Supporting Information

Spiga et al. 10.1073/pnas.1406768111

SI Results

In this study, ethanol dependence in rats was induced by chronic exposure to an ethanol-containing liquid diet, prepared daily from cow's milk with some additions (5,000 IU/L vitamin A and 17 g/L sucrose). Validation of the ethanol-containing liquid diet's ability to induce dependence was tested by objective evaluation of the ethanol withdrawal syndrome. Chronically ethanol-treated rats were tested immediately (EtOH-CHR) or 12 h after suspension of ethanol exposure [ethanol withdrawal (EtOH-W)].

Daily ethanol consumption during ethanol exposure (from 2.4 to 7.2%) ranged from 11.3 ± 0.26 to 18.8 ± 1.01 g/kg. One-way ANOVA confirmed a significant increase in ethanol consumption with exposure time ($F_{1,32} = 5.23$; $P < 0.0001$). The increase in ethanol consumption was paralleled by an increase in blood ethanol levels, which reached 76.41 ± 16.41 mg/dL (measured at the end of treatment, within 30 min of liquid diet suspension) in EtOH-CHR and <1 mg/dL (12 h of liquid diet suspension) in EtOH-W.

Body weight showed a mild decrease from the beginning to the end of the study in EtOH-CHR, which reached a 7.3% peak ($P = 0.00015$, Tukey test), whereas in control (CTRL) rats, a 5.8% increase was observed. Mann–Whitney U tests, used to compare behavioral changes (scores), revealed a significant effect of withdrawal ($P < 0.001$). In particular, as shown in Fig. S1, analysis of withdrawal signs revealed a significant overall effect of ethanol withdrawal vs. the EtOH-CHR and CTRL groups.

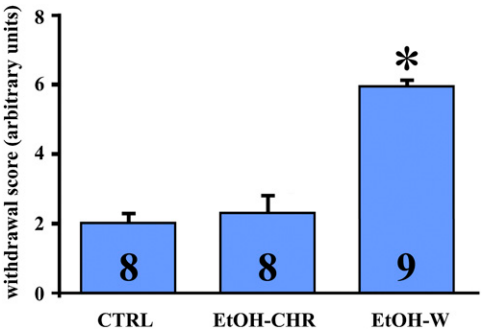


Fig. S1. Effect of ethanol liquid diet removal on ethanol withdrawal signs. Withdrawal scores for each item (vocalization, tail rigidity, body tremors, ventromedial limb retraction, number of entries in open arms, time spent in open arms) are summed and averaged. Each withdrawal sign was assigned a score of 0 to 2 (1). Values represent the mean (±SEM) per group. The number of subjects is indicated in each bar. *$P < 0.05$.

1. Uzbay IT, Kayaalp SO (1995) A modified liquid diet of chronic ethanol administration: Validation by ethanol withdrawal syndrome in rats. *Pharmacol Res* 31(1):37–42.

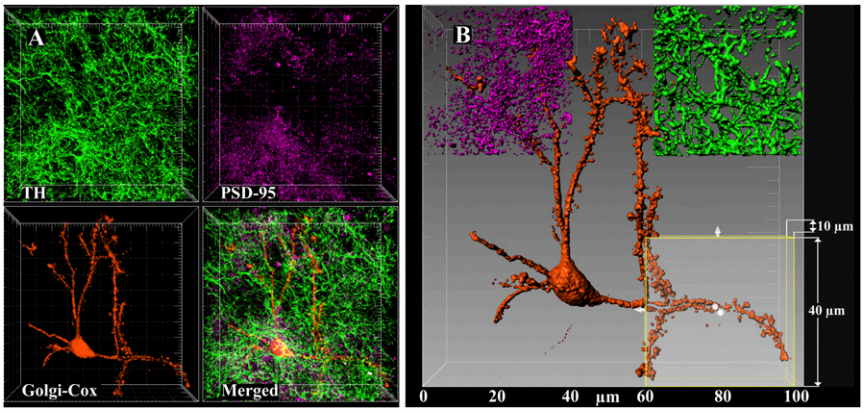


Fig. S2. (*A*) Three-dimensional reconstruction of an impregnated medium spiny neuron (MSN) of the nucleus accumbens (Nacc) shell (orange), surrounded by a dense tangle of tyrosine hydroxylase (TH)-positive fibers (green) and PSD-95 puncta (fuchsia) and merged picture. Golgi-Cox staining and immunofluorescence were obtained simultaneously in the same specimen (see ref. 1 for details). (*B*) An example of box dimensions (regions of interest) and TH and PSD-95 volume counts. An MSN (orange) is also represented. Dimensions are indicated.

1. Spiga S, et al. (2011) Simultaneous Golgi-Cox and immunofluorescence using confocal microscopy. *Brain Struct Funct* 216(3):171–182.

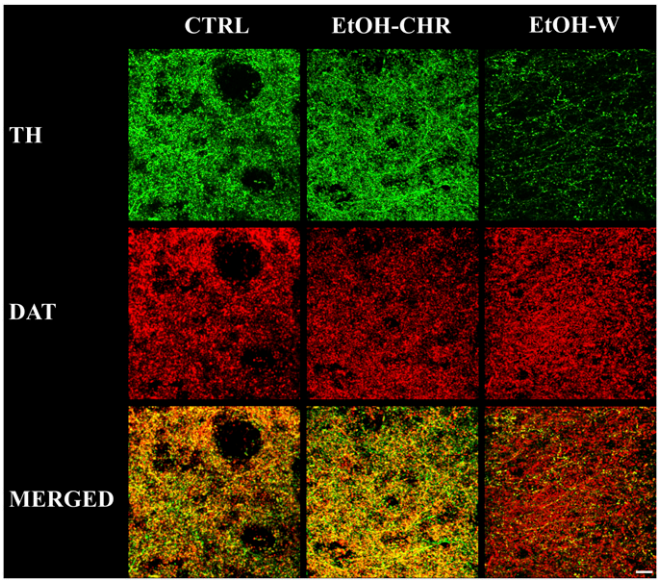


Fig. S3. Representative 3D (maximum-intensity) reconstruction of TH (green) and dopamine transporter (DAT) (red) immunofluorescent fibers and merged image in the three experimental conditions. (Scale bar, 10 μm.)

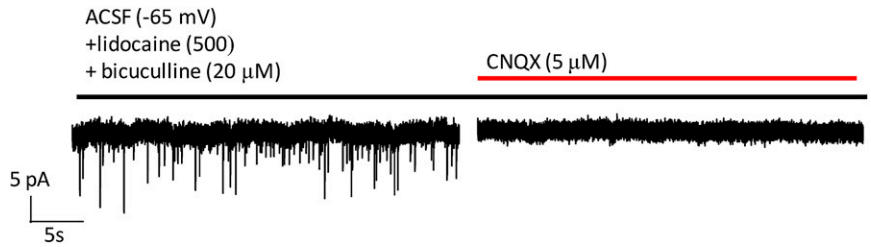


Fig. S4. CNQX (6-cyano-7-nitroquinoxaline-2,3-dione; 5 μM) suppresses miniature excitatory postsynaptic currents (mEPSCs) recorded in voltage-clamped Nacc shell MSNs from CTRL rats. The representative tracing was obtained from a single MSN in the presence of lidocaine and bicuculline, before and during bath application of the AMPA/kainate receptor antagonist CNQX. ACSF, artificial cerebrospinal fluid.

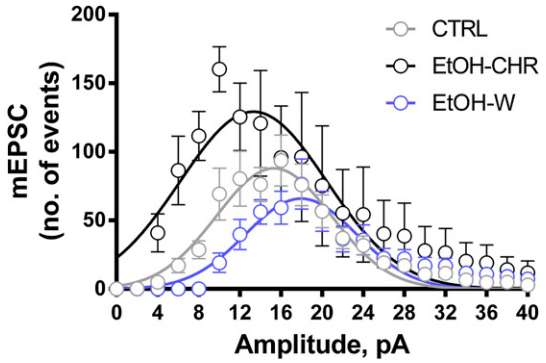


Fig. S5. Distribution analysis of AMPA receptor-mediated mEPSCs recorded from Nacc shell MSNs observed in different experimental conditions as shown. Data are expressed as the averaged number of events for varying current amplitudes. n = 25 (CTRL), 8 (EtOH-CHR), and 24 (EtOH-W). Error bars represent SEM.

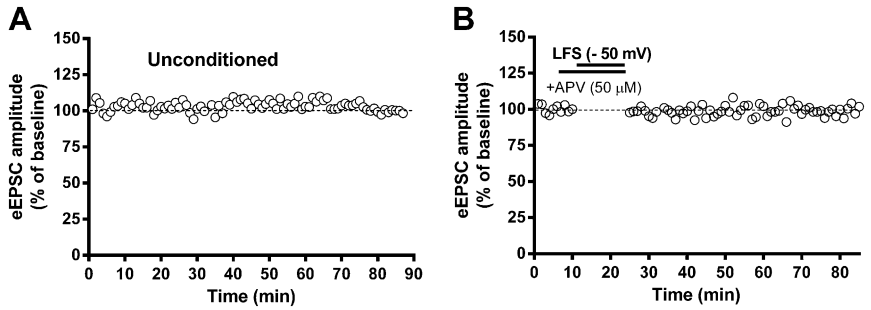
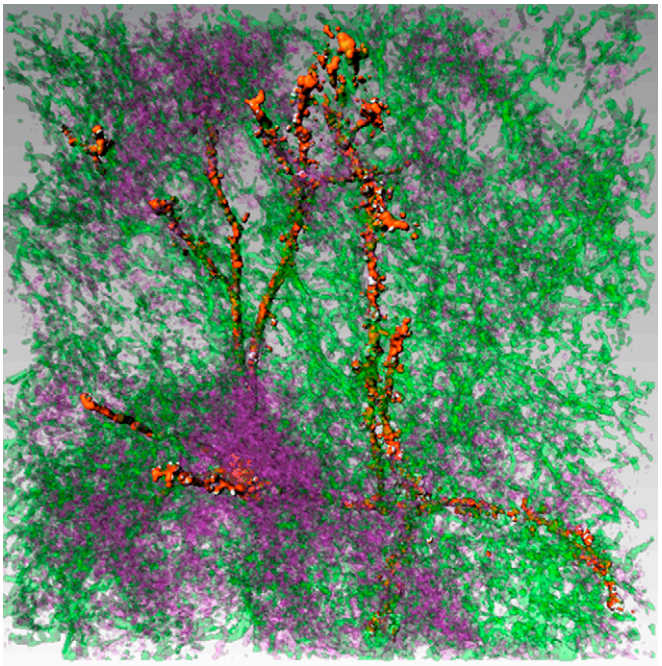


Fig. S6. Long-term depression (LTD) induction is prevented by the NMDA receptor antagonist (2*R*)-amino-5-phosphonopentanoate (APV). In voltage-clamped (−65 mV) MSNs of the Nacc shell from CTRL animals, bath application of the NMDA receptor antagonist APV (50 μM) completely abolished the formation of LTD following low-frequency stimulation (LFS) (500 stimuli at 1 HZ) paired with membrane depolarization (−50 mV). eEPSC, evoked excitatory postsynaptic current.



Movie S1. Video illustrates 3D reconstruction of Golgi-stained material (MSN) and immunofluorescent elements (TH, green; PSD-95, purple) in the same bloc of tissue. White indicates PSD-95 colocalized with Golgi stain.

Movie S1