Supporting Information

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SI Materials and Methods

Surgical Procedures. Male Sprague–Dawley rats (260–320 g; Harlan) were individually housed on a 12:12 light cycle (lights on at 07:00 h), in clear plastic cages with ad libitum access to food and water. They were anesthetized with xylazine hydrochloride (10 mg/kg, i.p.) and ketamine hydrochloride (100 mg/kg, i.p.) and placed in a stereotaxic frame. The rostral placement in the NAc shell was to minimize norepinephrine contributions (1) because dopamine and norepinephrine cannot be voltammetrically distinguished (2). Norepinephrine terminals do occur at more ventral locations than were used in this work. The vertical position of the stimulating electrode was adjusted until a characteristic behavioral reflex was evoked by an electrical stimulation (biphasic constant-current pulses, 2 ms each phase, 60 Hz, 60 pulses). Then the stimulating electrode, guide cannula, and reference electrode were permanently affixed to the skull with machine screws and cranioplastic cement, and the micromanipulator was removed.

ICSS. Rats were allowed to recover from surgery for at least 48 h before being placed in a behavioral chamber (Med Associates) that contained a retractable lever with a cue light above it. A carbon-fiber microelectrode was inserted into the NAc shell with the microdrive that was locked into the previously implanted guide cannula. The carbon-fiber electrode was connected to a locally constructed headstage amplifier, and cables to all of the electrodes were connected to a rotating commutator (Crist Instrument Co.) located at the top of the behavioral chamber that allowed unrestricted movement.

When the animal depressed the lever, a stimulus train (24 biphasic pulses, 60 Hz, 125–150 μA, 2 ms per phase) was delivered to the stimulating electrode on average 150 ms later. The clock for the detection of the TTL signal arising from the lever press was synchronized to the cyclic voltammogram repetition rate. Thus, the exact time of stimulus delivery was the time between cyclic voltammograms (100 ms) plus the time from the lever press to the detection of this event. The rats pressed the lever on a fixed ratio 1 (FR1) schedule. Animals initially (n = 9) underwent a training phase (30 trials) during which the lever was continuously extended. Criterion responding was established once the rats pressed the lever 30 consecutive times in rapid succession. Next, animals were trained on a variable time-out (VTO) schedule for ICS in two phases termed maintenance and maintenance-delay. In phase 1 (maintenance), lever extension was accompanied by a 67-dB (1 kHz) tone with the cue light off and the house lights on. Following lever depression, the stimulation train was delivered, the lever was retracted, the tone and house lights went off, and the cue light was illuminated. If the animal failed to press the lever within 15 s of extension, the same events occurred but the stimulation was not delivered. The timeout between trials varied from 5 to 25 s. This initial maintenance phase comprised 50 trials. When the latency to press was ≤0.3 s, cue-evoked dopamine could not be distinguished from dopamine release evoked by the electrical stimulation, hence no value was given to the cue-evoked dopamine response in those trials. In phase 2 (maintenance-delay) that immediately followed, conditions were identical (including the variable interval between trials) except that the lever extension was delayed by 2 s from the presentation of the tone/house light and simultaneous cue light off. This phase was comprised of 150 trials, and enabled a clear separation of dopamine events related to the predictive cues and electrical stimulation.

Fast-Scan Cyclic Voltammetry. Fast-scan cyclic voltammetry is a differential technique and therefore measures changes in analyte concentration. To remove the background and charging currents collected at the carbon-fiber electrode, ten cyclic voltammograms recorded before a stimulation were subtracted from ten cyclic voltammograms collected during the stimulation. Similarly, the background current before an event was subtracted to determine the species leading to the electrochemical signal.

The carbon-fiber microelectrode was placed into the NAc shell, and its position was adjusted until it was in a site where electrical stimulation evoked dopamine release. The electrode was further adjusted a few tens of micrometers so that it was at a site where naturally occurring dopamine transients were found (3). The presence of dopamine transients and stimulated release assured that the electrode was placed in a microenvironment with functional dopaminergic terminals that released dopamine.

Once the behavioral sessions started, data were collected in 2 min files.

Principal Component Regression. A calibration set of cyclic voltammograms of dopamine and pH changes was obtained *in vivo* by varying the number of stimulus pulses (n = 6, 12, or 24) and frequency (30 or 60 Hz). The dimensionality of the calibration data were reduced by principal component analysis. Principal component regression analysis was used to extract dopamine, pH, and the residual from the measured results during ICSS. To measure the time and amplitude of the maximal dopamine response to the compound cue, the individual trials were subsequently evaluated with Mini Analysis (Synaptosoft version 6.0.3).

Verification of Carbon-Fiber Microelectrode Placement. To determine the carbon-fiber electrode location, rats were anesthetized with a lethal dose of sodium urethane (2 g·kg^{-1}, i.p.). The carbon-fiber electrode causes little damage to brain tissue (4). Therefore, it was removed and a tungsten electrode was reinserted into the location where recordings had been made. A constant current of 500 μA was applied twice for 5 s through the tungsten electrode to generate electrolytic lesions. Animals were then transcardially perfused with 300 ml of saline followed by 300 ml of a 10% formalin solution. Brains were removed, cryoprotected and coronally sectioned at 40 μm on a cryostat. Slices were mounted on slides, stained with thionin, coverslipped, and viewed with bright field microscopy. All recording sites were verified to be located in the shell region of the NAc, as shown in supporting information (SI) Fig. S1.

1. Heien ML, Phillips PE, Stuber GD, Seipel AT, Wightman RM (2003) Overoxidation of carbon-fiber microelectrodes enhances dopamine adsorption and increases sensitivity. *Analyst* 128:1413–1419.
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3. Wightman RM, *et al.* (2007) Dopamine release is heterogeneous within microenvironments of the rat nucleus accumbens. *Eur J Neurosci* 26:2046–2054.
4. Peters JL, Miner LH, Michael AC, Sesack SR (2004) Ultrastructure at carbon fiber microelectrode implantation sites after acute voltammetric measurements in the striatum of anesthetized rats. *J Neurosci Methods* 137:9–23.
5. Paxinos G, Watson C (1986) *The Rat Brain in Stereotaxic Coordinates* (Academic Press, New York).

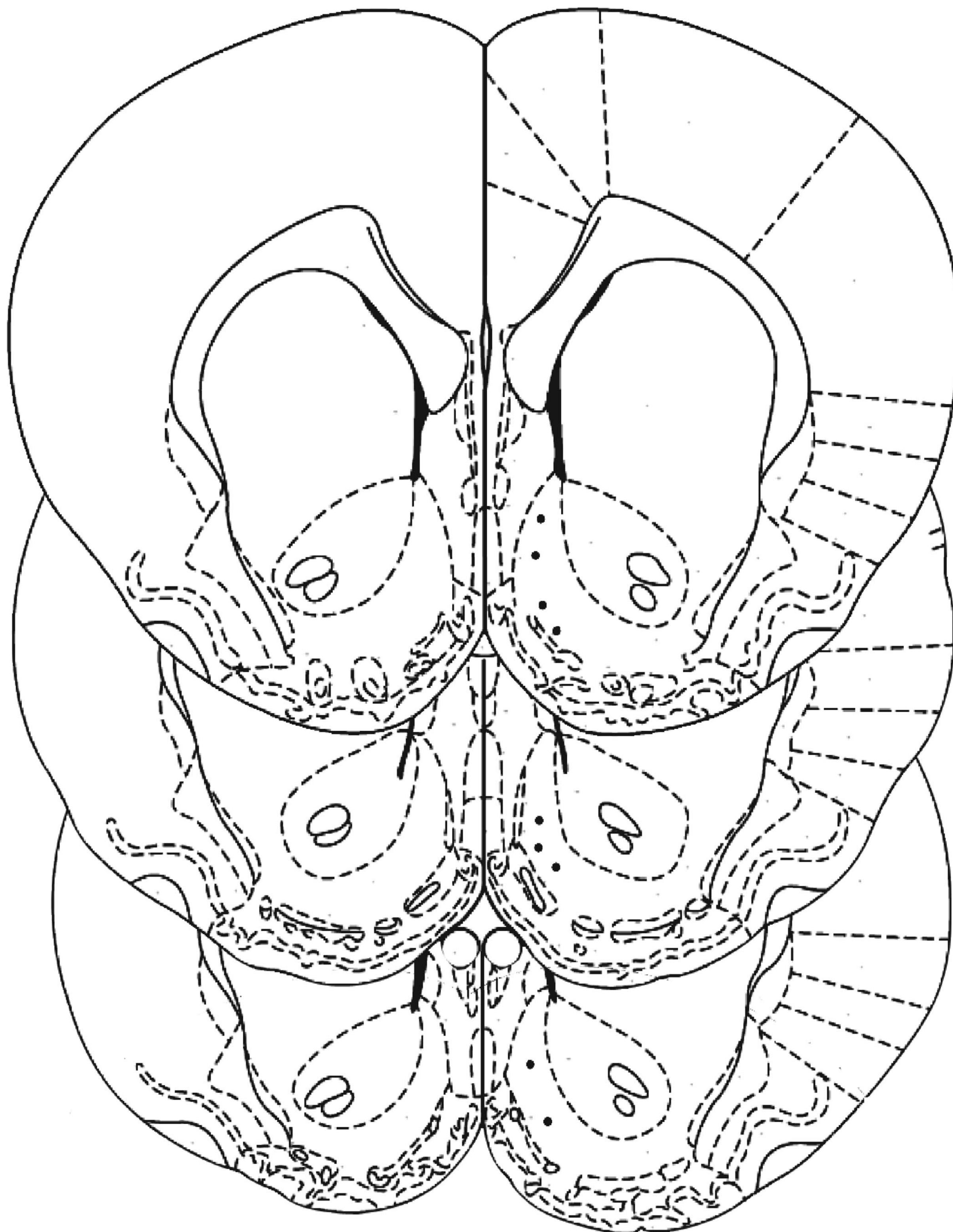

Fig. S1. Recording sites of carbon fiber electrodes (circles) reconstructed from the histological record indicated on a coronal section ≈1.7, 1.6, and 1.2 mm anterior to bregma (5).