Behavioral procedures:

Cocaine Treatment

FoodTraining

Sixteen male Sprague-Dawley rats (250-300 g) were trained to perform an operant response for food delivery. Training was conducted in operant-conditioning chambers enclosed within sound-attenuating cabinets. Chambers contained two levers, one active and one inactive, each with a stimulus light 5 cm above the lever, a food dispenser and a house light. Rats were trained to discriminate the active from the inactive lever. Presses on the active lever resulted in delivery of 45 mg food pellets on a fixed ratio schedule of reinforcement (FR1; one lever press resulted in delivery of one pellet). Presses on the inactive lever were without consequence. Each active lever press was followed by a one-second time out (TO). During the TO, the stimulus light above the active lever was illuminated and the levers retracted. TO was gradually increased to 20 sec over the course of about 5 days. When stable responses for food were achieved, the rats were implanted with an intravenous catheter. None of the animals used in these experiments were food or water restricted during the experiment.

Intra-jugular Catheter Implants

Rats were implanted with a 3 cm Silastic (*ID*: 0.30 mm, *OD*: 0.64 mm) catheter in the right jugular vein under ketamine (45 mg/kg)/xylazine (5.5 mg/kg)/acepromazine (1.1 mg/kg) anesthesia. Each catheter was connected to a 9 cm length tube (*OD*: 0.94 mm) passed subcutaneous to a point between the scapulae. The end of the tubing was fitted to an L-shaped stainless steel tube (22 gauge) that passed through the skin. The distal portion of the metal tube was mounted inside a threaded plastic connector that anchored a drug infusion tube inside the test chamber. Rats recovered for 5 days before the cocaine administration procedure.

Cocaine Self-administration and Passive Drug Exposure

In the operant-conditioning chambers, rats self-administered cocaine ($n = 7$) during daily 3 hours sessions for 11 days. During drug-access sessions, presses on the active lever (FR1) resulted in a 140 µl infusion of cocaine (0.75 mg/kg/infusion) over 4 sec. A 20 sec TO, signal by illumination of the light above the active lever followed the injection. Two other groups of rats received passive, intravenous injections of cocaine ($n = 5$) or saline ($n = 4$). Due to the limitations in recording electrophysiological responses at precisely the same time in each rat, animals in the passive drug group received the same amount of cocaine across the same time period as rats in the self-administration group. This was done by programming a computer to deliver cocaine (or saline in the control group) using inter-infusion intervals calculated from responses of animals in the self-administration group. Both levers were constantly retracted for passive cocaine and saline-treated rats. To compare our results in the BNST to previous studies on VTA cells, age-matched rats received a single intraperitoneal injection of cocaine (20 mg/kg/0.1ml, $n = 3$) or saline ($n = 3$). Cocaine hydrochloride (obtained from Research Triangle Institute under the NIDA

drug supply program) was dissolved in sterile saline (0.9 %) and pH was adjusted to 7.0 with NaOH.

Food Self-administration

Fourteen male Sprague-Dawley rats (250-300 gm) were used to test the effect of food reward on BNST recordings. These rats were trained to perform a lever-press operant for 45 mg food pellets as described above. Acquisition of the lever-press response was judged complete when rats self-administered 50 food pellets in a 60 min session. Following acquisition, and to maintain comparability of recordings in these rats to those obtained from cocaine treated rats, animals were anesthetized and implanted with a sham intra-jugular catheter. The sham catheter consisted of an L-shaped stainless steel tube (22 gauge) mounted inside a threaded plastic connector that passed through the skin between the scapulae. Animals recovered from surgery for 5 days and were then divided into 2 groups. One group (n = 6) continued to self-administer food on a FR reinforcement schedule in 2 hour daily sessions for 11 consecutive days. The value of the reinforcement ratio was set between FR3 and FR9 at a level where animals received on average 38 food pellets in a 2 hr session. The second group (n = 6) received food pellets independent of their behavior, in a quantity and pattern determined by the average response number and pattern of the food self-administration rats. As with the passive cocaine exposure group in the first experiment, a true-yoked procedure was not used because electrophysiological recordings needed to be carried out at the exact same time after the last food session. All animals had *ad libitum* access to laboratory chow and water in home cages throughout the study.

Whole-cell voltage clamp recordings:

Brain slices preparation

Twenty-four hours after the last treatment, rats were anesthetized with halothane for brain slices preparation. Coronal slices (250 µm) containing the BNST were prepared in a physiological solution containing (in mM) 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$ and, 11 D-glucose at 15°C. Slices were incubated at 34°C for 30 minutes and transferred to a chamber constantly perfused (1.5 ml/min) with physiological solution maintained at 34°C and equilibrated with 95%O_2/5%CO_2.

Evoked excitatory postsynaptic currents (EPSCs)

Recordings were made in the anterior part of the lateral BNST (Bregma –0.26 mm). At this level of the vlBNST, two subpopulations of neurons have been distinguished in a previous publication. The present study reported only recordings from neurons that share physiological properties with those projecting to the ventral tegmental area[1]. Whole-cell voltage-clamp recordings were made using microelectrodes filled with a solution containing (in mM) 130 Cs^+MeSO3^-, 1 EGTA, 5 HEPES, 2 MgATP, and 0.3 GTP. EPSCs were evoked by local fiber stimulation (0.1ms, 0.1 Hz) using bipolar tungsten electrodes. Neurons were first voltage-clamped at –60 mV and AMPA EPSCs were evoked. Stimulation intensity was adjusted to obtain approximately 80% of the maximum response.

Neurons were then voltage-clamped at +40mV to release Mg^{++}-induced blockade of NMDA receptors. After a stabilization period of 5 to 15 min, the NMDA receptor antagonist D-AP5 (5 min, 50 μM) was bath applied to isolate AMPA currents. Isolated AMPA currents were digitally subtracted offline from the total EPSCs to obtain the NMDA currents. AMPA to NMDA ratios were calculated and compared using a one-way analysis of variance across the 8 groups of rats. Multiple posthocs comparisons were done using a Bonferronni's test.

1. Dumont, E. C. & Williams, J. T. *J Neurosci* **24**, 8198-204 (2004).