Materials and methods

Surgery to implant the intravenous catheter

Rats received intravenous catheterization of the external jugular vein under anesthesia with isoflurane gas. Briefly, a sylastic catheter was inserted in the right external jugular vein, passed in the mid-scapular region, and secured with a pedestal mount on the back of the rat. The catheter was sealed with a sylastic cap, and covered by a metal tube to prevent group-housed rats from chewing the catheter port. Catheters were flushed with sterile saline (100-150 µL) to prevent them from clogging. We did not use systemic antibiotics, except for rats from experiment 4, which received daily antibiotic treatment (cefazolin, 50 mg/kg/0.3 mL, intravenously, Webster Veterinary Supply, Inc. Devens, MA) because they received two catheter surgeries.

Testing for footshock intensities that produce flinching

Pilot studies showed that behavioral reactivity to electric footshock varied across rats and correlated with body mass. Therefore, to control for possible differences in perceived sensitivity to electric footshock, we adjusted the shock intensity for each rat on the day prior to testing for footshock-induced seeking (experiments 1 and 3). In experiment 4, we extrapolated footshock intensity based on results from experiments 1 and 3. Footshock intensities used for each experiment are provided in Table S1.

To titrate for footshock intensity, the day prior to the reinstatement testing, rats were briefly shocked for 3-4 times (800 ms duration at about every 30 s). This was done in the self-administration chambers equipped with an electric footshock scrambler (Med Associates Inc., St Albans, VT). We appraised the reaction of each rat to the footshock and altered the

footshock intensities in increments to produce flinching while remaining below the threshold for freezing (Nielsen and Crnic, 2002). Rats in the sham footshock group were also given 3 electric footshocks to match the experience of the stress group.

Statistics

All variables (infusions, nose pokes, corticosterone levels, and electric footshock intensity) were analyzed with analysis of variance (ANOVA) using age (adolescents versus adults) as between-subjects factor. When rats self-administered either saline or cocaine (experiment 1), we also used self-administered drug (saline versus cocaine) as between-subjects factor. Days were analyzed as within-subject factor. Correlations were performed with Pearson's test and post-hoc analyses with Newman-Keul's test.

In all self-administration experiments, intake of cocaine was very high on days 1-2 of training, and stabilized from day 3 onward. This is similar to what we observed previously (Wong *et al*, 2013). Therefore, self-administration results include data from day 3 onward only.

Extinction and reinstatement were analyzed using nose pokes in the active hole as the dependent variable, and nose pokes in the inactive hole as a covariate (therefore these statistics have fewer degrees of freedom than other statistics that do not use a covariate).

In experiments using the within-session extinction/reinstatement procedure (experiments 1 and 2), separate groups of rats were tested with or without stress; therefore, the effects of stress were examined as a between-subject factor: footshock (sham electric footshock versus electric footshock), or corticosterone (saline versus corticosterone). In

experiments using the between-session extinction/ reinstatement procedure (experiment 3), the same subjects were tested during repeated extinction/reinstatement sessions; therefore the effects of stress were examined as a within-subject factor: average of 3 days of extinction prior to the reinstatement session (for extinction), versus saline, corticosterone, electric footshock, or yohimbine.

The between-session progressive ratio experiment was analyzed with fixed ratios (1-24) as within-subject factors. The within-session progressive ratio was analyzed as “breaking point” (the highest ratio reached to earn an infusion of cocaine); given that responses increased semi-logarithmically, data were log-transformed, to avoid artificial amplification).

Table

A. Within-session extinction/reinstatement (Experiment 1)

Age of onset of SA	Drug SA	n	Electric footshock intensity (mA)
Adolescent	Cocaine	12	0.37 ± 0.02 (range: 0.35-0.45)
Adult	Cocaine	10	0.39 ± 0.02 (range: 0.35-0.50)
Adolescent	Saline	6	0.36 ± 0.02 (range: 0.30-0.40)
Adult	Saline	5	0.38 ± 0.01 (range: 0.35-0.40)

B. Between-session extinction/reinstatement (Experiment 3)

Age of onset of SA	Drug SA	n	Electric footshock intensity (mA)
Adolescent	Cocaine	12	0.39 ± 0.01 (range: 0.35-0.40)
Adult	Cocaine	10	0.42 ± 0.02 (range: 0.35-0.45)

C. Within-session progressive ratio (Experiment 4)

Age of onset of SA	Drug SA	n	Electric footshock intensity (mA)
Adolescent	Cocaine	10	0.39 ± 0.01 (range: 0.35-0.40)
Adult	Cocaine	8	0.42 ± 0.02 (range: 0.35-0.45)

Table S1. Electric footshock intensities given to rats following prolonged withdrawal from drug self-administration (SA) and subsequently tested for (A) within-session extinction/reinstatement test, (B) between-session extinction/reinstatement test, or (C) self-administration of cocaine using within-session progressive ratios. Values are mean ± SEM.

References

Nielsen DM, Crnic LS (2002). Automated analysis of foot-shock sensitivity and concurrent freezing behavior in mice. *J Neurosci Methods* **115**(2): 199-209.

Wong WC, Ford KA, Pagels NE, McCutcheon JE, Marinelli M (2013). Adolescents are more vulnerable to cocaine addiction: behavioral and electrophysiological evidence. *J Neurosci* **33**(11): 4913-4922.