Supplement 1

Supplementary Methods

Animals

Male Sprague Dawley rats (280-330 g) were housed individually on a 12 h/12 h dark/light cycle and were handled according to the "Guide for the Care and Use of Laboratory Animals" (1). All procedures were approved by the Institutional Animal Care and Use Committee of Rosalind Franklin University of Medicine and Science. Rats were allowed to acclimate for 7-14 days prior to surgically implanting catheters for intravenous self-administration.

Self-administration

Rats were anesthetized with a ketamine/xylazine cocktail (65 mg/kg and 20 mg/kg, respectively) or with isoflurane gas (3%) and were administered flunixin meglumine as an analgesic (2 mg/kg/mL, S.C.). A silastic catheter was inserted in the right auricle through the external jugular vein as previously described (2). After 7-10 days of recovery from surgery, rats were allowed to self-administer cocaine (600 µg/kg/infusion, in a volume of 100 µL/kg/infusion) or saline (100 µL/kg/infusion) during 2 h daily sessions that started approximately 4 h prior to the onset of the dark period. The self-administration chamber (41 × 24 cm floor area; 26 cm high, MED Associates, St. Albans, VT) was outfitted with two holes. Nose pokes in the designated-active hole resulted in an infusion of cocaine or saline dispensed over a period of 3 seconds and illuminated the hole for 30 seconds; this served as a time-out period in which nose pokes in the active hole were counted but did not result in any infusion. Nose poking in the designated inactive hole had no consequences. The number of nose pokes in both holes was recorded using MED Associates software (Schedule Manager).

Extinction

Following the self-administration training, rats were submitted to an extinction phase. During this phase, the cocaine and saline solutions were removed so that nose-poking in the active hole activated the light cue and the sound of the infusion pump without resulting in drug delivery. Extinction lasted 16 days, and only rats that nose-poked in the active hole <25 times during each of the last three extinction sessions were included in these studies. Thus, we excluded one out of 19 rats in the saline self-administration group and four out of 23 rats in the cocaine group.

Swim Stress and Reinstatement

One to two hours following the last extinction session, each rat was transported to a cold room (4-5°C), to be subjected to a brief cold swim stress. Each rat was placed individually in a cylindrical container (37 cm wide, 56 cm tall) that was filled to a 40 cm depth with cold water (4-5°C). This swim stress procedure lasted 4.0-4.5 minutes (3,4). During this time, each rat was continuously monitored. If rats started to sink below the surface of the water they were assisted for several seconds to prevent drowning. As soon as each rat was removed from the water, it was thoroughly dried with a large towel, returned to its home cage, and transported back to the animal room. The next day (i.e. 16-18 hours following this stress procedure), rats were once again placed back in the self-administration chamber to assess possible reinstatement of seeking behavior, using procedures identical to the previous days. Behavior was monitored for a total of four days after the swim stress, to determine the duration of the effect. Two rats, one in the cocaine group and one in the saline group, were excluded from the study, because their nose-poking two days after the end of the stressor were >3 standard deviations above the group mean. Statistical analyses without excluding these rats remain unchanged, but the error bar on reinstatement day 2 is larger.

Statistical Analyses

Data were analyzed with analysis of variance (ANOVA) with Drug (saline vs. cocaine) as between factors, and the following within factors (i.e. repeated measures): Hole (active vs. inactive); Stress (pre-stress vs. post-stress days); Days (four days pre-stress and four days post-stress). Newman-Keul's tests were used for post-hoc comparisons.

The experiments were performed three times. In the first run, the same experimenter performing the swim stress procedure also performed the self-administration, extinction, and reinstatement tests. In order to control for possible conditioned responses to the experimenter performing the stress procedure, the second and third group of rats were stressed by a different experimenter, who had not participated in any other experimental procedure. Results were similar and thus were pooled.

References

1. Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council (1996): *Guide for the Care and Use of Laboratory Animals*. Washington, D.C.: The National Academies Press.
2. Marinelli M, Cooper DC, Baker LK, White FJ (2003): Impulse activity of midbrain dopamine neurons modulates drug-seeking behavior. *Psychopharmacology (Berl).* 168:84-98.
3. Huber J, Darling S, Park K, Soliman KF (2001): Altered responsiveness to stress and NMDA following prenatal exposure to cocaine. *Physiol Behav*. 72:181-188.
4. Saal D, Dong Y, Bonci A, Malenka RC (2003): Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron.* 37:577-582.