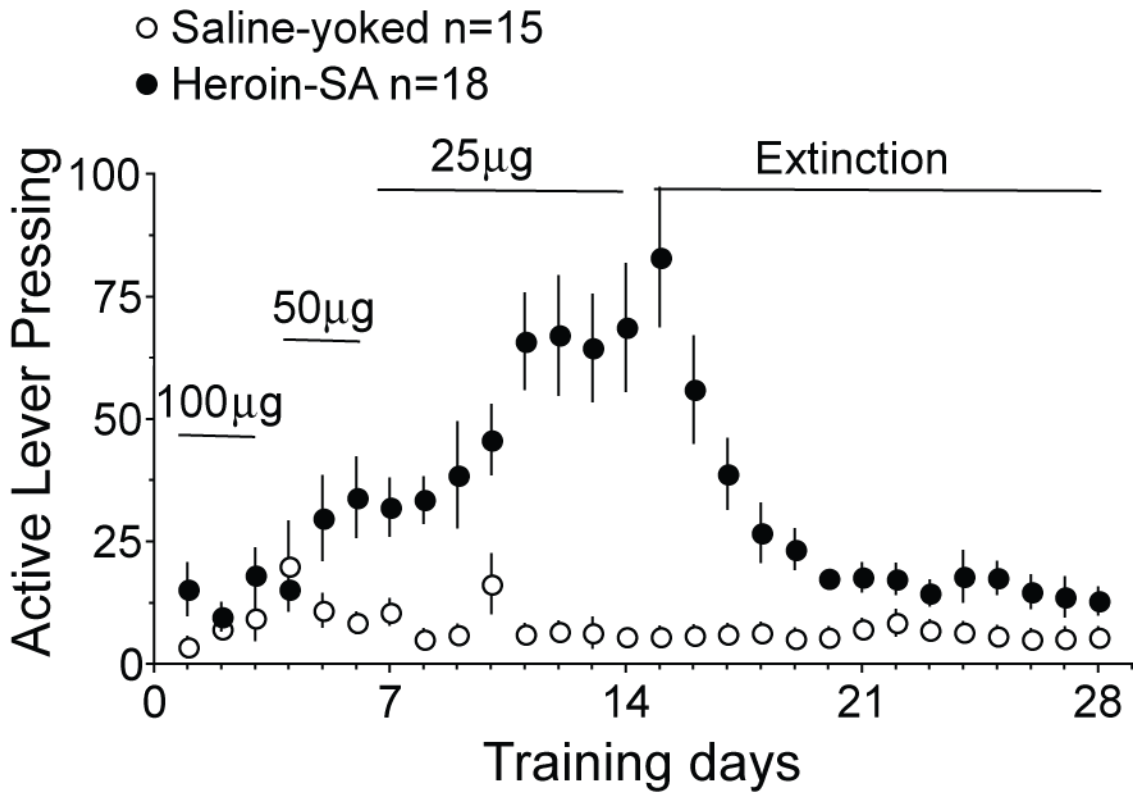
Supplemental Figure

Figure S1. Active lever pressing in rats trained to self-administer heroin and in yoked saline controls. The dose of heroin during self-administration training was reduced from 100 to 25 µg per infusion as shown.



Supplemental Methods

Animal and surgery

Male Sprague Dawley rats (250 g on arrival; Charles River Laboratories, Wilmington, MA) were individually housed in a temperature- and humidity-controlled environment with a 12 hr dark/light cycle (6:00 P.M. lights on). Experiments were conducted during the rats' dark cycle. Rats received food ad libitum until 1 d before behavioral training, after which food restriction procedures (20 g of rat chow per day) were implemented and maintained throughout the duration of the experiment. Rats were allowed 1 week to acclimate to the vivarium before inducing anesthesia and implanting jugular catheters. The surgical details have been described in a previous study (LaLumiere and Kalivas, 2008). In brief, the rats were anesthetized with ketamine HCl (87.5 mg/kg, i.m.) and xylazine (5 mg/kg, i.m.). Ketorolac (3 mg/kg, i.p.) was administered before surgery to provide analgesia. Silicone tubing was inserted subcutaneously between the shoulder blades and exited the skin via a dermal biopsy hole. The other end of the tubing was threaded under the skin, inserted 3 cm into the right jugular vein, and then sutured to the vein behind a silicone ball to secure its placement. The rats were then returned to their home cages for 7 days for recovery, and were flushed with heparin daily via the catheter until the last day of self-administration.

Heroin self-administration procedures

All self-administration experiments occurred in standard operant chambers with two retractable levers, a house light, and a cue light and tone generator (Med Associates, Fairfield, VT). All active lever presses, including those during the timeout, were

recorded and are reported as "active lever presses." During 3-hour sessions on 14 consecutive days, rats were trained to press the active lever on a fixed ratio 1 (FR-1) schedule with 20 sec timeout for an infusion of heroin-hydrochloride (100 μg/infusion for day 1–2, 50 μg/4 s infusion for day 3–4, 25 μg/infusion for day 5–14. Heroin was kindly provided by National Institute on Drug Abuse). Concurrent with the drug infusion, a cue tone (2900 Hz) and cue light immediately above the active lever turned on. Any rats that did not obtain 15 infusions per day for the last 3 d of self-administration were excluded from analysis. After 14 days of self-administration, rats began extinction training for two weeks Active lever presses produced no drug infusion or light/tone cues. Before the electrophysiological recording, cocaine-extinguished rats were required to meet a criterion of an average of <10 lever presses for 3 consecutive days. During self-administration, some rats were yoked to heroin self- administering rats and received noncontingent saline (yoked-saline) in the same temporal pattern as their heroin self-administering partners.

Electrophysiology in vivo

The rats were anesthetized with urethane (1.5 g/kg, i.p.), and mounted in a stereotaxic apparatus (Narishige). Subcutaneous atropine methylbromide (0.3 mg/kg) was used to minimize secretions and improve ventilation as needed, and core temperature was maintained using a heating pad. Concentric bipolar stimulating electrodes (Rhodes Medical Instruments) were placed in the prelimbic medial prefrontal cortex [anteroposterior (AP), +3.0 mm; mediolateral (ML), +0.6 mm; dorsoventral (DV), –3.3 mm from brain surface]. Glass recording electrodes were pulled using a Narishige PE-2

puller (1–2 MΩ). Filling solution consisted of 0.5M sodium acetate and 2% pontamine sky blue. Recording electrodes were aimed at the dorsomedial region of the NAc (AP, +1.8 mm; ML, +1.3–1.5 mm; DV, –6.2 to –6.4 mm from brain surface). The preparation was further stabilized with agar. Extracellular field potentials were amplified by a NPI Instruments SEC-05LX amplifier, and the data bandpass filtered at 300 Hz, then digitized by a National Instruments PCM-C1016E4 board feeding into a computer. Custom Labview Software (Lee Campbell, Salk Institute, La Jolla, CA) was used for data collection and analysis. To ensure the accuracy and stability of the recordings, baseline measurements were obtained 1 h after surgery. Field potential amplitude was estimated as the difference between the mean of a 2–4 ms window before the stimulation artifact and the mean of a 1 ms window around 15 ms after the stimulation artifact (corresponding to the negative peak of the field potential). The LTP protocol involved tetanic stimulation at the minimum current intensity that evoked a maximum field response (from an input-output curve) using two bursts of 100 pulses at 50 Hz with a 10- to 20-s interburst interval. The LTD protocol involved stimulation at the minimum current intensity that evoked a maximum response using three trains of 900 pulses at 5 Hz with a 5-min intertrain interval. The data were normalized to baseline field amplitude in each group and evaluated using a two-way ANOVA with repeated measures over time.

LaLumiere RT, Kalivas PW. (2008). Glutamate release in the nucleus accumbens core is necessary for heroin seeking. *Journal of Neuroscience* **28**, 3170-3177.