Archival Report

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Cocaine Use Reverses Striatal Plasticity Produced During Cocaine Seeking

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ABSTRACT

BACKGROUND: Relapse is a two-component process consisting of a highly motivated drug-seeking phase that, if successful, is followed by a drug-using phase resulting in temporary satiation. In rodents, cue-induced drug seeking requires transient synaptic potentiation (t-SP) of cortical glutamatergic synapses on nucleus accumbens core medium spiny neurons, but it is unknown how achieving drug use affects this plasticity. We modeled the two phases of relapse after extinction from cocaine self-administration to assess how cocaine use affects t-SP associated with cue-induced drug seeking.

METHODS: Rats were trained to self-administer cocaine (n = 96) or were used as yoked-saline control animals (n = 21). After extinction, reinstatement was initiated by 10 minutes of cue-induced drug seeking, followed by 45 minutes with contingent cocaine access, after which cocaine was discontinued and unreinforced lever pressing ensued. Three measures of t-SP were assayed during reinstatement: dendritic spine morphology, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) to *N*-methyl-D-aspartate (NMDA) ratios, and matrix metalloproteinase activity.

RESULTS: We found that cocaine use for 10 minutes collapsed all three measures of cue-potentiated t-SP back to baseline. Moreover, when cocaine use was discontinued 45 minutes later, dendritic spine morphology and AMPA to NMDA ratios were restored as animals became motivated to engage unrewarded lever pressing. Nonreinforced drug seeking was positively correlated with changes in spine morphology, and cocaine access reversed this relationship.

CONCLUSIONS: Using a novel modification of the reinstatement paradigm, we show that achieving cocaine use reversed the synaptic plasticity underpinning the motivation to seek the drug.

Keywords: Cocaine, Drug abuse, Motivation, Nucleus accumbens, Reinstatement, Synaptic plasticity

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Drug addiction is a chronic illness defined by enduring vulnerability to relapse to drug use after extended abstinence (1). A relapse consists of two sequential phases: a highly motivated drug-seeking state, and if seeking is successful, subsequent drug use (2,3). The circuit and cellular substrates underpinning the high motivation associated with the drug-seeking phase of relapse is studied in the rodent self-administration and reinstatement models using contingent presentation of Pavlovian cues previously associated with drug infusions to recapitulate the powerful motivation to seek produced by drug-associated cues in addicts (4). In this model, motivation is operationally defined as nonreinforced lever pressing in pursuit of drug reward. Cued reinstatement of cocaine, nicotine, and heroin seeking requires inducing rapid, transient synaptic potentiation (t-SP) at glutamatergic synapses on nucleus accumbens core (NAcore) medium spiny neurons (MSNs) (5–8). t-SP is quantified using standard electrophysiological and morphological measures that are consistent with increasing synaptic strength, including increases in the ratio of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) to *N*-methyl-D-aspartate (NMDA) currents (AMPA:NMDA) and dendritic spine head diameter (dh) or density (9–11). Recently, we found that t-SP requires catalytic cleavage of the extracellular matrix surrounding corticoaccumbens synapses by activated matrix metalloproteinase-2 and -9 (MMP-2,9), and increased MMP-2,9 activity constitutes a third t-SP measure (6). The importance of t-SP in cue-induced drug seeking is revealed in studies where interfering with glutamatergic transmission from prelimbic afferents to NAcore (5,12) or blocking MMP-9 activity (6) prevents t-SP induction and drug seeking. Correspondingly, the intensity of drug seeking is positively correlated with the extent of t-SP (5).

Recent studies show that cued drug seeking depends on t-SP at glutamate synapses in NAcore (5,6), but it is unknown what happens when the highly motivated state of drug seeking culminates in drug use that produces temporary satiation (defined as the process by which drug consumption causes a transient decrease in motivation for drug seeking) (2,3). Understanding the mechanism whereby drug use temporarily reduces drug seeking could reveal cellular mechanisms of satiation as useful targets for inhibiting the excessive desire to relapse. We postulated that if drug seeking successfully

SEE COMMENTARY ON PAGE e47

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culminates in drug use, switching from the drug-seeking phase to drug-using phase of relapse would reverse t-SP. Moreover, when drug availability is discontinued, rats reenter a robust drug-seeking phase (13,14), and we hypothesized that the return to highly motivated drug seeking would renew t-SP. Thus, we adapted a reinstatement paradigm to model distinct phases of relapse and measure t-SP: 1) cued reinstatement of lever pressing (nonreinforced drug seeking), 2) cue-induced reinstatement leading to cocaine use (drug using), and 3) discontinuing cocaine availability (return to nonreinforced drug seeking). We found that t-SP underpinning cocaine seeking was collapsed by cocaine use and when drug seeking was renewed, inputs to NAcore MSN synapses were repotentiated.

METHODS AND MATERIALS

Subjects and Surgery

Male Sprague Dawley rats (250–300 g; Charles River Laboratories, Wilmington, MA) were maintained on a 12 hour–12 hour reverse light/dark cycle with ad libitum food and water. After 1 week of vivarium acclimation, rats were implanted with jugular catheters. Rats used for in vivo zymography additionally underwent stereotaxic surgery delivering 23-gauge cannula to NAcore (see the Supplemental Experimental Procedures). Food was temporarily restricted to 25 g/day standard chow prior to food training. After one food training session, rats were restored to ad libitum feeding. Experimental procedures were approved by the Animal Care and Use Committee of the Medical University of South Carolina and performed in accordance with National Institutes of Health guidelines.

Self-administration

Rats were trained to acquire operant responding for food in a single 2-hour session prior to beginning 2-hour daily self-administration sessions or were yoked saline control animals receiving noncontingent saline infusions paired with light and tone cues according to a preprogrammed pattern of average cocaine responding (Figure 1A). Rats self-administered cocaine (0.2 mg/0.05 mL infusion, ~0.5–0.67 mg/kg/infusion; National Institute of Drug Abuse, Bethesda, MD) until reaching criterion of ≥10 days with >10 infusions, followed by 1 to 2 weeks of extinction (15). Training was performed in standard operant chambers containing house and cue lights, tone cues, and two retractable levers (Med Associates, St. Albans, VT). Subjects were trained on a fixed-ratio 1 schedule of reinforcement paired with light and tone cues (78 dB, 4.5 kHz) and followed by a 20-second timeout period signaled by absence of house light. During extinction, active lever presses no longer resulted in cocaine or cues. Extinction criteria were met when animals averaged <25 active lever presses for 2 days prior to reinstatement testing.

Reinstatement and Obtaining Brain Tissue

The protocol and timeline for obtaining tissue is illustrated in Figure 1A. Two groups (yoked-saline [Yoked-Sal]; cocaine-extinction [Coc-Ext]) were killed 24 hours after the last extinction session and did not enter reinstatement. Two groups entered a reinstatement session (cocaine-cue-saline [Coc-Cue-Sal]; cocaine-cue-cocaine [Coc-Cue-Coc]). During the first 10 minutes of all reinstatement sessions, responses on the active lever resulted in contingent delivery of previously drug-paired light and tone cues, akin to standard cue-induced reinstatement (0–10 minutes). Between 11 and 55 minutes, saline infusions accompanied cues with each lever press in the Coc-Cue-Sal group, whereas cocaine plus cues was delivered in the Coc-Cue-Coc group using self-administration conditions outlined above. Cocaine was delivered at 0.2 mg/0.05 mL infusion during this phase of the reinstatement session, except for in a control experiment where 0.06 mg/0.05 mL infusion was used. Brain cocaine concentrations were estimated in accordance with the method described by Pan *et al*. (16) (see the Supplemental Experimental Procedures). During the last 65 minutes of the trial (56–120 minutes), infusions and cue delivery ceased for both the Coc-Cue-Sal and Coc-Cue-Coc groups. Reinstatement groups were killed at various times after initiating the session (20, 50, or 65 minutes) to capture the effect of cocaine delivery on cue-induced t-SP (20 minutes), the effect of continued saline versus cocaine delivery (50 minutes), and the effect of removing cocaine and saline delivery (65 minutes).

Dendritic Spine Quantification

A confocal microscope (Leica, Wetzlar, Germany) was used to image DiI-labeled sections (see the Supplemental Experimental Procedures) using the helium-neon 543-nm laser line. DiI-labeled neurons and dendrites (Figure 2A, B) were imaged via optical sectioning by a 63× oil immersion objective (numerical aperture = 1.4) with pixel size 0.01 μm in the xy plane and 0.13-μm intervals along the z axis. Images were deconvolved by AutoQuant (Media Cybernetics, Rockville, MD), and a three-dimensional perspective was rendered by the Surpass module of the Imaris software (Bitplane, Concord, MA). Spines on dendrites beginning at >75 μm and ending at ≤200 μm distal to the soma and after the first branch point were quantified from NAcore MSNs. Three to 12 segments (45–55 μm each) were analyzed per animal (mean = 8 segments/rat). Minimum dh was set at ≥0.143 μm to reflect the Nyquist frequency resolution limits of the microscope.

Slice Preparation and Whole-Cell Recordings

Coronal slices were collected into a vial containing artificial cerebrospinal fluid. Recordings were collected at 32°C in dorsomedial NAcore. Inhibitory synaptic transmission was blocked with picrotoxin (100 μmol/L). AMPA and NMDA currents were recorded in whole-cell patch-clamp configuration. Glass microelectrodes (1.5–2.5 MΩ) were filled with cesium-based internal solution. To evoke postsynaptic currents, we placed a bipolar stimulating electrode (FHC, Bowdoin, ME) ~300 μm dorsomedial of the recorded cell to maximize chances of stimulating prelimbic cortex afferents. Stimulation intensity was chosen to evoke excitatory postsynaptic currents of 200 to 400 pA, whereas the cell was voltage clamped at −80 mV, which typically represented