Protracted Withdrawal from Cocaine Self-Administration Flips the Switch on 5-HT$_{1B}$ Receptor Modulation of Cocaine-Abuse Related Behaviors

Supplemental Information

Supplemental Methods and Materials

Subjects

Male Sprague–Dawley rats weighing 268-308 g at the time of surgery were individually housed in a climate-controlled colony room with a 12-h reversed light/dark cycle (lights off at 6 AM). Animal care and housing adhered to the conditions set forth in the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996).

Surgery

Rats (n = 74) were handled daily for 7-10 days prior to surgery. Rats were deeply anesthetized using isoflurane (3-4% for induction, 2-3% for maintenance) and catheters were implanted into their jugular veins. Next, the rats were placed into a stereotaxic alignment system (Kopf Instruments, Tujunga, CA) and two small burr holes were drilled into the skull in order to implant stainless steel guide cannulae (26G, Plastics One, Roanoke, VA) bilaterally into the nucleus accumbens shell (NAcsh). Guide cannulae were implanted 1.0 mm dorsal to the desired injection site using a David Kopf micromanipulator with the following coordinates obtained from the Rat Brain Atlas (1): +1.60 mm anterior to bregma, +/-1.1 mm from the midline and -6.6 mm ventral from the surface of the skull; coordinates were based on previous research (2). The guide cannulae and the metal end of the catheter were secured to the skull and 4-anchor screws drilled into the skull using dental acrylic cement. Stainless steel stylets (30G) were inserted into

the guide cannulae to maintain patency. Following surgery, rats were returned to their home cages for 7-10 days recovery prior to the start of self-administration training. In order to prevent infection and maintain patency, catheters were flushed daily with 0.1 ml saline containing heparin sodium (70 U/ml; APP Pharmaceuticals, Schaumburg, IL), Abbokinase (20 mg/ml; ImaRx Therapeutics, Tucson, AZ) and Timentin (66.7 mg/ml; GlaxoSmithKline, Research Triangle Park, NC). Proper catheter function was tested periodically by administering 0.05 ml methohexital sodium (16.7 mg/ml; JHP Pharmaceuticals, Rochester, MI), a dose that produces brief anesthetic effects only when administered intravenously (i.v.).

Drugs/Viral Vectors

Cocaine hydrochloride (RTI International, Research Triangle Park, NC) was dissolved in sterile saline and filtered through 0.2 μm membrane filters. The herpes simplex virus (HSV) vector system utilized in the present study (see Figure 1B) incorporated a replication-deficient helper virus for packaging that has been used and reviewed previously (3-5). Two plasmid amplicons were packaged into viral vectors. 5-HT_{1B}R–green fluorescent protein (GFP) expresses both hemagglutinin-tagged 5-HT_{1B}R and GFP from separate transcriptional cassettes, whereas GFP-only expresses only the GFP transcript. The GFP-only vector serves as a control for all nonspecific aspects of the viral gene transfer procedure, and this HSV GFP-only vector system does not alter drug reward or behavior compared with sham or vehicle microinfusions (3, 4, 6). This 5-HT_{1B}R–GFP vector system produces a 3-fold increase in 5-HT_{1B}R mRNA in neuronal (5), but not glial (2) cells, and hemagglutinin epitope tagging does not alter the function of 5-HT_{1B}Rs (5).

Self-Administration Training

Self-administration occurred in operant conditioning chambers (28 x 10 x 20 cm; Med Associates, St. Albans, VT) equipped with an active lever, an inactive lever, a cue light 4 cm above the active lever, a tone generator (500 Hz, 10 dB above background noise) and a house light on the top center of the wall opposite the levers. Each operant conditioning chamber was housed within a larger ventilated sound-attenuating chamber. Infusion pumps (Med Associates) were connected to liquid swivels (Instech, Plymouth Meeting, PA) located above the chambers. The swivels were fastened to the catheters via polyethylene 20 tubing encased inside a metal spring leash (Plastics One, Roanoke, VA).

To facilitate acquisition of cocaine self-administration (7), rats were initially food-restricted to 16 g of food/day beginning 2 days prior to training. Subjects were maintained on food restriction (16 -28 g) throughout self-administration training and testing in order to maintain a weight ≥ 90% of their free feeding weight. Rats were trained to self-administer cocaine (0.75 mg/kg/0.1 ml, i.v.) progressing from a FR1 to a FR5 schedule of reinforcement 6 days/week during 2-h sessions, which occurred during their dark cycle at the same time each day. Schedule completions on the active lever resulted in the simultaneous activation of the cue light, house light, and tone generator followed 1 s later by a 6-s cocaine infusion. All stimuli were inactivated with the termination of the infusion except the house light, which remained activated signaling a 20-s timeout period, during which lever presses were recorded but produced no consequences. Inactive lever presses were recorded throughout training but produced no consequences. Once self-administration infusion rates stabilized, defined as less than 10% variability of infusions/session across 3 consecutive days of self-administration with no upward or downward trends (16–22 sessions), rats included in the cocaine dose-response experiments

were given 30 min access to varying doses of cocaine presented in ascending order (0.0, 0.032, 0.1, 0.32 and 1.0 mg/kg/0.1 ml, i.v.) on a FR5 schedule with a 10-min timeout period between successive doses (8). Within session dose-response training sessions occurred every 2 days alternating with access to the training dose of cocaine for 2-h sessions. For the reinstatement and progressive ratio (PR) experiments during protracted withdrawal, once self-administration infusion rates stabilized on the training dose of cocaine (0.75 mg/kg/0.1 ml, i.v.; 18 sessions), rats began extinction training (17 total sessions across 21 days of abstinence) or were placed into forced abstinence (21 days), respectively.

Effects of Viral-Mediated Gene Transfer (VMGT) on Cocaine Intake During Maintenance

Testing procedures began once within-session cocaine dose-effect functions stabilized to less than 10% variability across 3 consecutive sessions. Average cocaine intake during these last 3 training sessions was used as a baseline. Rats were assigned to groups counterbalanced for previous total cocaine intake and received bilateral microinfusions (2.0 µl/side over 10 min) containing approximately 200,000 infective units of either 5-HT_{1B}R-GFP (n = 10) or GFP-only (n = 9) into the medial NAcsh under light isoflurane (1-2%) anesthesia. The microinjectors extended 1 mm beyond the guide cannulae into the NAcsh. Injection cannulae remained in place for an additional 10-min period to allow diffusion away from the cannulae tips prior to removal, and then the stylets were replaced. To allow for maximal viral expression prior to testing, rats were given access to their training dose of cocaine for the next 3 days and were then tested on the within-session cocaine dose-effect function on day 4 post-infection; a subset of rats were tested for break points on a PR schedule on day 5. Testing occurred on the fourth and fifth days following infection as this time course corresponds with peak viral-mediated 5-HT_{1B}R

expression for the vector utilized in this study (2, 3). Break points were defined as the highest ratio attained once rats failed to receive a cocaine infusion during a 1-h period on a schedule that progressed exponentially from an FR1 according to the formula 5*e^(0.2n)-5 rounded to the nearest integer, with n reflecting the number of reinforcers the rat received during the session (9).

Extinction Training

Rats in the reinstatement experiments began extinction training the day after the last self-administration session consisting of daily 1-h (6 days/wk) exposures to the self-administration environment. During extinction, rats were connected to the tethers, and active and inactive lever responses were recorded but produced no consequences (i.e., the cocaine infusion pump was not activated, and the discrete light and tone cues were not presented). Responding on the active lever in the absence of cocaine reinforcement is the operational definition of cocaine-seeking behavior. Extinction training continued until response rates on the active lever declined to less than 20% of the highest rate observed during extinction (17 sessions). Once responses declined to criterion levels, cocaine-seeking behavior was considered extinguished.

Effects of VMGT on Cue- and Cocaine-Primed Reinstatement of Extinguished Cocaine-Seeking Behavior

Upon reaching the extinction criterion delineated above (i.e., 14 days of extinction), rats (n = 29) received microinfusions of their assigned viral vector [5-HT_{1B}R-GFP (n = 15) or GFP-only (n = 14)] into the medial NAcsh as detailed above. To allow for maximal viral expression prior to testing, rats continued extinction training for the next 3 days and were then tested for cue- and cocaine-primed reinstatement of extinguished cocaine-seeking behavior on day 4 post-

infection. For cue-elicited reinstatement, responses on the active lever during the 1-h test session resulted in response-contingent presentations of the same stimulus complex that had been previously paired with cocaine infusions on a FR1 schedule of reinforcement, but no cocaine was available. If a rat did not respond within the first 5 min, a non-contingent cue was presented.

Immediately following completion of the cue-elicited reinstatement test phase, rats received an intraperitoneal (i.p.) saline injection and were exposed to the operant chambers for a 1-h extinction session in order to control for injection stress and to re-establish low baseline response rates. Lever responses during this test phase produced no consequences and served as the baseline for the cocaine-primed reinstatement test. Immediately after the saline-primed extinction phase, rats were tested for the effects of VMGT on cocaine-primed reinstatement of extinguished cocaine-seeking behavior. Immediately after receiving a cocaine-priming injection (10 mg/kg, i.p.), rats were placed into the operant conditioning chambers for a 1-h test session during which responses produced no consequences.

Effects of VMGT on Cue-Elicited Cocaine-Seeking Behavior and Cocaine Intake During Protracted Withdrawal

For these experiments, a period of forced abstinence (21 days) began the day after the last self-administration session. During abstinence, rats were handled and flushed daily in order to maintain catheter patency, but were not exposed to the self-administration environment. On the 17^{th} day of abstinence, rats (n = 20) received microinfusions of their assigned viral vector [5-HT_{1B}R-GFP (n = 11) or GFP-only (n = 9)] into the medial NAcsh as detailed above. Tests for cue-elicited cocaine seeking and cocaine intake were conducted on day 4 and 5 post-infection, respectively. Parameters for both the cue-elicited cocaine seeking and the PR self-administration

tests were identical as those listed above, except testing commenced following 21 days of forced abstinence.

Effects of VMGT on Anxiety-Like Behavior in the Elevated Plus-Maze (EPM)

The day after reinstatement testing (day 5 post-infection), rats were tested for anxiety-like behavior in the EPM as described previously (10). Briefly, the EPM apparatus consisted of four Plexiglas arms configured as a cross and elevated 75 cm above the floor. The arms were 10 cm wide and 50 cm long, and each arm was joined at the center by a 10 cm square platform. The two opposite "open" arms contained no walls, while the other two "closed" arms contained 40 cm high sides. Rats were individually placed in the center of the apparatus facing one of the two closed arms and were allowed to explore freely for 5 min under dim light. Entries and time spent in the open and closed arms, and total locomotor activity were later analyzed from videotapes by a highly trained observer blind to group assignment. The apparatus was cleaned between each test trial using 5% ethanol.

Histology

After testing, rats were anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. Brains were then harvested and placed into 4% paraformaldehyde for 24 h and then transferred sequentially to 15% and 30% sucrose solutions (24 h each), and were then sectioned using a cryostat (Leica, Buffalo Grove, IL). A series of coronal sections (40 μm) were taken throughout the entire rostral–caudal extent of the NAc and ventral tegmental area (VTA). The sections were immediately cover slipped using Vectashield (Santa Cruz Biotechnology, Santa Cruz, CA) and

were analyzed using a dual laser confocal microscope (Zeiss, Peabody, MA) in order to verify cannulae tip placement and GFP expression by an observer blind to experimental conditions. Rats with misplaced cannulae or that lacked GFP expression in both the NAcsh and VTA were excluded from the analyses.

Supplemental Results

Histology and Attrition

The histological boundaries of injector tip placements for rats included in the analyses are shown in Figure 1A and representative photomicrographs within the medial NAcsh and VTA are shown in Figure 1C, E and 1D, F, respectively. For the maintenance experiments, 1 rat was excluded from both viral groups due to placements outside the NAcsh, 2 were eliminated from the GFP-only group due to catheter non-patency, 1 was eliminated from the GFP-only group due to signs of cocaine toxicity, and 2 were eliminated from the 5-HT_{1B}R-GFP group due to failure to demonstrate stable within-session cocaine self-administration, yielding a final n = 9 and 8 for the 5-HT_{1B}R-GFP and the GFP-only groups, respectively. For the reinstatement experiments, 1 rat from the GFP-only group failed to acquire self-administration and was eliminated, yielding a final n = 15 and 14 for the 5-HT_{1B}R-GFP and the GFP-only groups, respectively. For the abstinence experiment, 2 rats were excluded from both viral groups due to placements outside the NAcsh, yielding a final n = 9 and 7 for the 5-HT_{1B}R-GFP and the GFP-only groups, respectively.

Supplemental References

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