Supporting Information

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SI Materials and Methods

Animals. All behavioral testing and voltammetry experiments were approved by the University of Washington Animal Care and Use Committee. All mice were 8–16 wk of age at the time of behavioral testing. C57BL/6 mice were obtained from Jackson Labs (stock #000664). D1-receptor KO (D1R-KO) mice were generated as described (1). Mice in which NMDA receptor (NMDAR) subunit NR1 is knocked out (NR1-KO) were generated by crossing mice with a floxed region of the *Grin1* gene (2) with mice expressing Cre recombinase from the DAT promoter (*Slc6a3*) (3) as described (4). All mice were extensively backcrossed to the C57BL/6 background. During training, mice were housed individually in environmentally enriched cages and maintained at ≈85% normal body weight on LabDiet 5053 chow.

Pavlovian Conditioning. All behavioral testing was done in operant conditioning chambers (ENV-307W; Med Associates, Inc.). Mice were trained to retrieve food pellets in a single magazine training session on the first day in which 10–20 food pellets (20 mg; BIO-SERV) were delivered randomly. On subsequent days, mice underwent Pavlovian conditioning in which an 11-s lever presentation (CS) was paired with delivery of a 20-mg food pellet (US). Although a lever was used as the CS in this paradigm, there was no instrumental contingency for food delivery. The CS and US overlapped, with US delivery occurring 10 s after CS onset. Animals received 7–10 sessions with 25 trials per session and a variable intertrial interval (ITI) of 60 s. The unpaired group underwent the same training except the CS was never paired with US delivery. Learning in both groups was assessed by comparing anticipatory magazine head entries (HEs) during CS presentation and HEs during the ITI. HEs were detected by an infrared photobeam within the food dispenser. Med-PC software (Med Associates, Inc.) was used for all behavioral programs and all data acquisition. SCH23390 (Sigma) was prepared in 0.9% saline and injected i.p. (0.01 mL/g) 30 min before each training session.

Fast-Scan Cyclic Voltammetry in Vivo. Fast-scan cyclic voltammetry (FSCV) procedures were modified from those described by Clark et al. (5). Carbon-fiber microelectrodes were constructed by threading carbon fibers (7-μm diameter) into polyimide-coated fused silica (90-μm outer and 20-μm inner diameter; Polymicro). Once threaded, the fiber and silica were sealed with epoxy (ITW Devcon). The electrodes then were cut to a length of 150–175 μm. Reference electrodes were Teflon-coated Ag/AgCl wires. Carbon-fiber microelectrodes were implanted into 8- to 12-wk-old anesthetized male mice based on stereotaxic alignment. Coordinates for the nucleus accumbens core in millimeters from bregma were Anteroposterior = 1.52, Mediolateral = 1.15, and Dorsoventral = −3.75; DV coordinates were approximated, and exact location was optimized (±0.15 mm) by incrementally lowering the working electrode and monitoring the dopamine release evoked by stimulation of the median-forebrain bundle using a bipolar stimulating electrode (50 μA, 60 Hz, and 400 ms; 0.15-mm in diameter) as described (6). Reference electrodes were placed in an arbitrary position in the contralateral hemisphere. Once positioned, the electrodes were secured to the skull using four set screws (Small Parts, Inc.) and dental acrylic. A custom-built connector made from a modified bipolar stimulating electrode (Plastics One) was used. After surgery, animals were allowed to recover for 4 wk. Recordings were obtained in a modified operant chamber using a custom-built headstage and an electric commutator (Dragonfly, Inc.). A triangular waveform of −0.4 to +1.3V was generated and applied to the carbon-fiber electrode using custom software and data acquisition cards (National Instruments) in a PC HP dc7700. On training days, the microelelectrodes were preconditioned by cycling at 60 Hz for ≈1 h and then at 10 Hz for another hour before training. Data were acquired at a scan rate of 10 Hz. Before each training session, the capacity of the electrode to detect dopamine was assessed by delivering a random food reward. The cyclic voltammogram (CV) obtained after reward delivery in each animal before each session was compared with a CV obtained by electrical stimulation of the median-forebrain bundle in an anesthetized mouse. If there was good correlation between the behaviorally evoked and anesthetized CVs ($r^2 \geq 0.80$), the signal was determined to be dopamine. The success rate for observing phasic dopamine release in response to food delivery before all seven sessions was comparable in the control (6/13) and KO (5/11) groups. During training, digital signals indicating event times were recorded. Data files were aligned to digital time stamps of CS delivery or reward retrieval using custom LabView software. dopamine concentrations were extracted from voltammetric signals using principle component regression with a training set based on stimulated dopamine release and a calibration factor determined from electrode calibration in vitro (7). The training set and calibration factors were determined from an independent set of electrodes that were previously implanted and subjected to the same conditions used here (8, 9). Because of variable reward retrieval latencies on the first day, phasic dopamine release at the time of retrieval rather than reward delivery was used to quantify US-evoked dopamine in the first session. Statistical analysis was performed on either peak (random pellet) or area under the curve (Pavlovian conditioning) by repeated-measures ANOVA using Statistica software (Statsoft). Electrode placement was confirmed by electrolytic lesion after applying 300 V for 30 s to the recording electrode upon termination of the experiment. Sections (30-μm) of paraformaldehyde (4% in PBS)-fixed brain slices were mounted on slides and stained using cresyl violet to visualize the lesion site. Figures were prepared using Prism and MatLab software. In the 3D plots, data were averaged in five-trial blocks and then smoothed across time using a single iteration of a five-point moving average.

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Fig. S1. HE rates (HE/min) during CS presentation indicate the animals anticipated reward delivery without temporal selectivity. HE rates during CS increased uniformly across sessions when analyzed in 2-s bins. Rates did not increase within the CS in any session.

Fig. S2. D1R antagonism decreased overall food-receptacle HE rates. (*A*) Overall HE rates were decreased in mice treated with the higher dose of SCH23390 (mean ± SEM, two-way ANOVA, saline vs. SCH0.3; $F_{(1,10)} = 12.6$; $^{++}P < 0.01$). This decrease resulted in an overall HE rate that was comparable to that in the D1R-KO mice (mean ± SEM, two-way ANOVA, saline vs. D1-KO; $F_{(1,10)} = 16.4$; $^{++}P < 0.01$). (*B*) Mice treated with the higher dose had an increased overall HE rate when tested in the absence of the D1R antagonist in session 7 (Fisher post hoc analysis, session 6 vs. session 7; $^{\#\#\#}P < 0.001$). All groups of mice had a decrease in overall HE rate when tested in the presence of the higher dose of D1R antagonist in session 8 (Fisher post hoc analysis, session 7 vs. session 8; $^{*}P < 0.05$; $^{**}P < 0.01$).

Fig. S3. Overtraining in a subset of recorded mice did not affect the magnitude of the US-evoked DA response. (*A*) Average conditioned approach behavior by control mice on days 7 and 10 (mean ± SEM). (*B*) Average DA current trace for a subset of mice ($n = 3$) on days 7 and 10.