Supporting Information

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SI Experimental Procedures

Animal Housing and Surgery. Male Sprague-Dawley rats (225–250 g on arrival; Charles River Laboratories) were individually housed in a temperature- and humidity-controlled environment and were kept on a 12-h/12-h light/dark cycle (6:00 PM lights on). All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Assessment and Accreditation of Laboratory Animal Care. All surgical details have been described elsewhere (1). Intracranial guide cannulas were bilaterally implanted stereotaxically into the core subcompartment of the nucleus accumbens (NAcore) for the microinjection and microdialysis experiments.

Nicotine Self-Administration and Reinstatement Procedures. After acclimation to facilities, male Sprague-Dawley rats underwent one overnight food-training session, followed by indwelling jugular catheterization surgery. Rats used in the microdialysis experiment were also implanted with bilateral intracranial guide cannulae immediately after catheterization. Stereotaxic coordinates for rats were derived from the stereotaxic atlas (2); cannulae were angled at 6°; anteroposterior (AP) +1.2, mediolateral (ML) ±1.8, dorsoventral (DV) −4.5. After recovery, animals began daily 2-h nicotine self-administration sessions, in which one response (fixed ratio 1) on the active lever yielded one i.v. nicotine infusion [0.02 mg/kg per infusion (pH 7.4), followed by a 20-s timeout period], paired with two white cue lights above both the active and inactive lever and a discrete tone cue (2,900-Hz tone). An inactive lever was available throughout the duration of each session to control for nonspecific responding. After 10 consecutive sessions of self-administration (≥10 infusions per day), rats were placed into daily extinction training sessions (no nicotine delivery or cues) for at least 14 sessions or until extinction criteria were met (≤25 active lever responses for a minimum of two sessions). Reinstatement was elicited by cues (tone + light delivery after an active lever press). A computer controlled the experimental sessions using Med-IV software (Med-Associates). Food-trained yoked saline controls were used as a comparison group. Previous research has shown that food-trained yoked saline animals will reinstate to nicotine and unpaired cues (3), thus it was imperative to examine neurobiological changes in this control group to ensure that any changes found are due to reinstatement of nicotine-seeking but not food-seeking behavior. For the ifenprodil experiment, animals were withdrawn from nicotine self-administration as in previous experiments and were then given a 120-min cue-induced reinstatement test after injection. Animals were allowed to reextinguish active lever responding for a minimum of three sessions or until responding decreased to prereinstatement levels between reinstatement test sessions. Ifenprodil and TCN-201 dose–responses were generated such that each animal received both vehicle and one dose of either TCN-201 (0.01 or 0.1 nmol) or ifenprodil (either 1.0 or 3.0 mg/kg), in a randomized order.

Drug Treatment. For i.v. self-administration and systemic nicotine priming injections, S(-)- nicotine bitartrate (purchased from Sigma-Aldrich) was dissolved in sterile saline (0.9% wt/vol NaCl). The solution was adjusted to pH 7.4 using sodium hydroxide (1 mol/L). Nicotine doses are expressed as free base. Ifenprodil (purchased from Abcam) was dissolved in sterile water for systemic injection. TCN-201 (purchased from Tocris Biosciences) was dissolved in 100% (vol/vol) DMSO and then diluted to 2% (vol/vol) DMSO in artificial cerebrospinal fluid (aCSF). Vehicle for the microinjection study was aCSF with 2% DMSO.

Microdialysis Procedures. In-house probe construction procedures and aCSF content are described elsewhere (4, 5). The night before collecting samples the probes were inserted into the accumbens and perfused with aCSF (0.2 μL/min). Flow rate was increased to 2.0 μL/min the next morning, 2 h before baseline collection. Six baseline collections were then taken at 20-min intervals. Immediately before sample 7, animals were either injected with a nicotine priming stimulus (nicotine-induced reinstatement) or not (cue-induced reinstatement), and levers were extended for a 2-h reinstatement session. Lever presses for the cue-induced reinstatement sessions resulted in the presentation of the compound cue stimulus, as described above. For nicotine-induced reinstatement, lever presses led to no programmed consequence. Dialysate samples were stored at −80 °C before being analyzed for glutamate using HPLC with electrochemical detection, as described elsewhere (6).

Microinjection Procedures. For microinjections, rats were stereotaxically implanted immediately after catheterization with bilateral guide cannulae aimed above NAcore [AP +1.5, ML ±1.8, DV −5.5 (2)]. Intracranial cannulae were secured to the skull using jeweler's screws and dental acrylic. Obturators were placed into the guide cannulae during the experimental protocol to prevent outside debris from entering the cannulae and were removed during microinfusions. Bilateral injection cannulae were lowered into the final site (2 mm below) through implanted guide cannulae both the day before injection (sham) and the day of injection, where 0.5 μL TCN-201 (0, 0.01, or 0.1 nmol) was infused over 2 min using a syringe pump. Injectors were kept in place for 1 min after injection to allow for diffusion. Rats were placed in the operant chamber for cue reinstatement 15 min after removal of injection cannulae. Obturators were again placed in the guides after injection. Coronal slices (100 μm thick) of NAcore were mounted and stained via cresyl violet to verify guide cannulae placement (Fig. S1*B*).

Quantification of Dendritic Spines. All dendritic spine quantification procedures have been described previously (7). Briefly, a confocal microscope (Zeiss LSM 510) was used to image DiI-labeled sections, and DiI was excited using the Helium/Neon 543-nm laser line. The micrograph of DiI-labeled dendrite (Fig. 1) was acquired via optical sectioning by a 63× oil immersion objective (Plan-Apochromat, Zeiss; N.A. = 1.4, working distance = 90 μm) with pixel size 0.07 μm at *xy* plane and 0.1-μm intervals along the *z* axis. A saturation limit of 5% along the dendrite was used. Images were deconvoluted by Autoquant before analysis (Media Cybernetics), and then a 3D perspective was rendered by the Surpass module of the Imaris software package (Bitplane). Only spines on dendrites beginning at >75 μm and ending at ≤200 μm distal to the soma and after the first branch point were quantified from cells localized to the NA core. Additionally, only spines that did not have saturation at the head were included in analysis. Any spine head that showed saturation was manually deleted from analysis. The length of quantified dendrites was 45–55 μm. For each of the animals examined in each group, 5–12 neurons were analyzed. A protocol that quantifies spine density and head diameter based on the Filament module of Imaris was used. The minimum end segment diameter (spine head) was set at ≥0.143 μm. We determined this sampling interval in the *x*/*y*/*z* according to the minimum size of a diffraction-limited spot of light, which can be estimated using the Rayleigh criteria (8, 9).

Slice Preparation. Rats were anesthetized with ketamine HCl (1 mg/kg Ketaset; Fort Dodge Animal Health) and decapitated. The

brain was removed from the skull, and coronal accumbens brain slices (220 μm) (VT1200S Leica vibratome; Leica Microsystems) were collected into a vial containing aCSF (in mM: 126 NaCl, 1.4 NaH_2PO_4, 25 NaHCO3, 11 glucose, 1.2 MgCl2, 2.4 CaCl2, 2.5 KCl, 2.0 NaPyruvate, and 0.4 ascorbic acid, bubbled with 95% O_2 and 5% CO_2) and a mixture of 5 mM kynurenic acid and 50 μM D-(-)-2-Amino-5-phosphonopentanoic acid (D-AP5). Slices were stored at room temperature until recording.

In Vitro Whole-Cell Recording. All recordings were collected at 32 °C (TC-344B; Warner Instrument) in the dorsomedial NAcore, where the prefrontal inputs are most dense (10, 11). Neurons were visualized with an Olympus BX51WI microscope (Olympus America). Inhibitory synaptic transmission was blocked with picrotoxin (50 μM). Multiclamp 700B (Axon Instruments) was used to record excitatory postsynaptic currents (EPSCs) in whole-cell patch-clamp configuration. Glass microelectrodes (1–2 MΩ) were filled with cesium-based internal solution (in mM: 124 cesium methanesulfonate, 10 Hepes potassium, 1 EGTA, 1 MgCl2, 10 NaCl, 2.0 MgATP, and 0.3 NaGTP, 1 QX-314, pH 7.2–7.3, 275 mOsm). Data were acquired at 10 kHz and filtered at 2 kHz using AxoGraph X software (AxoGraph Scientific). To evoke EPSCs a bipolar stimulating electrode (FHC) was placed ∼300 μm dorsomedial of the recorded cell to maximize chances of stimulating prelimbic afferents. The stimulation intensity chosen evoked a ∼50% of maximal EPSC. Recordings were collected every 20 s. Series resistance (Rs) measured with a 2-mV depolarizing step (10 ms) given with each stimulus and holding current were always monitored online. Recordings with unstable Rs or when Rs exceeded 10 MΩ were aborted.

Measuring the AMPA/NMDA Ratio. Recordings started no earlier than 10 min after the cell membrane was ruptured, to allow diffusion of the internal solution into the cell. AMPA currents were first measured at −80 mV to ensure stability of response. Then the membrane potential was gradually increased until +40 mV. Recording of EPSCs was resumed 5 min after reaching +40 mV to allow stabilization of cell parameters. EPSCs containing both AMPA and NMDA currents were then obtained. Then AP5 was bath-applied (50 μM) to block the NMDA currents, and recording of AMPA currents at +40 mV was started after at least 2 min. NMDA currents were obtained by subtracting the AMPA currents from the total current at +40 mV. To estimate the time course of the decay of the NMDA current we measured the time in which the NMDA current decayed to 37% of its peak. Note that some cells from yoked saline animals included in the NMDA decay (Fig. 4*B*) were used for AMPA/NMDA ratio in a previously published study (9).

Membrane Fractionation and Western Blotting. Crude membrane fractionation was prepared for assessment of change in protein expression as described previously (12). Animals were rapidly decapitated at $t = 0$, and NAcore tissue was dissected and homogenized in ice-cold 0.2 mL buffer containing Na Hepes and sucrose (pH 7.4). All buffers were supplemented with 1:100 protease and phosphatase inhibitor mixtures (Thermo Scientific). Homogenates were centrifuged at $1,000 \times g$ for 10 min at 4 °C, and the pellet was homogenized with an additional 0.2 mL homogenization buffer and centrifuged again. Supernatants were centrifuged at $12,000 \times g$ for 20 min. The resultant pellet was resuspended in 30 μL radioimmunoprecipitation assay (RIPA) buffer (Thermo Scientific) supplemented with 1.0% SDS as well as protease and phosphatase inhibitors. A final centrifugation step at $10,000 \times g$ for 5 min was performed to remove insoluble material. Protein concentration was determined using the bicinchoninic acid (BCA) method (Thermo Scientific), and equal microgram quantities were loaded per lane. Corresponding antibodies were used [GLT-1, glutamate aspartate transporter (GLAST), GluA1, GluA2 (Abcam); GluN2A, GluN2B (Millipore); Calnexin (Chemicon International)] Western blotting was performed onto nitrocellulose membranes, using standard techniques as described previously (13). To maximize use of tissue, individual gel lanes were used to probe multiple proteins of different molecular weights. For the proteins in Fig. 5, 10–20 μg of protein was loaded in each lane. Calnexin was used as an unchanged loading control (12), and data were normalized to yoked saline controls.

Statistics. All spine density and diameter data were statistically analyzed by averaging the values for all of the neurons in each animal. The number of determinations in each group was established using an analysis of statistical power based on previous data from our laboratory (1, 14, 15). Behavioral and Western data were analyzed using appropriate repeated-measures ANOVAs, and t tests were used to compare Westerns, d_h, and A/N. Post hoc comparisons were conducted using Bonferroni-corrected t tests. All statistical tests were conducted using Graphpad or SPSS software packages. Rats that did not meet criteria for acquisition of nicotine self-administration and/or extinguished lever pressing were eliminated.

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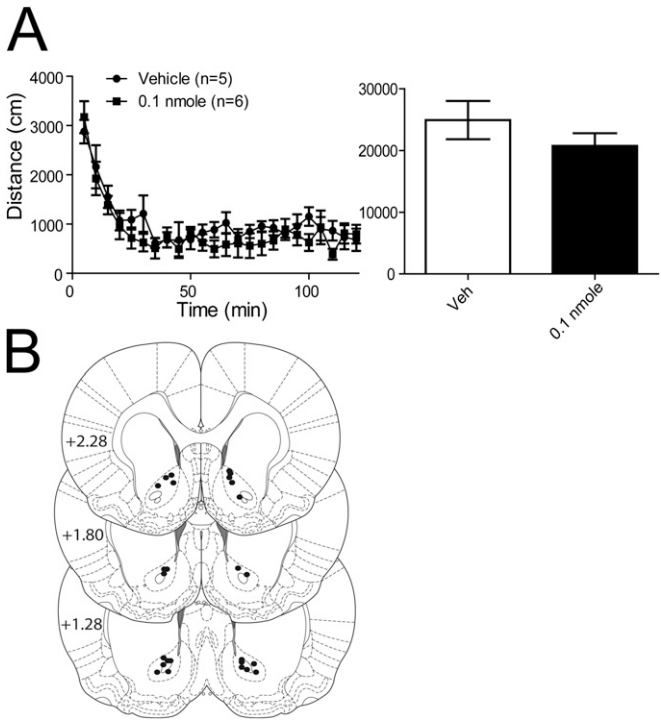

Fig. S1. TCN-201 into NAcore does not decrease locomotor activity in a novel open field. (*A*) No difference in novel open field locomotor activity was found after intra-NAcore administration of either vehicle or TCN-201 (0.1 nmol). (*B*) Histological verification of NAcore guide cannula. Circles drawn on the modified stereotaxic atlas (1) indicate the location of the tip of the microinjector (2 mm below the end of the guide cannula). Numbers refer to mm rostral to Bregma.