Supporting Information

Willuhn et al. 10.1073/pnas.1213460109

SI Experimental Procedures

Animals. Adult male Wistar rats from Charles River weighing 300–400 g were housed individually and kept on a 12-h light/12-h dark cycle (lights on at 0700) with controlled temperature and humidity and with food and water available ad libitum. All animal use was approved by the University of Washington Institutional Animal Care and Use Committee, and surgical procedures were performed under aseptic conditions. For the first voltammetry experiment, 34 animals underwent surgery; of these animals, 18 maintained catheter patency throughout the experiment, had at least one functional and histologically verified electrode, and passed behavioral criteria (see below). For the pharmacological experiment, 32 of 39 rats that underwent cannulation had histologically verified bilateral cannula placements in the dorsolateral striatum (DLS), maintained i.v. catheter patency, and were used in the study. Of the 21 rats prepared for the lesion experiment, 17 maintained catheter patency, had a histologically verified lesion of the nucleus accumbens core, had at least one functional and histologically verified electrode in the DLS, and passed behavioral criteria.

Stereotaxic Surgery. Rats were anesthetized with isoflurane and placed in a stereotaxic frame. The scalp was swabbed with alcohol and Betadine, bathed with a mixture of lidocaine (0.5 mg/kg) and bupivacaine (0.5 mg/kg), and incised to expose the cranium. Holes were drilled in the cranium, and dura mater was cleared for targeting of the DLS [1.2-mm anterior, 3.1-mm lateral, and 4.8-mm ventral to bregma (1)] and, in some animals, the nucleus accumbens core of the ventral medial striatum (VMS) (1.3-mm anterior, 1.3-mm lateral, and 7.2-mm ventral to bregma). For the first set of animals, one carbon-fiber microelectrode made in-house (2) was positioned in the nucleus accumbens core and another in the DLS, and an Ag/AgCl reference electrode was implanted in a separate part of the forebrain. For the next set of animals, guide cannulas (26 gauge; Plastics One) occluded by dummy cannulas of equal length were implanted bilaterally to target the DLS. In a final set of animals, quinolinic acid (0.09 M; 0.5 μL) was infused unilaterally into the nucleus accumbens core to induce an excitotoxic lesion (3), and carbon-fiber microelectrodes were implanted bilaterally into DLS. Electrodes and guide cannula were secured with cranioplastic cement anchored to the skull by screws. After surgery, rats were placed on an isothermal pad to maintain body temperature until ambulatory and were allowed to recover for at least 5 d. Rats were administered a long-acting, nonsteroidal anti-inflammatory, either meloxicam (1 mg/kg, s.c.) before surgery or carprofen (2 mg/kg, s.c.) just after surgery. All animals were implanted with i.v. catheters during a separate surgery.

Implantation of i.v. Catheters. Rats were anesthetized with isoflurane. Catheters were made of Silastic tubing with an outer diameter of 0.6 mm and were attached to a "hub" at one end (distal to vein insertion) (Plastics One) for connection to an infusion pump. Catheters were pushed s.c. through an incision on the back between the shoulders to the front of the body and were anchored into the right jugular vein aided by a silicon rubber bead near the proximal end of the catheter. Optimal positioning of the catheter was verified by drawing blood into it with negative pressure. The hub then was secured by a piece of Teflon mesh sutured to surrounding tissue, and incisions were closed, leaving the hub protruding from the rat's back. The catheter was flushed with a heparin solution (80 U/mL in saline) and was filled with a viscous solution of polyvinylpyrolidone (PVP) and heparin (1,000 U/mL). The catheter hub was capped with a short, crimped piece of polyethylene tubing, and the PVP solution remained in the catheter to ensure patency. After surgery, rats were allowed to recover for at least 5 d.

Cocaine Self-Administration. Self-administration sessions were conducted between 1000 and 1600 h. Rats learned to self-administer cocaine (Sigma) in a modular operant chamber (Med Associates) equipped with two nose-poke response devices (with integrated cue lights) located on adjacent panels of the same wall, a house light, and speakers to provide pure-tone and white-noise stimuli. The operant chamber was housed within a sound-attenuated outer chamber. Rats were trained to obtain cocaine following an operant response on a continuous reinforcement (FR-1) schedule. Nose-poking in the active port (the side was counterbalanced between animals) resulted in an immediate i.v. infusion of cocaine (0.5 mg/kg over about 10 s) paired with a 20-s presentation of an audiovisual stimulus [illumination of the light inside the nose poke port and tone; conditioned stimulus (CS)]. During CS presentation, a 20-s time out was imposed during which nose poking did not result in further drug infusion or any other programmed consequences. Drug availability during the session was signified by white noise and illumination of the house light. To control for response specificity, nose-poking of the second (inactive) port was monitored but was never reinforced. A criterion for inclusion in the study was five or more active responses per session on two successive sessions. The number of precriterion sessions varied among animals from none to seven sessions. After pretraining sessions, rats were given daily access to cocaine for 1 h, 6 d/wk, for 3 wk.

Infusion of Flupenthixol into the DLS. The effects on drug-taking behavior of bilateral infusion of the nonspecific dopamine receptor antagonist flupenthixol [5 μg dissolved in 0.5 μl artificial cerebrospinal fluid (ACSF) (Sigma) into each side; 0.5 μl/min] and ACSF (vehicle) into DLS were examined in single sessions during the first or third weeks of self-administration. The dose of flupenthixol used did not inhibit general performance, as determined in pilot studies and previous studies conducted by other investigators (3, 4). To avoid potentially confounding effects of repeated flupenthixol administration, one group of rats received flupenthixol and ACSF in the first week of cocaine self-administration, counterbalanced on 2 d, and a separate group received counterbalanced infusions in the third week ($n = 16$ each). On infusion days, the dummy cannula was replaced with a 33-gauge infusion cannula that protruded 1.0 mm beyond the guide cannula. Infusions were given 5 min before session start. After the infusion, the infusion cannulas were left in place for 2 min to allow diffusion of the drug.

Voltammetric Measurements and Analysis. For dopamine detection by fast-scan cyclic voltammetry during experimental sessions (recordings were performed during two sessions per week), chronically implanted carbon-fiber microsensors were connected to a head-mounted voltammetric amplifier, interfaced with a PC-driven data-acquisition and analysis system (National Instruments) through an electrical swivel (Med Associates) that was mounted above the test chamber. Voltammetric scans were repeated every 100 ms to achieve a sampling rate of 10 Hz. During each voltammetric scan, the potential at the carbon-fiber electrode was ramped linearly from −0.4 V versus Ag/AgCl to +1.3 V and back at 400 V/s (total scan time, 8.5 ms) and held at −0.4 V between scans. When dopamine is present at the surface of the electrode, it is oxidized during the anodic sweep to form dopamine-*o*-quinone (peak

reaction detected at approximately +0.7 V), which is reduced back to dopamine in the cathodic sweep (peak reaction detected at approximately −0.3 V). The ensuing flux of electrons is measured as current and is directly proportional to the number of molecules that undergo electrolysis. The background-subtracted, time-resolved current obtained from each scan provided a chemical signature characteristic of the analyte, allowing resolution of dopamine from other substances (5). Voltammetric data were band-pass filtered at 0.025–2,000 Hz. Dopamine was isolated from the voltammetric signal using chemometric analysis using a standard training set (2) based on electrically stimulated dopamine release detected by chronically implanted electrodes. Dopamine concentration was estimated based on the average postimplantation sensitivity of electrodes (2). Before analysis of average concentration, all data were smoothed with a five-point within-trial running average. The concentration of dopamine was averaged over the 7 s (approximate duration of the observed phasic signal) following the operant response (postresponse) or noncontingent presentation of the CS and was compared with the average concentration over the 2 s prior to the response or CS (baseline). The CS was presented noncontingently (twice per session for 20 s each) during every recording session conducted in the second and third weeks but not during the first week to avoid interference with the associative conditioning between drug delivery and the cue during the period when this association presumably was still developing.

Statistical Analysis. Individual signals were averaged across session and then across animals and weeks to increase statistical power. Averages then were compared using one-, two-, and three-way ANOVAs with response, brain region, and week as factors. For comparison with voltammetric results, behavioral data were grouped into weeks. For the flupenthixol-infusion experiment, baseline data grouped by weeks were computed by averaging the data over 3 d during the week without infusions. Behavioral data were analyzed using one- and two-way ANOVAs with drug and weeks as factors. In case of significant main effects or interactions, post hoc analyses were conducted, and *P* values were adjusted according to the Holm–Bonferroni correction method for multiple testing (6). Plots were made using Prism (GraphPad Software). All statistical analyses were carried out using SPSS, version 17.0.

Histological Verification of Recording Sites. On completion of experimentation, animals were anesthetized with i.p. ketamine (100 mg/kg) and xylazine (20 mg/kg). In animals with electrode implants, recording sites were marked with an electrolytic lesion (300 V) before transcardial perfusion with saline followed by 4% paraformaldehyde (40 g/l). Brains were removed and postfixed in paraformaldehyde for 24 h and then were rapidly frozen in an isopentane bath (~5 min), sliced on a cryostat (50-μm coronal sections, −20 °C), and stained with cresyl violet to aid in visualization of anatomical structures and the electrode-induced lesion or infusion sites.

SI Discussion

In most of the previous work that measured phasic dopamine release in the nucleus accumbens core during cocaine self-administration, recording sites were optimized for maximal stimulated dopamine release (7–10) and therefore sampled maximum dopamine responses. Only one previous study (experiment 2 in ref. 10) selected recording sites by electrophysiological recording rather than by neurochemical responses and thus provided an unbiased sample of the nucleus accumbens core, measuring the average dopamine responses across this region which exhibits neurochemical heterogeneity (11). In the current work, implantation sites were based entirely on stereotaxic coordinates without functional feedback and so also provide an unbiased sample of the nucleus accumbens core. Notably, there is remarkable concordance between these two studies with unbiased sampling in both the concentration and the dynamics of the phasic dopamine response to cocaine self-administration (Fig. S3).

1. Paxinos G, Watson C (1998) *The Rat Brain in Stereotaxic Coordinates* (Academic, New York).
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3. Belin D, Everitt BJ (2008) Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. *Neuron* 57(3):432–441.
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7. Phillips PEM, Stuber GD, Heien ML, Wightman RM, Carelli RM (2003) Subsecond dopamine release promotes cocaine seeking. *Nature* 422(6932):614–618.
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10. Owesson-White CA, et al. (2009) Neural encoding of cocaine-seeking behavior is coincident with phasic dopamine release in the accumbens core and shell. *Eur J Neurosci* 30(6):1117–1127.
11. Wightman RM, et al. (2007) Dopamine release is heterogeneous within microenvironments of the rat nucleus accumbens. *Eur J Neurosci* 26(7):2046–2054.

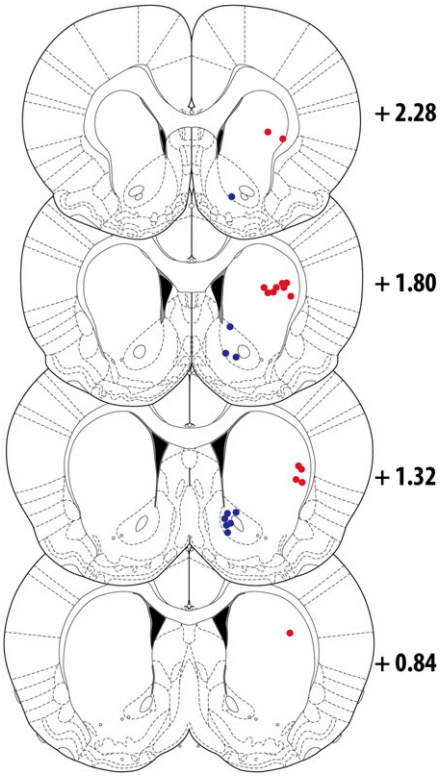

Fig. S1. Histological verification of recording sites in the VMS and DLS (first experiment). VMS recording sites (blue circles) were confirmed to be within the nucleus accumbens core, and DLS recording sites (dark red circles) were in the lateral half of the dorsal striatum. The numbers on each plate indicate the distance in millimeters anterior from bregma (1).

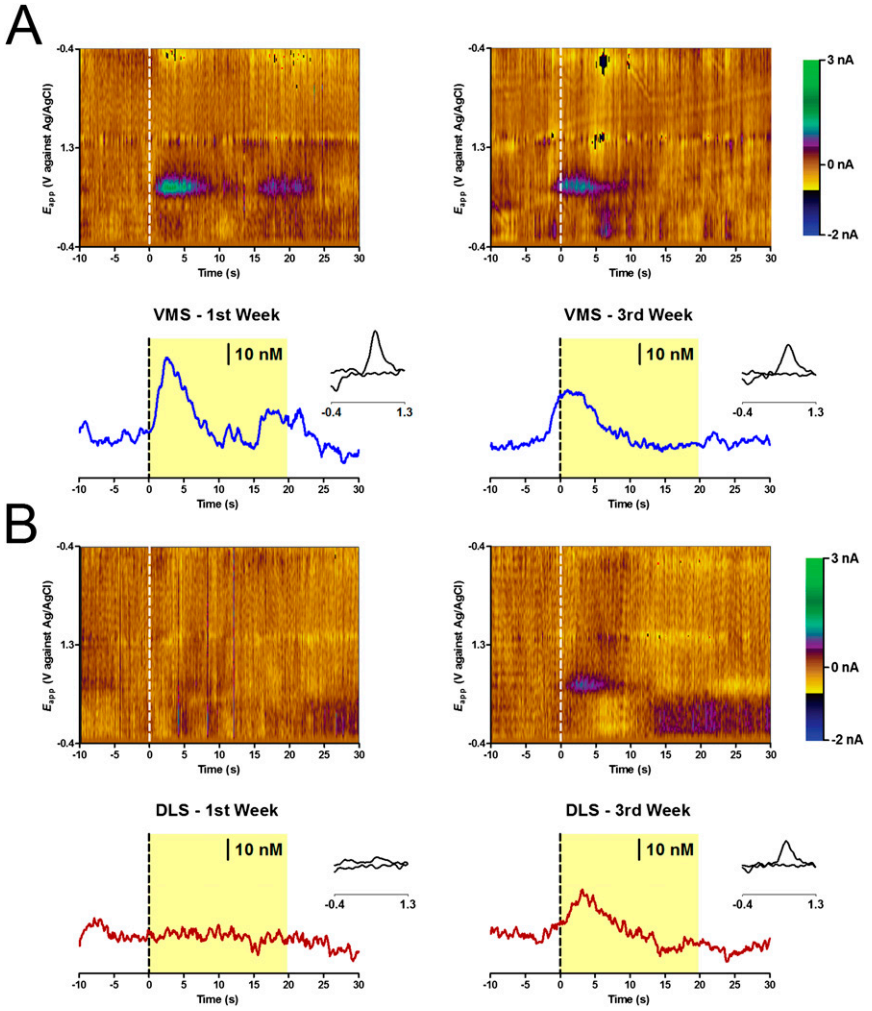

Fig. S2. Examples of phasic dopamine release in the VMS and DLS associated with an individual nose poke (single trial) into the active port. (*A*) Pseudocolor plots (*Upper*), dopamine traces (*Lower*), and cyclic voltammograms (*Insets* in lower panel) for representative current fluctuations recorded in the VMS for the period 10 s before an operant response (dashed line), during the subsequent 20-s presentation of the CS (yellow box), and 10 s after the offset of the CS during the first (*Left*) and third (*Right*) weeks of cocaine self-administration. (*B*) Pseudocolor plots (*Upper*), dopamine traces (*Lower*), and cyclic voltammograms (*Insets* in lower panel) for representative current fluctuations recorded in the DLS during the first (*Left*) and third (*Right*) weeks of self-administration. The color plots show current changes across the applied voltages (E_{app}; *y* axis) over time (*x* axis).

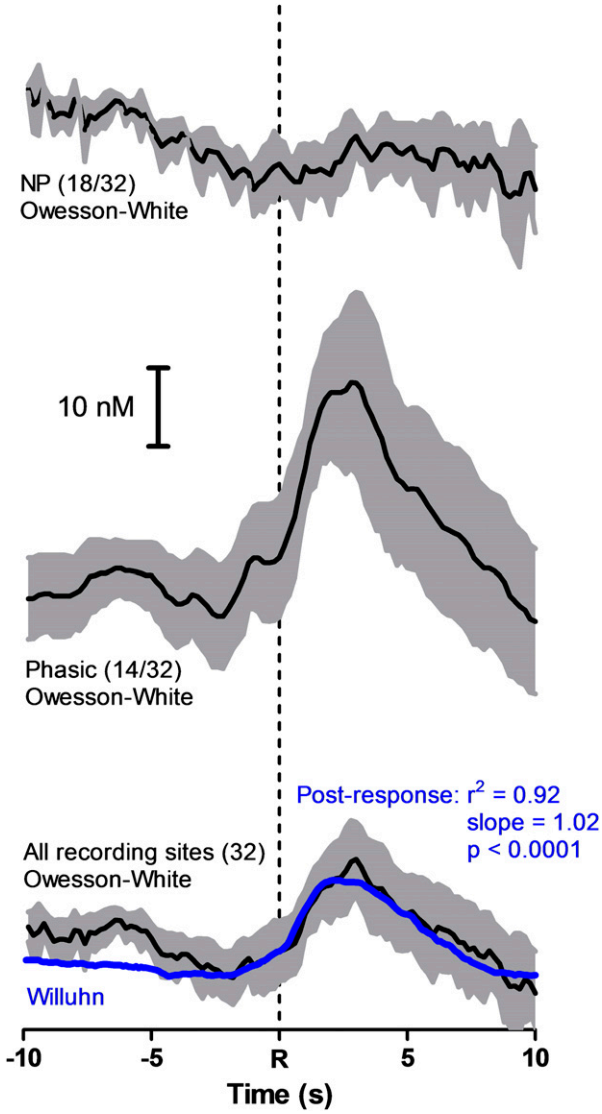

Fig. S3. Comparison of data from Owesson-White et al. (10) and the current findings. Phasic dopamine release (digitized using GetData Graph Digitizer, http://getdata-graph-digitizer.com) from recording sites adjacent to neurons whose firing rates were responsive (*Middle*) or unresponsive (*Top*) to cocaine self-administration were combined to produce a weighted average (*Bottom*). This average response was similar to the average response in the current study obtained from the nucleus accumbens core in the first week of cocaine self-administration (*Bottom*, blue trace). The phasic responses that follow an operant response (*R*) for cocaine from the two studies have concentration profiles that match closely in shape (r^2 = 0.92) and amplitude (slope = 1.02).

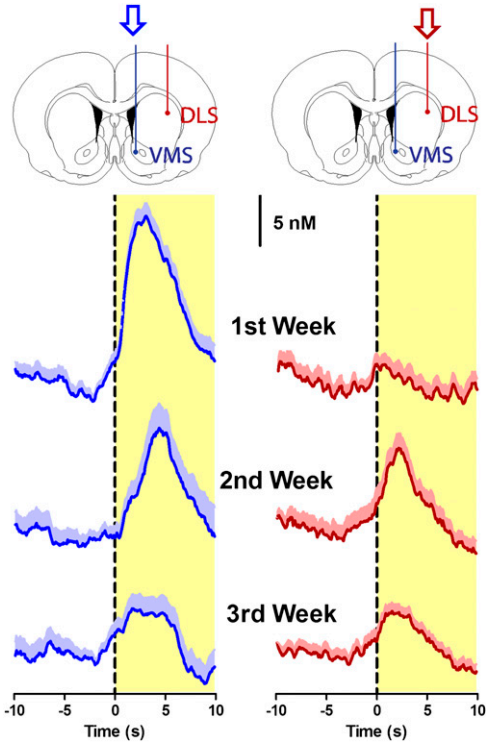

Fig. S4. Phasic dopamine signaling in the VMS and DLS for an individual animal. The pattern of average phasic dopamine release in the VMS (*Left*) and DLS (*Right*) is depicted 10 s before and 10 s after responses into the active nose-poke port across 3 wk of cocaine self-administration in an individual animal. Data are expressed as mean + SEM per week.

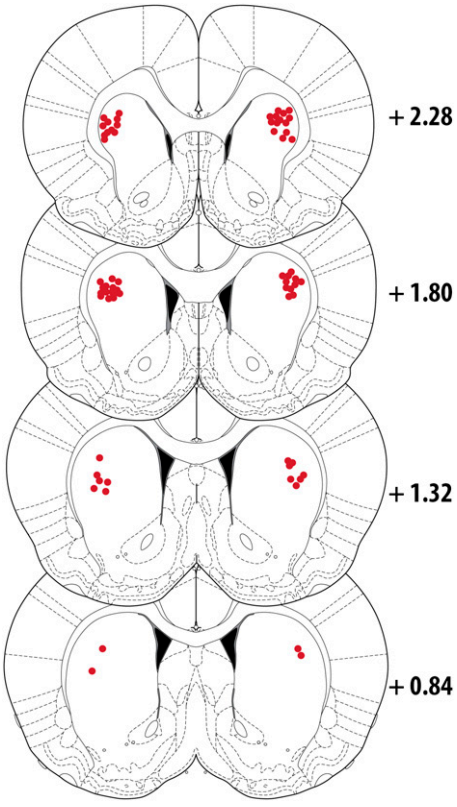

Fig. S5. Histological verification of infusion sites in the DLS (pharmacological experiment). DLS infusion sites (dark red circles) were verified to be in the lateral half of the dorsal striatum. The numbers on each plate indicate the distance in millimeters anterior from bregma (1).

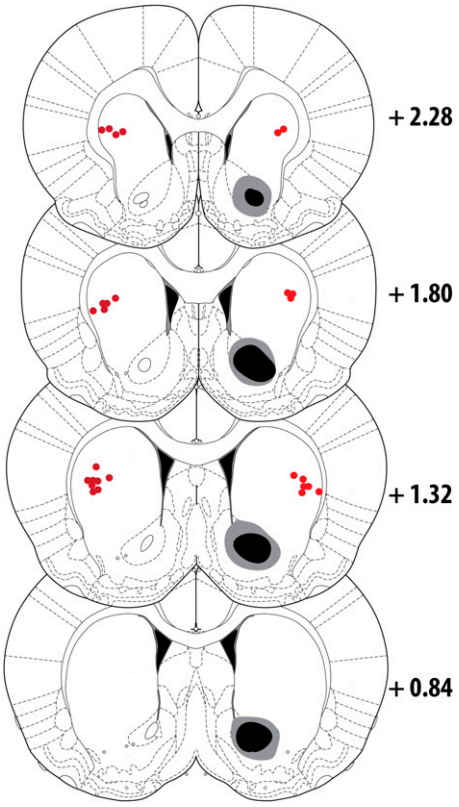

Fig. S6. Histological verification of lesion sites in VMS and recording sites in the DLS (lesion experiment). DLS recording sites were verified to be in the lateral half of the dorsal striatum, and infusions of quinolinic acid produced lesions in the nucleus accumbens core (VMS). Recording sites in the DLS ipsilateral to the lesion are shown in light red (*Right*). DLS recording sites in the contralateral hemisphere are shown in dark red (*Left*). Areas shaded in gray and black represent the largest and smallest extent of neuronal damage, respectively. The numbers on each plate indicate the distance in millimeters anterior from bregma (1).

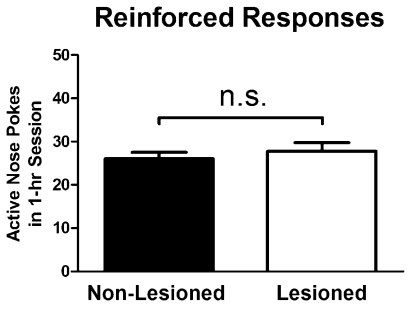

Fig. S7. Responses to obtain cocaine infusions by animals with intact VMS (first experiment) and by animals with unilateral lesion of the VMS (lesion experiment). The average total number of reinforced nose pokes (mean + SEM) did not differ significantly ($P > 0.05$) in nonlesioned rats (filled bar) and rats with unilateral lesion of the VMS (open bar). n.s., not significant.

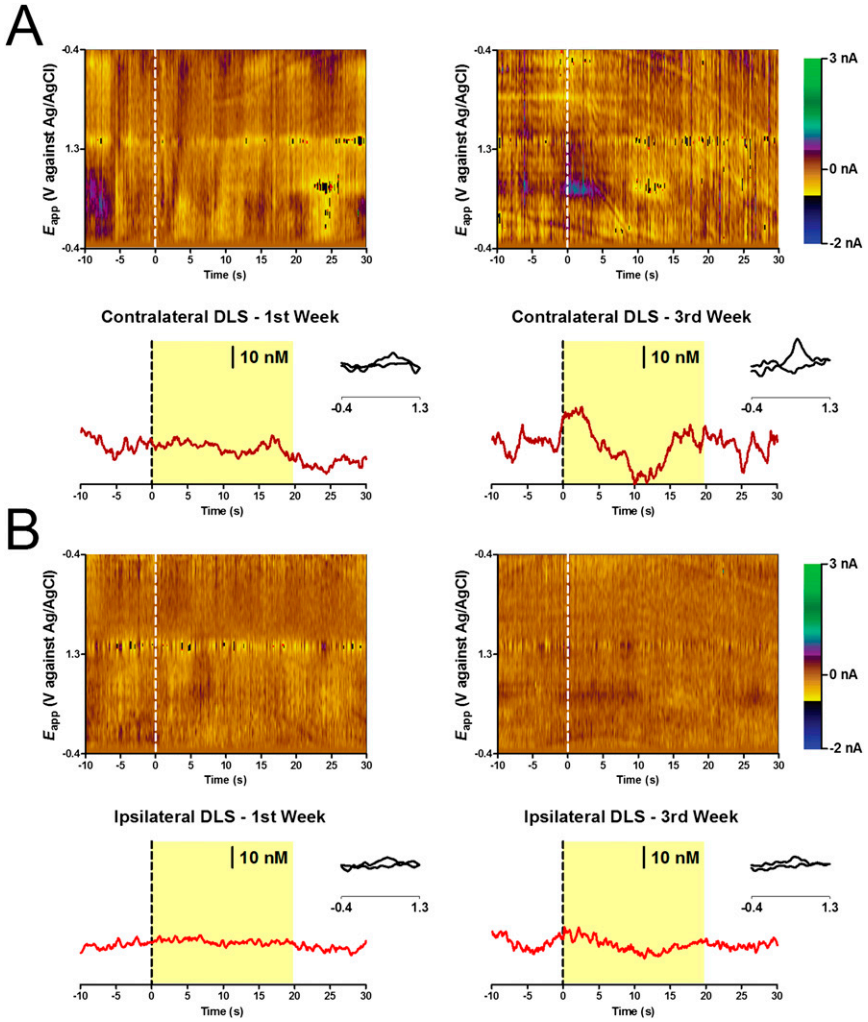

Fig. S8. Examples of phasic dopamine release in the DLS contralateral and ipsilateral to lesioned VMS associated with an individual nose poke (single trial) in the active port. (*A*) Pseudocolor plots (*Upper*), dopamine traces (*Lower*), and cyclic voltammograms (*Insets* in lower panel) for representative current fluctuations recorded in the DLS of the hemisphere contralateral to the VMS lesion for the period 10 s before an operant response (dashed line), during the subsequent 20-s presentation of the CS (yellow box), and 10 s after the offset of the CS during the first (*Left*) and third (*Right*) weeks of cocaine self-administration. (*B*) Pseudocolor plots (*Upper*), dopamine traces (*Lower*), and cyclic voltammograms (*Insets* in lower panel) for representative current fluctuations recorded in the DLS of the hemisphere ipsilateral to the VMS lesion during the first (*Left*) and third (*Right*) weeks of self-administration. The color plots show current changes across the applied voltages (E_{app}; *y* axis) over time (*x* axis).

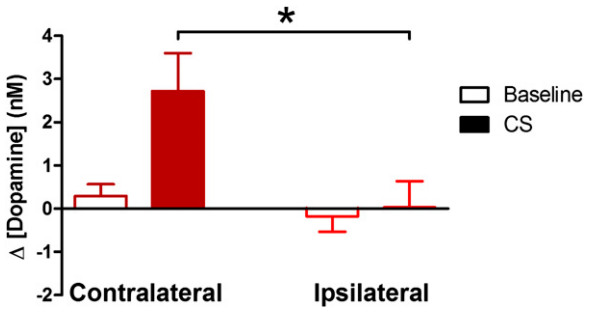

Fig. S9. CS-induced dopamine signaling in the DLS contralateral and ipsilateral to lesioned VMS (n = 17 and 11 electrodes, respectively, in 17 rats). The average phasic release of dopamine in response to the noncontingent presentation of the drug CS is greater in the DLS of the hemisphere contralateral to the VMS lesion than in the ipsilateral DLS ($P_{Holm} < 0.05$). Data are expressed as mean + SEM.

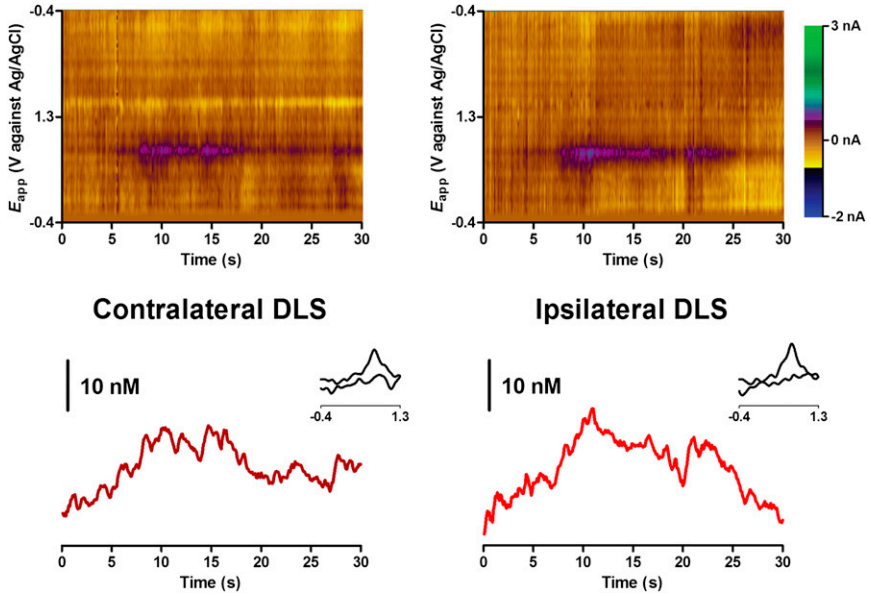

Fig. S10. Spontaneous release of dopamine in the DLS contralateral and ipsilateral to the lesioned VMS. Averaged pseudocolor plot (*Upper*), dopamine traces (*Lower*), and voltammograms (*Insets* in lower panel) for spontaneous current fluctuations (n = 7 electrodes in 4 rats) recorded in the DLS of the hemisphere contralateral (*Left*) or ipsilateral (*Right*) to the VMS lesion for a 30-s period. Dopamine release in the and ipsilateral DLS did not differ significantly [$t(11) = -0.913$, $P = 0.381$], indicating that spontaneous dopamine release (i.e.,during time periods free of operant responding and CS presentation) in the DLS is not affected by the VMS lesion.