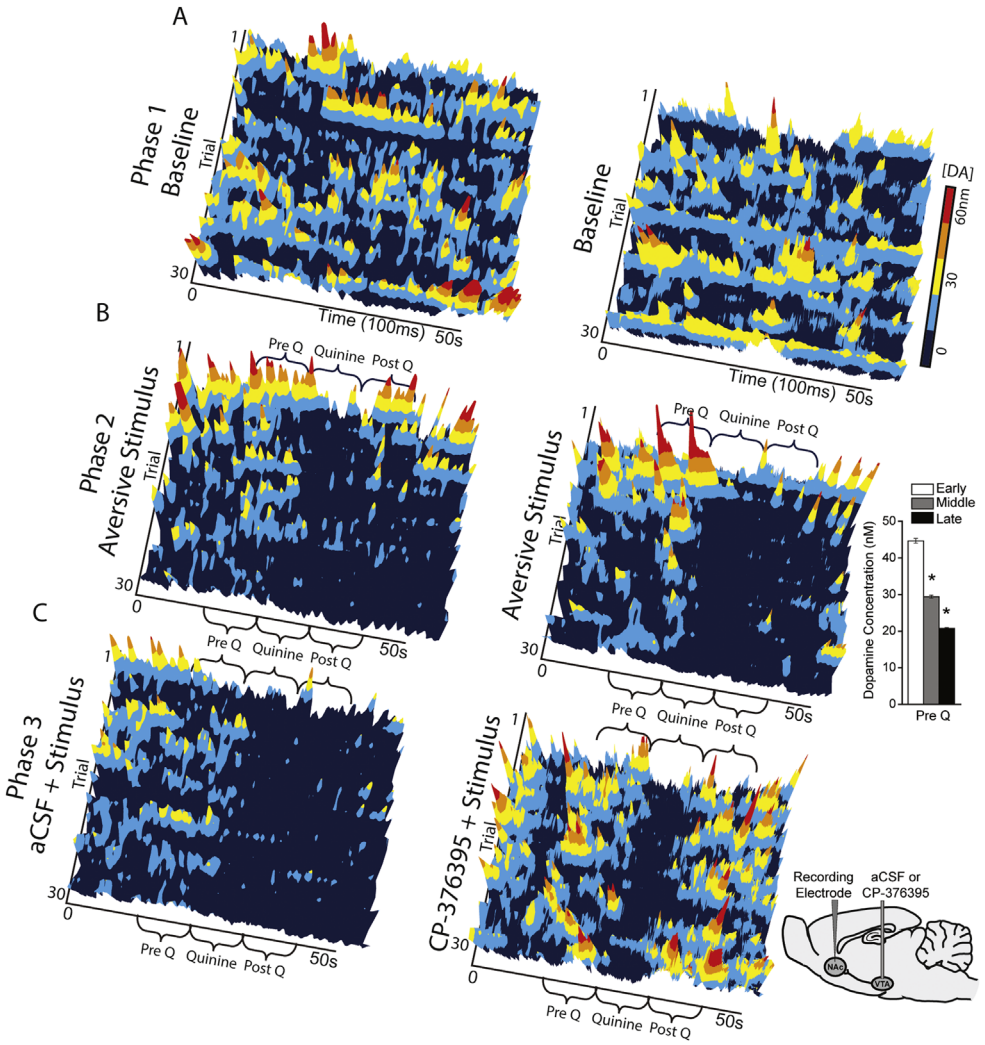

Figure 1. Corticotropin-releasing factor regulation of dopamine signaling during the experience of an unavoidable aversive stimulus. **(A)** Representative fluctuations in naturally occurring dopamine concentration in the shell of the nucleus accumbens in a behaving control (left) and experimental (right) rat in the baseline phase (phase 1). **(B)** Altered dopamine signaling in response to the intraoral administration of quinine (phase 2). Reductions can be observed both acutely in response to quinine (x axis) and also broadly across trials (y axis, prequinine [Pre Q] period). **(B)** (far right) Intraoral delivery of quinine reduced tonic dopamine concentration measured across trials in the prequinine period of phase 2 (analysis of variance main effect: trials $F_{2,22} = 11.73$, $p < .01$; Tukey's post hoc, $^{*}p < .05$, significant reduction in middle and late trials compared with early trials). **(C)** Quinine-induced reductions in dopamine signaling were attenuated by intraventral tegmental area injections of the corticotropin-releasing factor antagonist, CP-376395 (phase 3). aCSF, artificial cerebrospinal fluid; DA, dopamine; Post Q, postquinine.

latter effect was quantified as a significant reduction in the middle (trials 11–15) and late (trials 26–30) trials, compared with the early (first 5) trials in the prequinine period 10 seconds before quinine infusion (Figure 1B, right). These data confirm the ability of aversive stimuli to lower terminal dopamine concentration and reveal a temporal complexity to this response.

We next asked whether this aversion-induced drop in terminal dopamine is influenced by CRF signaling in the VTA (21). In phase 3, animals received intra-VTA microinjections of the CRF antagonist CP-376395 (.3 μg/.3 μL/min) or aCSF (.3 μL/min), while intraoral quinine delivery and fast-scan cyclic voltammetry recordings continued (Figure 1C, right; Figure S1 in Supplement 1). CRF antagonism in the VTA had no effect on the ability of quinine to cause a rapid, transient decrease in dopamine concentration during the quinine intraoral infusion period (Figure 1C). In contrast, CRF antagonism in the VTA abolished the inhibitory effect of quinine on non-time-locked dopamine tone during the prequinine and postquinine periods (Figure 1C). By averaging across trials, a time-averaged dopamine concentration can be visualized (Figure 2A,B), along with the acute reduction that results from quinine infusion. An attenuation of this response can be visualized following CRF antagonism (Figure 2D) and quantified following chemometric analysis (Figure 3A,B).

Changes in terminal dopamine concentration in behaving animals could be driven by alterations in either the frequency or amplitude of dopamine release events (38). Here, we observed that quinine reduced dopamine tone by selectively reducing release frequency, and this effect was reversed by blocking CRF receptors in the VTA (Figure 4A). Combined, these data indicate that upon aversive stimulation, CRF signaling in the VTA suppresses dopamine tone in the NAc by modulating the frequency of dopamine release events.

Aversive stimuli potently regulate not only affective state but also the maladaptive motivated behavior of drug seeking (3,9,39,40), which is intimately tied to midbrain dopamine signaling (41,42) and regulated by CRF (43–46). We therefore tested whether quinine exposure and its attendant drop in NAc dopamine are sufficient to drive drug seeking in a reinstatement paradigm. Rats were trained to press a lever for an IV cocaine infusion. After a period of stable self-administration,

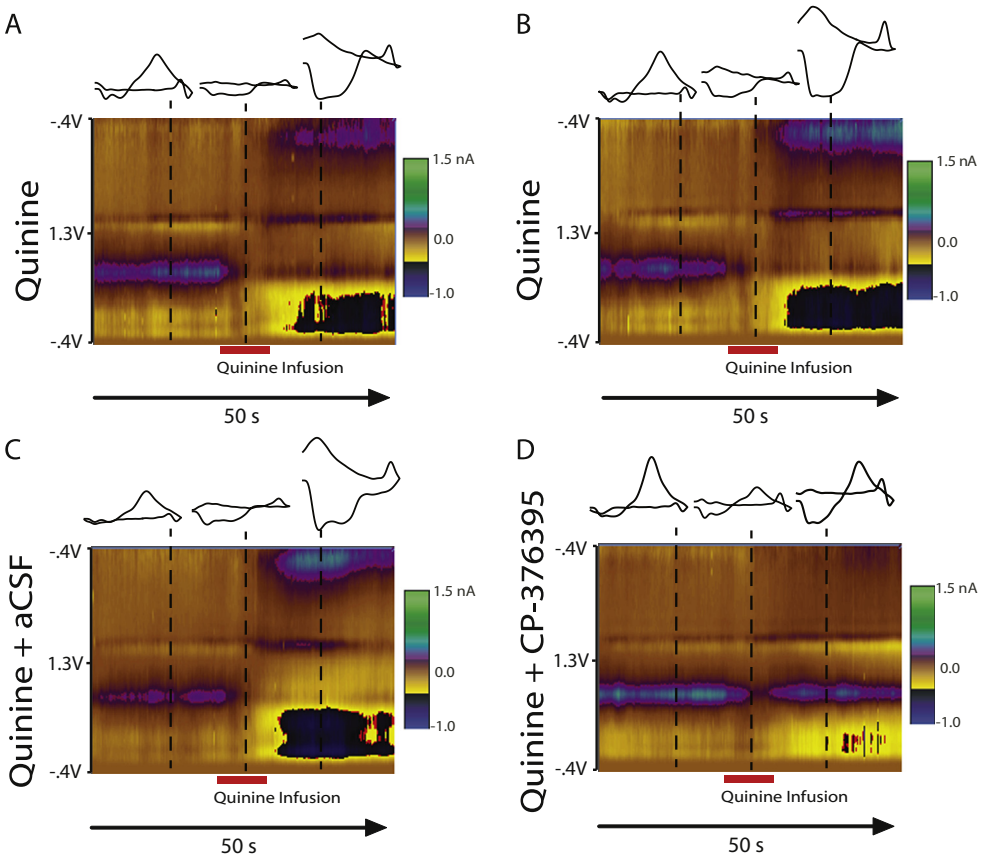

Figure 2. Time-averaged dopamine concentration change during quinine infusion and following corticotropin-releasing factor receptor blockade. Two-dimensional color representations of cyclic voltammetric data collected for 50 seconds around quinine infusions, averaged across trials for each phase of the experiment. The ordinate is the applied voltage (Eapp) and the abscissa is time (seconds [s]). Changes in current at the carbon fiber electrode are indicated in color. In phase 2, quinine infusion reduced the time-averaged dopamine concentration in control **(A)** and experimental **(B)** animals. **(C)** This reduction persisted in rats that received artificial cerebrospinal fluid (aCSF) infusions bilaterally into the ventral tegmental area. **(D)** Bilateral infusions of the corticotropin-releasing factor antagonist CP-376395 attenuated this reduction. Vertical dashed lines indicate time points in which cyclic voltammograms are plotted to illustrate the presence of dopamine (left), its reduction by quinine (center), and the pH change following intraoral infusion (right).

the lever-pressing behavior was extinguished by discontinuing cocaine availability. After extinction, rats received inescapable intraoral quinine infusions (1 infusion/min for 15 minutes) followed by the opportunity to press the lever that previously provided cocaine. Quinine administration increased lever pressing only on the active lever (Figure 5A; Figure S4 in Supplement 1), demonstrating that an aversive stimulus that suppresses dopamine tone can also reinstate drug-seeking behavior. Moreover, reinstatement behavior was fully prevented by blocking CRF receptors in the VTA (Figure 5A; Figure S2 in Supplement 1). Intriguingly, although CRF antagonism blocked reinstatement behavior, it spared the perceived aversive properties of quinine, as indicated by the persistent expression of aversive taste reactivity (Figure 5B). Taken

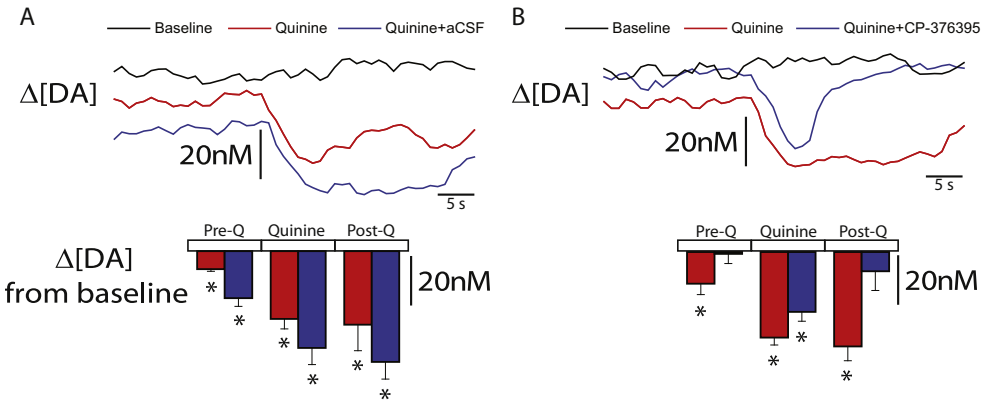

Figure 3. Intraoral delivery of the aversive taste, quinine, reduced dopamine concentration in a corticotropin-releasing factor dependent manner. Changes in dopamine (DA) concentration, determined via principal component analysis, are plotted in **(A)** and **(B)**. **(A)** Quinine reduced dopamine concentration significantly from baseline (phase 1) in artificial cerebrospinal fluid (aCSF)-injected rats (analysis of variance period × drug interaction; $F_{4,20} = 10.683$, $p < .001$; Tukey's post hoc, $^*p < .05$). **(B)** The quinine-induced dopamine reduction was attenuated in CP-376395-injected rats (analysis of variance period × drug interaction; $F_{4,20} = 6.77$, $p < .01$; Tukey's post hoc, $^*p < .05$, significant reduction in CP-376395-treated animals only in quinine period). The dopamine reduction was reversed by intraventral tegmental area injections of CP-376395 but only in the prequinine (Pre-Q) and postquinine (Post-Q) periods in which quinine was not present. Data are presented as mean + SEM.

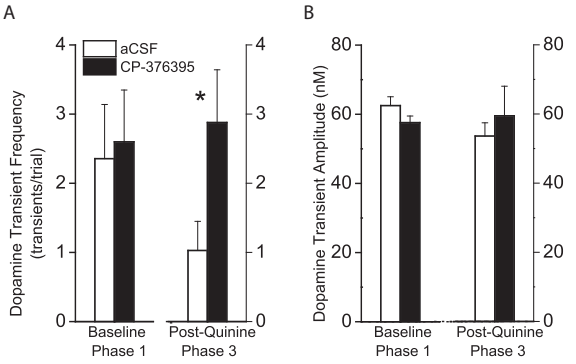

Figure 4. Quinine reduced the frequency of dopamine release events. **(A)** The aversive quinine stimulus reduced dopamine transient frequency in the period following the intraoral infusion, and this effect was reversed by the corticotropin-releasing factor antagonist [artificial cerebrospinal fluid (aCSF) baseline compared with aCSF postquinine ($F_{1,10}$ = 10.21, Tukey post hoc, *p < .05]. **(B)** The quinine infusion had no effect on release amplitude during this same period ($F_{1,10}$ = .75, p > .05). Data are presented as mean + SEM.

together, these data demonstrate that an aversive stimulus that suppresses dopamine tone can also reinstate drug-seeking behavior and both of these responses are preventable by blocking CRF receptors in the VTA.

DISCUSSION

This report highlights a mechanism by which aversive stimuli can drive the motivational circuit to induce drug-seeking behavior. Intraoral infusions of the aversive tastant, quinine, caused both phasic and tonic reductions in terminal dopamine signaling (i.e., reductions across seconds and across minutes). Previous reports have described rapid, phasic, aversion-induced decreases in dopamine release (16). However, in the absence of direct aversive stimulation, the transient release events that comprise a tonic signal also can be time-averaged over minutes (47). Using this approach, we found that cocaine-predictive stimuli can induce negative affect while simultaneously reducing both phasic and tonic dopamine signaling in the NAc (19). Interestingly, we observed that the tonic, but not the phasic, reduction was reversed by blocking CRF receptors in the VTA. While this manipulation did not affect the perceived aversive properties of quinine, it did reverse quinine-induced cocaine seeking, demonstrating that aversive stimuli can drive drug seeking in a state of low dopamine tone. Additional studies will be necessary to characterize both the mechanism and potential behavioral significance of the phasic decrease in dopamine in response to aversive stimuli.

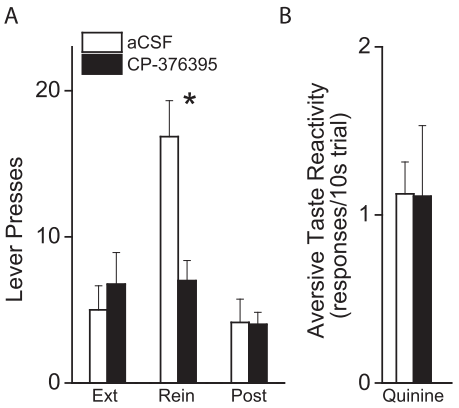

Figure 5. Motivational processes, but not hedonic expression, were regulated by corticotropin-releasing factor. **(A)** Following extinction (Ext), intraoral infusions of quinine caused cocaine seeking in the reinstatement test (Rein) in artificial cerebrospinal fluid (aCSF)-treated rats, an effect that was reversed by intraventral tegmental area injections of CP-376395 (analysis of variance drug × day interaction; $F_{2,16}$ = 5.83, p < .05; Tukey's post hoc, *p < .05). **(B)** Intraoral delivery of the aversive taste, quinine, caused the expression of aversive taste reactivity in aCSF-treated rats. This effect was not altered by intraventral tegmental area injections of CP-376395 ($t_{1,9}$ = .98, p > .05). Data are presented as mean + SEM. Post, postquinine.

These findings underscore the need to scrutinize the apparently complex manner by which aversive stimuli act on reward circuitry to motivate behavior, elevating dopamine signaling in some situations and decreasing dopamine signaling in others. For example, aversive electric footshock has been shown to increase CRF activity in the VTA (45,46), which, in turn, can increase dopamine neuron activity (25,44,45,48) and reinstate drug seeking (43,45,46). While these findings appear to be at odds with the current report, they are consistent with terminal measures of dopamine signaling using microdialysis that typically report elevations in dopamine concentration for several minutes during and after aversive stimulation that promotes dug seeking (49–52). The current data are provocative because they demonstrate that aversive stimuli that decrease dopamine signaling can also drive drug seeking and that both phenomena are under the control of CRF.

One possible explanation for how increases and decreases in dopamine signaling could both lead to drug seeking can be found in the cellular organization of dopamine target regions. Phenotypically distinct striatal neuron populations are tuned to be differentially sensitive to either increases or decreases in dopamine concentration. In the dorsal striatum, low-affinity D1-receptor-expressing MSNs that comprise the direct motor output pathway are activated by elevations of dopamine that promote voluntary movement. Correspondingly, high-affinity D2-receptor-expressing MSNs, comprising the indirect motor output pathway, are inhibited by high dopamine tone but are sensitive to, and activated by, phasic pauses in dopamine that suppress behavior [for review, see (26)]. There is mounting evidence that this organization is paralleled to a significant degree in the ventral striatum. In the NAc, phasic increases in dopamine signaling activate low-affinity dopamine receptor-expressing MSNs that promote reward learning. Conversely, decreases in dopamine signaling activate high-affinity dopamine receptor-expressing MSNs and promote aversion (27,30,53). In the current studies, quinine likely engaged the latter circuit, serving as an aversive environmental stressor that decreased dopamine signaling in a CRF-dependent manner and promoted drug seeking. Other ethologically relevant environmental stimuli may increase dopamine signaling in the NAc and could lead to the same behavioral result by engaging different circuitries.

NAc dopamine signaling has been heavily implicated in the mechanisms that promote addiction. NAc dopamine is essential for reward-related learning (54) and the proper responses to incentive cues (55), supporting the idea that dopamine signaling provides incentive for or stamps in the motivational value of reinforcing stimuli (42) and contributes significantly toward compulsive drug seeking (41,56). Accepting this, it may

be intuitive to imagine dopamine-elevating stimuli causing drug seeking but less so to imagine how aversive stimuli that decrease dopamine signaling accomplish this. However, some of the earliest theories of substance abuse suggested that drug withdrawal acts through negative reinforcement mechanisms to promote relapse in substance abusers attempting to remain abstinent (57–59). Although subsequent tests of these theories questioned whether acute withdrawal could contribute to a disorder characterized by chronic relapse following extended periods of drug abstinence (60–63), negative reinforcement mechanisms clearly have a role. The host of neuroadaptations that accompany chronic drug use and promote reward insensitivity and tolerance (7,56,64–66) could make the sensitivity to environmental stressors an even more important factor in promoting relapse in drug-abstinent populations. In fact, even moment-to-moment cocaine self-administration appears to involve negative reinforcement learning mediated by striatal dopamine signaling. As dopamine concentration falls, self-administration reliably resumes and animals titrate cocaine intake to maintain the desired brain dopamine concentration (67,68). During self-administration, animals might learn to respond to avoid a state of lowered dopamine, and the product of this negative reinforcement learning could be subsequently engaged by aversive stimuli that lower dopamine tone. The current report identifies a potential dopaminergic mechanism of this aversive motivation that involves CRF, a stress-activated neuromodulator. Aversive stimuli reduce dopamine signaling, engaging this mechanism and its attendant emotional states (e.g., negative affect or craving) in the absence of drug availability. Indeed, the current findings suggest that substance abusers learn to correct stress-induced decreases in dopamine signaling in the most efficient manner by self-administering cocaine.

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ARTICLE INFORMATION

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