Stress Promotes Drug Seeking Through Glucocorticoid-Dependent Endocannabinoid Mobilization in Prelimbic Cortex

Supplemental Information

Supplemental Methods and Materials

Subjects: Male Sprague-Dawley rats (275-300g at arrival) obtained from Envigo (Indianapolis, IN) were individually housed in a humidity- and temperature-controlled, AAALAC-accredited facility with *ad libitum* access to food and water. Rats were housed under a 12-h/12-h reverse light/dark cycle (0700-1900 lights off). For electrophysiology experiments, male Sprague-Dawley rats (200-250g at arrival) were obtained from Charles River laboratories (Wilmington, MA). All behavioral procedures were completed in the dark phase of the light cycle. All experimental procedures were carried out in compliance with National Institutes of Health Guidelines and were approved by Institutional Animal Care and Use Committees (Marquette University and Medical College of Wisconsin).

Surgery: For intravenous cocaine self-administration, rats were anesthetized with ketamine HCl (100 mg/kg, i.p.; Henry Schein, Melville, NY) and xylazine (2 mg/kg, i.p.; Henry Schein, Melville, NY) and had an indwelling venous catheter implanted surgically. The polyurethane (0.6 mm i.d. x 1.1 mm o.d.; Access Technologies, Skokie, IL) catheters were connected to a back-mounted cannula (Plastics One, Roanoke, VA) attached to polypropylene mesh (500 microns; Small Parts, Logansport, IN). Catheters were implanted into the superior vena cava with the back-mount placement situated approximately 1 inch behind the scapula. For rats that received intra-prelimbic cortical (PL) infusions prior to reinstatement testing, guide cannula were surgically implanted immediately following catheter surgery. For guide cannula implantation, the skull was positioned

in a stereotaxic frame (Stoelting Inc; Wood Dale, IL) and two 11-mm stainless steel guide cannula (26 gauge; Plastics One, Roanoke, VA) were positioned 0.5 mm above the PL [coordinates (in mm): anteroposterior (A/P): +2.8 from Bregma; mediolateral (M/L): +1.0 from midline; dorsoventral (D/V): -3.5 from the skull surface; incisor bar: -3.3 from interaural line (Paxinos and Watson, 2005)]. Guide cannula were fixed in place with acrylic dental cement and four small anchoring screws. Internal dummy cannula were inserted into each cannula to maintain patency. Rats were given Bio-Serv Rimadyl tablets (5 g; Fisher Scientific; Hampton, NH) in their cage for 3 days and antibiotic treatment (100 mg/kg, iv; Cefazolin; Henry Schein, Melville, NY) for at least 5 days following surgery. All rats recovered for a minimum of one week before the initiation of self-administration.

Cocaine self-administration, extinction, and reinstatement: Self-administration procedures were conducted in computer-interfaced operant conditioning chambers equipped with retractable levers and stimulus lights above each lever in sound-attenuating cubicles (MED-Associates; Fairfax, VT). Following recovery from surgery, rats were food deprived to 90% of their body weight and were then trained to receive sucrose pellets by pressing a lever under a fixed-ratio (FR) 1 schedule. Once rats successfully acquired lever pressing for food, rats were then switched over to self-administer cocaine (0.5 mg/kg/infusion) by pressing a lever under a FR1 schedule during daily 2-h sessions. Pressing the lever resulted in an infusion (200 μL over 5-sec) followed by a 10-sec time-out period, during which lever presses were recorded but not reinforced and the stimulus light above the lever was extinguished and the house light turned on. Responding on a second, inactive lever was also recorded but not reinforced. Following stable responding, the FR value was increased gradually to FR4. Once stable responding on FR4 was maintained (<10% change over 3 days), rats were provided access to cocaine for self-administration during daily 2-h sessions for 14

days. Rats then underwent extinction training, wherein the cocaine syringe was replaced with saline, until the extinction criterion was met (<10 lever presses/2 h). Once rats met this criterion, a reinstatement test was conducted the following day.

Reinstatement conditions: The 2-h reinstatement tests were preceded by footshock, corticosterone, and cocaine and/or drug delivery but were otherwise identical to extinction conditions. A complete within-subjects design was used wherein all rats underwent multiple reinstatement tests within each experiment in a counterbalanced fashion for a maximum of 6 reinstatement tests. Rats that did not complete all reinstatement tests were excluded from analysis. Rats were given additional extinction/washout sessions between reinstatement tests to reestablish extinction criterion (<10 lever presses/2 h) before receiving additional tests. For stress-potentiated reinstatement of cocaine seeking, the electric footshock stress (3 x 0.5 mA, 200-msec duration, mean intershock interval 40-sec, range 10-70-sec over a 15-min period) was administered through stainless steel grid floors. Immediately following cessation of the electric footshock stress, a subthreshold dose of cocaine (2.5 mg/kg, i.p.) or saline was given followed by the 2-h reinstatement session. For corticosterone-potentiated reinstatement of cocaine seeking, a systemic injection of corticosterone (2 mg/kg, i.p) or vehicle (10% EtOH) was administered 40-min prior to an injection of low-dose cocaine (2.5 mg/kg, i.p) or saline, followed by the 2-h reinstatement session.

Intra-cranial drug administration: HBC-conjugated corticosterone (50 ng/0.3 µL; Sigma-Aldrich, St. Louis, MO), the CB1R antagonist AM251 (300 ng/0.3 µL; Sigma-Aldrich, St. Louis, MO), the CB1R agonist WIN 55,212-2 (50 ng/0.3 µL; Sigma-Aldrich, St. Louis, MO), the MAGL inhibitor URB602 (300 pmol/0.3 µL; Tocris, Bristol UK), or the DAGL inhibitor DO34 (0.1, 1.0 µg/0.3 µL; Cravatt Lab, Scripps Research Institute) were micro-infused directly into the PL at various times prior to reinstatement testing as described in the Results section. Infusion needles were

comprised of 11.5 mm 30-gauge stainless steel injectors (Plastics One, Roanoke, VA) attached to polyethylene-20 tubing and Hamilton syringes. The vehicle or drug was backfilled into the infusion needle and all drugs were infused at a volume of 0.3 µL and at a rate of 0.3 µL/min using a syringe pump. The needles remained in place for an additional 1-min to allow for diffusion.

Trunk blood collection and plasma corticosterone analysis: To confirm that the systemic dose of corticosterone used in the study recapitulates stress-evoked increases in blood levels of corticosterone, plasma corticosterone levels were quantified by radioimmunoassay (RIA) following either electric footshock stress or systemic corticosterone administration. Some rats received the same 15-min intermittent electric footshock stress parameters used for behavioral testing, and were sacrificed immediately after cessation of the footshock. Other rats were assigned to a No Shock control wherein rats were placed in the self-administration chamber for 15 min and were sacrificed immediately afterward. Another group of rats were given a systemic injection of corticosterone (2 mg/kg, i.p.) or vehicle (10% EtOH) and were sacrificed 40-min after the injection. All animals were sacrificed by rapid decapitation, and trunk blood collected for analysis. Trunk blood was collected into centrifuge tubes containing heparin and the blood was spun in a centrifuge at 1000 x *g* for 10-min to separate the plasma. The plasma was transferred into a new tube and stored at −80°C for long-term storage. Corticosterone levels in plasma were determined by using a ^{3}H-Corticosterone RIA kit (MP Biomedicals, Santa Ana, CA) according to the manufacturer protocol.

Slice electrophysiology: Drug-naïve rats were anaesthetized by isoflurane inhalation and decapitated. Cortical slices were cut using a vibrating slicer (Leica, Wetzler, Germany). The slices were transferred into artificial cerebrospinal fluid (ACSF) containing (in mM): 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose, and allowed to recover at least

one hour at room temperature. All solutions were saturated with 95% O_2 and 5% CO_2. Whole-cell voltage clamp recordings were made using a patch clamp amplifier (Multiclamp 700B; Molecular Devices, Sunnyvale, CA) under infrared-differential contrast interference microscopy. Data acquisition and analysis were performed using a digitizer (DigiData 1440A; Molecular Devices, Sunnyvale, CA) and analysis software pClamp 10 (Molecular Devices, Sunnyvale, CA). Pyramidal neurons in layer V of the PL were identified visually based upon pyramidal-shaped soma with a prominent apical dendrite. Additionally, the pyramidal neurons exhibit spike frequency adaptation in response to depolarizing current injections (1). For recording of evoked IPSCs, layer V pyramidal neurons were voltage-clamped at −60 mV, and IPSCs were evoked at 0.05 Hz by a tungsten stimulation electrode placed near the apical dendrites. The pipettes were filled with an internal solution containing (in mM): 80 K-gluconate, 60 KCl, 10 HEPES, 0.2 EGTA, 2 $MgCl_2$, 2 Mg-ATP, 0.3 Na_2GTP, and 10 Na_2-phosphocreatine (pH 7.2 with KOH). Spontaneous miniature IPSCs (mIPSCs) were recorded from pyramidal neurons at a holding potential of −70 mV. Action potential generation was blocked with tetrodotoxin (TTX; 0.5 μM). Glutamate receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) and D-2-amino-5-phosphonovaleric acid (D-AP-5, 20 μM) were present in the ACSF throughout the experiments. Series resistance (15-30 MΩ) was monitored throughout the recordings, and data were discarded if the resistance changed by more than 20%. All recordings were performed at 32 ± 1°C by using an automatic temperature controller.

Mass spectrometry: To assess effects of the DAGL inhibitor DO34 on corticosterone-induced 2-arachidonoylglycerol (2-AG) content, rats received intra-PL infusions of DO34 (1 μg/0.3μL) or vehicle (70% DMSO) 30 min prior to a corticosterone (2 mg/kg, ip) or vehicle (10% EtOH) injection. Rats were rapidly decapitated 45-min following the injection and brains were removed

and flash frozen by submersion in liquid nitrogen (total time from decapitation was approximately 90-sec) and stored at −80°C. For tissue dissection, 1 mm prelimbic prefrontal cortical sections (approx. 3.72 mm to – 2.76 mm from Bregma) were isolated from coronal sections on dry ice as described previously (2) and stored at −80°C. Placement of guide cannula was visually confirmed to be in the prelimbic cortex during dissection. Tissue samples were weighed and homogenized in acetonitrile containing 67 pmol [$^2H_8$]AEA and 8 nmol [$^2H_8$]2-AG (Cayman Chemical Company, Ann Arbor, MI), sonicated for 60 min, and frozen overnight at −20°C to precipitate proteins. Particulates were then removed by centrifugation at 1500 x *g* for 2 minutes at 4°C, after which the supernatant was rapidly extracted and concentrated under N_2 gas re-suspended in 100% methanol and AEA and 2-AG were isolated and quantified by tandem liquid chromatography-mass spectrometry (LC/MS/MS; Agilent Technologies 6460 Triple Quad LC/MS), and concentrations were calculated by isotope dilution as previously described (3).

Histology*:* To determine the accuracy of guide cannula placements, rats were sacrificed and brains were post-fixed in 4% paraformaldehyde, cryoprotected in 30% sucrose, and finally 50 µm sections were taken from the level of the prelimbic cortex using a cryostat. Tissue sections were mounted onto gelatin-coated slides and stained using a cresyl violet nuclear stain. Guide cannula placement was determined to be accurate if the injection needle terminated in the prelimbic region of the medial prefrontal cortex. A limited number of rats (n=5) did not have bilateral hits for cannula placement and were excluded from the study. In each case, drug effects were not observed in rats that had injections outside of the prelimbic cortex.

Drugs*:* Cocaine HCl was obtained from the National Institute on Drug Abuse (NIDA) through the NIDA Drug Supply Program. Cocaine was dissolved in saline (0.9% bacteriostatic saline). Corticosterone (Steroloids, Newport, RI) was dissolved in a 10% EtOH in saline solution, and 2-

hydroxypropyl-β-cyclodextrin (HBC)-conjugated corticosterone (Sigma-Aldrich, St. Louis, MO) was dissolved in saline. AM251 (Sigma-Aldrich, St. Louis, MO), WIN-55,212 (Tocris, Bristol, UK), URB502 (Tocris, Bristol, UK), and DO34 (0.1 μg) were made in a vehicle containing 10% DMSO in saline. DO34 (1 μg) was made in a vehicle containing 70% DMSO in saline.

Statistical analysis: Statistical analyses were conducted using SPSS (IBM Analytics, Armonk, NY) or Sigmaplot (Systat Software, San Jose, CA) statistics software. For behavioral experiments, lever responding was analyzed using two-way repeated measures ANOVA followed by post-hoc testing using Bonferroni-corrected t-tests. For electrophysiology experiments, IPSC amplitude was normalized to the baseline. The depression (%) of IPSCs by corticosterone was calculated as follows: 100 × [mean amplitude of IPSCs during the last 5 min treatment/mean amplitude of baseline IPSCs]. Data sets were compared with Student's *t*-test. For mass spectrometry, endocannabinoid content was analyzed using a two-way ANOVA followed by post-hoc testing. For all analyses, statistical significance was defined as $p<.05$.

Supplemental Data

Table S1. Cocaine self-administration and extinction responding. Data represent total active lever responses/2-h session for Figures 1, 3, 4 and 5 during days 1 and 14 of cocaine self-administration (SA) and total active lever responses/2-h session on the first day of extinction (Ext) and the last day of extinction before reinstatement testing for experiments 1 and 2. The number of days to reach extinction criteria for each experiment is also reported. Data are represented as mean ± SEM. (EFS, Electric footshock stress; COC, cocaine; CORT, corticosterone; PL, prelimbic cortex; AM251, CB1R antagonist; WIN, WIN 55,212-2 CB1R agonist; URB602, monoacylglycerol lipase inhibitor; DO34; diacylglycerol lipase inhibitor).

	Cocaine SA		Extinction		Range of Days to Ext	Average Days to Ext
Experiment no. (n)	SA Day 1	SA Day 14	First Ext	Last Ext	# Days	# Days
1 EFS/COC; (6)	106.00 ± 8.10	123.83 ± 7.48	68.50 ± 13.57	8.67 ± 1.43	5-10	8.00 ± 0.85
1 CORT/COC; (5)	121.40 ± 10.18	114.00 ± 13.57	33.2 ± 13.35	6.80 ± 1.56	5-15	8.80 ± 1.69
1 PL CORT/ COC; (7)	115.86 ± 10.98	137.71 ± 24.25	68.85 ± 7.53	7.57 ± 1.57	5-17	8.28 ± 1.76
3 PL AM251/ EFS/COC; (6)	123.67 ± 8.38	128.33 ± 6.95	76.00 ± 19.24	7.17 ± 1.49	4-12	7.17 ± 1.19
3 PL AM251/ CORT/COC; (6)	131.00 ± 15.18	132.50 ± 14.32	71.83 ± 12.59	8.83 ± 1.54	5-20	8.83 ± 2.30
4 PL WIN/ COC; (6)	146.33 ± 17.95	149.50 ± 28.00	68.50 ± 10.13	5.17 ± 2.52	5-9	6.67 ± 0.56
4 PL URB602/ COC (6)	112.67 ± 8.46	117.33 ± 6.45	77.67 ± 15.08	8.17 ± 1.72	3-9	6.17 ± 0.98
5 PL DO34/ CORT/COC (5)	112.60 ± 11.44	122.60 ± 18.99	32.00 ± 12.41	5.00 ± 1.58	5-13	9.00 ± 1.52

Table S2. Detailed statistics for experiments depicted in Figure 1. Two-way repeated measures (RM) ANOVAs were conducted with reinstatement condition and day (extinction, reinstatement) as factors. These were followed by separate one-way RM ANOVAs examining responding during extinction and reinstatement testing. Post-hoc testing was conducted using Bonferroni-corrected t-tests. * denotes significance. (Ext=extinction; Rst=reinstatement; Coc=cocaine, Cort=corticosterone; Sal=saline; Veh=vehicle).

Figure 1A			
2-way RM ANOVA		Post hoc test: Ext vs Rst	
Condition	$F_{(3,15)}$=18.05, p<.001*	No Shock/Sal	p>.05
Day	$F_{(1,5)}$=29.59, p<.01*	Shock/Sal	p>.05
Condition X Day	$F_{(3,15)}$=17.11, p<.001*	No Shock/Coc	p<.05*
		Shock/Coc	p<.01*
1-way RM ANOVA		Post hoc test: Rst – Shock/Coc vs	
Extinction	$F_{(3,15)}$=.05, p>.05	No Shock/Sal	p=.06
Reinstatement	$F_{(3,15)}$=19.26, p<.001*	Shock/Sal	p<.05*
		No Shock/Coc	p<.05*
Figure 1B			
2-way RM ANOVA		Post hoc test: Ext vs Rst	
Condition	$F_{(3,12)}$=7.38, p<.01*	Veh/Sal	p>.05
Day	$F_{(1,4)}$=5.50, p=.07	Cort/Sal	p>.05
Condition X Day	$F_{(3,12)}$=10.30, p<.001*	Veh/Coc	p>.05
		Cort/Coc	p<.05*
1-way RM ANOVA		Post hoc test: Rst – Cort/Coc vs	
Extinction	$F_{(3,12)}$=.55, p>.05	Veh/Sal	p>.05
Reinstatement	$F_{(3,12)}$=9.07, p<.01*	Cort/Sal	p>.05
		Veh/Coc	p>.05
Figure 1C			
2-way RM ANOVA		Post hoc test: Ext vs Rst	
Condition	$F_{(2,12)}$=3.99, p<.05*	Cort/Sal	p>.05
Day	$F_{(1,6)}$=6.78, p<.05*	Veh/Coc	p>.05
Condition X Day	$F_{(2,12)}$=4.89, p<.05*	Cort/Coc	p<.05*
1-way RM ANOVA		Post hoc test: Rst – Cort/Coc vs	
Extinction	$F_{(2,12)}$=1.01, p>.05	Cort/Sal	p>.05
Reinstatement	$F_{(2,12)}$=4.80, p<.05*	Veh/Coc	p>.05

Table S3. Detailed statistics for experiments depicted in Figure 3. Two-way repeated measures (RM) ANOVAs were conducted with reinstatement condition and day (extinction, reinstatement) as factors. These were followed by separate one-way RM ANOVAs examining responding during extinction and reinstatement testing. Post-hoc testing was conducted using Bonferroni-corrected t-tests. * denotes significance. (Ext=extinction; Rst=reinstatement; Coc=cocaine, Cort=corticosterone; Sal=saline; Veh=vehicle).

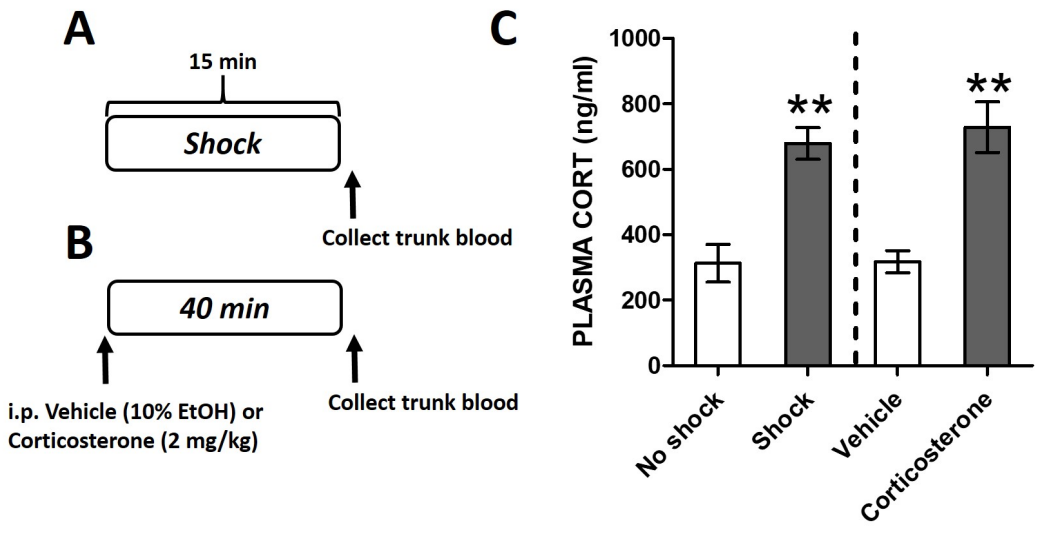
Figure 3A			
2-way RM ANOVA		Post hoc test: Ext vs Rst	
Condition	$F_{(3,15)}=10.64$, $p<.001$*	Veh/Shock/Sal	$p>.05$
Day	$F_{(1,5)}=8.80$, $p<.05$*	Veh/No Shock/Coc	$p>.05$
Condition X Day	$F_{(3,15)}=15.71$, $p<.001$*	Veh/Shock/Coc	$p<.01$*
		AM251/Shock/Coc	$p>.05$
1-way RM ANOVA		Post hoc test: Rst – Veh/Shock/Coc vs	
Extinction	$F_{(3,15)}=.61$, $p>.05$	Veh/Shock/Sal	$p<.05$*
Reinstatement	$F_{(3,15)}=19.26$, $p<.001$*	Veh/No Shock/Coc	$p<.05$*
		AM251/Shock/Coc	$p=.06$
Figure 3B			
2-way RM ANOVA		Post hoc test: Ext vs Rst	
Condition	$F_{(3,15)}=9.52$, $p<.001$*	Veh/Cort/Sal	$p>.05$
Day	$F_{(1,5)}=11.14$, $p<.05$*	Veh/Veh/Coc	$p>.05$
Condition X Day	$F_{(3,15)}=6.94$, $p<.01$*	Veh/Cort/Coc	$p<.05$*
		AM251/Cort/Coc	$p>.05$
1-way RM ANOVA		Post hoc test: Rst – Veh/Cort/Coc vs	
Extinction	$F_{(3,15)}=1.41$, $p>.05$	Veh/Cort/Sal	$p>.05$
Reinstatement	$F_{(3,15)}=14.39$, $p<.001$*	Veh/Veh/Coc	$p>.05$
		AM251/Cort/Coc	$p>.05$

Table S4. Detailed statistics for experiments depicted in Figure 4. Two-way repeated measures (RM) ANOVAs were conducted with reinstatement condition and day (extinction, reinstatement) as factors. These were followed by separate one-way RM ANOVAs examining responding during extinction and reinstatement testing. Post-hoc testing was conducted using Bonferroni-corrected t-tests. * denotes significance. (Ext=extinction; Rst=reinstatement; Coc=cocaine; Sal=saline; Veh=vehicle; WIN=WIN 55,212-2).

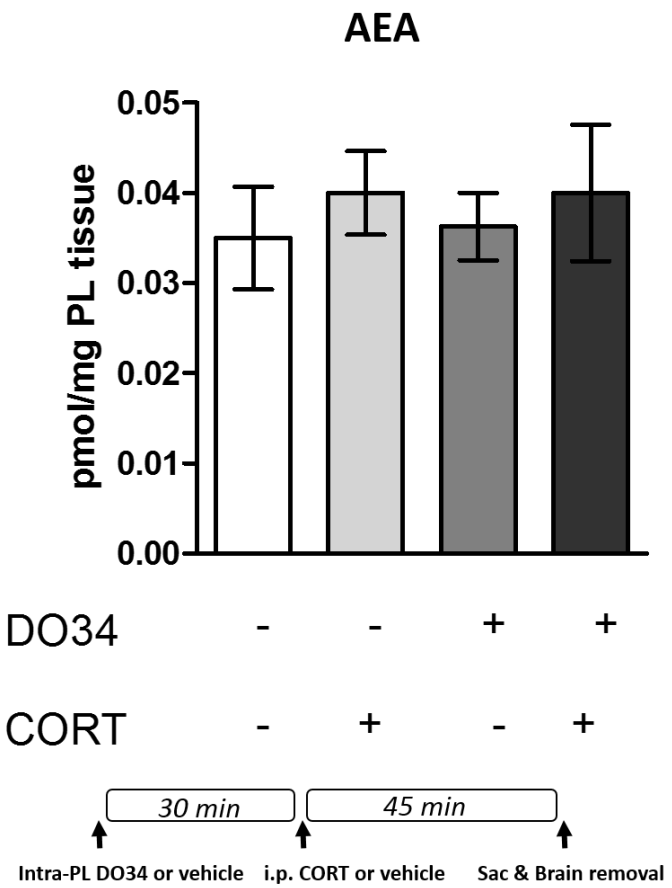
Figure 4A			
2-way RM ANOVA		Post hoc test: Ext vs Rst	
Condition	$F_{(2,10)}$=4.01, p<.05*	WIN/Sal	p>.05
Day	$F_{(1,5)}$=6.24, p<.05*	Veh/Coc	p>.05
Condition X Day	$F_{(2,10)}$=7.09, p<.01*	WIN/Coc	p<.05*
1-way RM ANOVA		Post hoc test: Rst – WIN/Coc vs	
Extinction	$F_{(2,10)}$=0.18, p>.05	WIN/Sal	p>.05
Reinstatement	$F_{(2,10)}$=5.39, p<.05*	Veh/ Coc	p>.05
Figure 4B			
2-way RM ANOVA		Post hoc test: Ext vs Rst	
Condition	$F_{(2,10)}$=5.26, p<.05*	URB602/Sal	p>.05
Day	$F_{(1,5)}$=8.12, p<.05*	Veh/Coc	p>.05
Condition X Day	$F_{(2,10)}$=5.89, p<.05*	URB602/Coc	p<.05*
1-way RM ANOVA		Post hoc test: Rst – URB602/Coc vs	
Extinction	$F_{(2,10)}$=2.94, p>.05	URB602/Sal	p>.05
Reinstatement	$F_{(2,10)}$=7.47, p<.01*	Veh/Coc	P<.01*

Table S5. Detailed statistics for experiments depicted in Figure 5. Two-way repeated measures (RM) ANOVAs were conducted with reinstatement condition and day (extinction, reinstatement) as factors. These were followed by separate one-way RM ANOVAs examining responding during extinction and reinstatement testing. Post-hoc testing was conducted using Bonferroni-corrected t-tests. * denotes significance. (Ext=extinction; Rst=reinstatement; Coc=cocaine; Sal=saline; Veh=vehicle; DO=DO34).

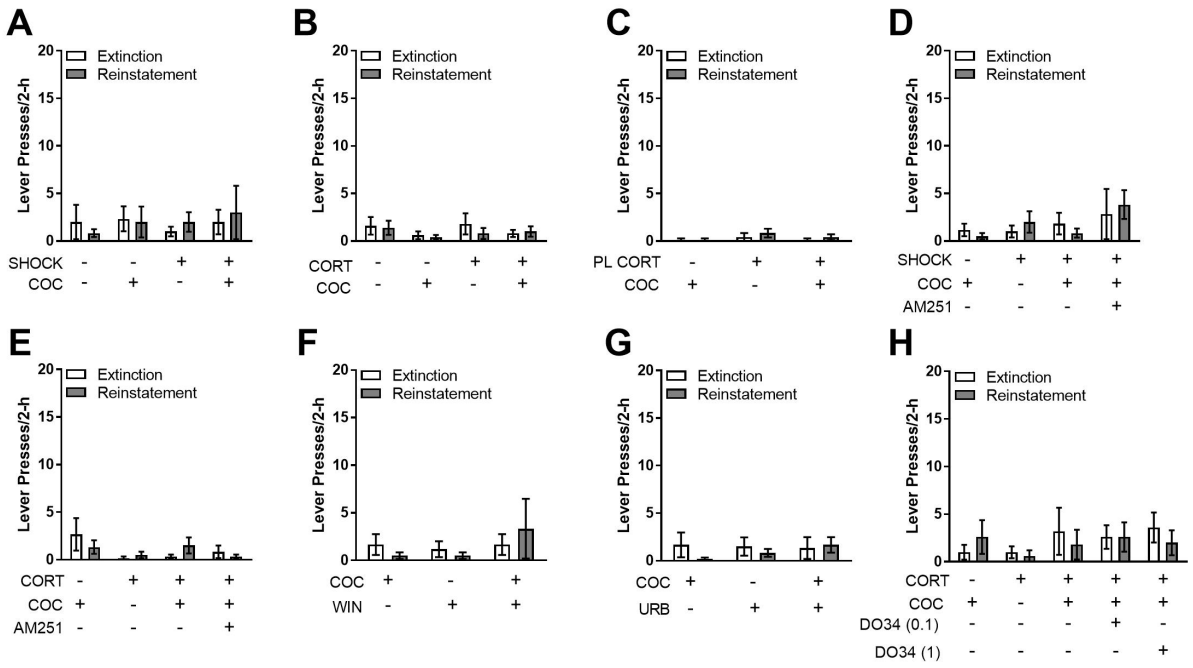
Figure 5C			
2-way RM ANOVA		Post hoc test: Ext vs Rst	
Condition	$F_{(4,16)}=5.28$, $p<.01$*	Veh/Cort/Sal	$p>.05$
Day	$F_{(1,4)}=7.24$, $p<.05$*	Veh/Veh/Coc	$p>.05$
Condition X Day	$F_{(3,16)}=3.84$, $p<.05$*	Veh/Cort/Coc	$p<.05$*
		DO(0.1)/Cort/Coc	$p>.05$
		DO(1.0)/Cort/Coc	$p>.05$
1-way RM ANOVA		Post hoc test: Rst – Veh/Cort/Coc vs	
Extinction	$F_{(4,16)}=2.11$, $p>.05$	Veh/Cort/Sal	$p>.05$
Reinstatement	$F_{(4,16)}=4.62$, $p<.01$*	Veh/Veh/Coc	$p>.05$
		DO(0.1)/Cort/Coc	$p>.05$
		DO(1.0)/Cort/Coc	$p>.05$



Supplemental Figure S1. Plasma levels of corticosterone following footshock or systemic corticosterone administration. A) Timeline of blood collection. Time points were selected to correspond to the start of the reinstatement session in rats tested for behavior. B) 15-min of electric footshock stress (Shock) significantly increases plasma levels of corticosterone (n=7-8, **p<.01 compared to No Shock). Systemic administration of corticosterone (n=5-11, 2 mg/kg, i.p.) significantly increases plasma levels of corticosterone similar to levels observed with footshock (** p<.01, compared to Vehicle). Data are represented as mean ± SEM.



Supplementary Figure S2. Direct administration of the diacylglycerol lipase inhibitor DO34 (1 µg/0.3 µL) into the prelimbic cortex (PL) 30-min prior to a systemic injection of corticosterone (CORT; 2 mg/kg, i.p) has no effect on PL anandamide (AEA) content (n=7-8, $p>.05$).



Supplementary Figure S3. Inactive lever responding during extinction and reinstatement tests. There was no significant effect of reinstatement condition on lever responding under any condition. A) Inactive lever responding corresponding to Fig 1A. B) Inactive lever responding corresponding to Fig 1B. C) Inactive lever responding corresponding to Fig 1C. D) Inactive lever responding corresponding to Fig 3A. E) Inactive lever responding corresponding to Fig 3B. F) Inactive lever responding corresponding to Fig 4A. G) Inactive lever responding corresponding to Fig 4B. H) Inactive lever responding corresponding to Fig 5C. (COC, cocaine; CORT, corticosterone; PL, prelimbic cortex; AM251, CB1R antagonist; WIN, WIN 55,212-2 CB1R agonist; URB602, monoacylglycerol lipase inhibitor; DO34; diacylglycerol lipase inhibitor).

Supplemental References

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