Supporting Information

Zhou et al. 10.1073/pnas.0915173107

SI Materials and Methods

Animals. The mice with a mixed background, 129/Sv X C57BL/6, were maintained five per cage with ad libidum access to food and water on a 7:00 AM–7:00 PM light cycle. Only male mice, 3–9 months old, were used for behavioral and pharmacological studies unless otherwise mentioned. tetO-CK1δ/CaMKIIα-tTA double positive mice were used as the CK1δ OE and tetO-CK1δ positive/CaMKIIα-tTA negative were used as the WT littermate controls. For a given compound, when several doses were tested, the results for each dose were obtained using a different animal group.

In Situ Hybridization. Frozen sections (12–14 μm) were fixed in 4% paraformaldehyde, acetylated, dehydrated, and air dried. A riboprobe, approximately 550 bp spanning exon II of CK1δ, labeled with 33p-UTP, was used for in situ hybridization to detect CK1δ mRNA. The autoradiographic films were exposed overnight. For five coronal sections, an identical surface of the same brain region was scanned and quantified using a National Institutes of Health image software.

Immunoblotting. Mice were killed by focused microwave irradiation and brain tissues were rapidly dissected and homogenized in Tris-EDTA buffer containing 2% SDS. Proteins were separated by SDS-polyacrylamide gel electrophoresis following standard procedures (see *SI Materials and Methods* for details). Total protein (35 μg) was separated by SDS-polyacrylamide gel electrophoresis (Nu-PAGE 4–12%). Proteins were transferred to nitrocellulose membranes and the membranes were blocked with 5% nonfat milk in TBS buffer and then incubated with primary antibody overnight at 4 °C. Antibodies were CK1δ (1:8000, gift from Dr. DeMaggio, ICOS Corp., Bothell, WA), dopamine D1R (Sigma), D2R (Millipore), $G_{\alpha olf}$ (gift from Dr. Herve, INSERM U114, Paris, France), $G_{\alpha i}$ (Calbiochem), and beta-actin (AbCam).

DLC. A dark plastic box was placed inside the open field chambers to divide the chamber equally into a bright compartment and a dark compartment. Mice were placed in the dark compartment, and the time and ambulatory exploration were recorded in both areas for 30 min.

EPM. The mice were placed in the middle of the elevated plus maze (50 cm from the floor) and allowed to explore the maze for 6 min. Time and number of entries into open and closed arms were scored.

NSF. Mice were food-deprived (no food—water only) for 24 h before the test. At the time of testing, each mouse was placed in one corner of the test box and allowed to explore for a maximum of 15 min. The test box, 50 × 50 × 10 cm, was filled with 3 cm of fresh bedding and three food pellets were placed on a white filter paper in the middle of the box. The time necessary to bite a food pellet was measured. Immediately after an eating event, the mouse was transferred back to its home cage and allowed to free-feed for 5 min. The amount of home cage food consumption was measured.

SI. The mice were housed individually for one week. Then, an intruder (one WT mouse) was added to the isolated mouse (resident) cage and the interaction was tape-recorded and scored for 6 min. The following parameters were scored: aggressive behavior (e.g., hind biting, lateral attack, frontal attack), social initiation/investigation (e.g., nape, pin, chasing, anogenital investigation, pouncing), and social avoidance (e.g., quick movement away from intruder).

FC. The experiments were performed in a Plexiglas mouse-conditioning chamber enclosed within a sound attenuation box. On the first day of the experiment, mice were habituated for 1 h. The mice were then transferred individually into the conditioning chamber and exposed to the following testing procedure a: (*i*) habituation (60 s), (*ii*) cue light blinking (120 s), (*iii*) tone beeping (85 dB, 20 s) with light blinking, (*iv*) shock (0.8 mA, 1 s) paired with the tone-conditioned stimulus, (*v*) and light off after 120 s. During the second day, the mice went through the same procedure but without the shock. The animal freezing time, defined as cessation of all except respiration-related motion and by a crouching posture, in the chamber was scored.

Pharmacological Studies. Mice (7–10 mice per test group) were i.p. injected with the following drugs: d-amphetamine (1, 2, and 4 mg/kg, Sigma-Aldrich); methylphenidate, threo-Methyl α-phenyl-α-(2-piperidyl)acetate hydrochloride (1, 2.5, 5, and 10 mg/kg, Sigma-Aldrich); SKF81297, 6-Chloro-2,3,4,5-tetrahydro-1-phenyl-^{1}H-3-benzazepine hydrobromide (1 mg/kg Tocris); SCH23390, R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-^{1}H-3-benzazepine hydrochloride (0.1, 0.25, and 0.5 mg/kg, Sigma-Aldrich); quinpirole, (–)-quinpirole monohydrochloride, LY-171,555, trans(–)-(4aR)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-^{1}H-pyrazolo[3,4-g] quinoline monohydrochloride (0.1 and 1 mg/kg, Sigma-Aldrich); haloperidol, 4-[4-(p-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone, 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone, (0.25, 0.5, and 1 mg/kg, Sigma-Aldrich); and MK801, dizocilpine hydrogen maleate, (5R,10S)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (0.2 mg/kg, Sigma-Aldrich).

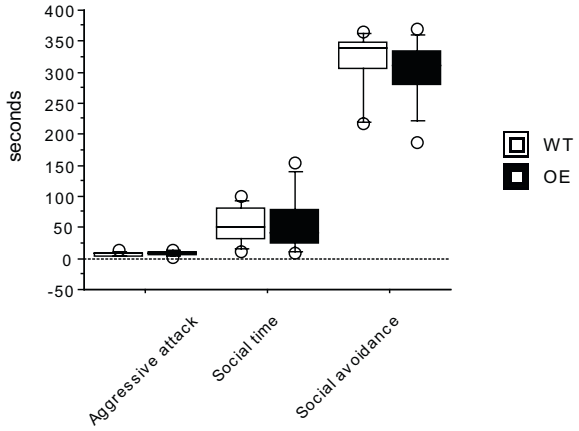

Fig. S1. Analysis of social interactions. Animals were recorded during a test period of 6 min. Social behavior was scored for aggressive attack (biting), social time (napping, sniffing, pinning, and chasing), and social avoidance (avoidance of the intruder). Whiskers represent the 1.5 interquartile range. ANOVA Fisher's PLSD test (n = 9 each genotype).

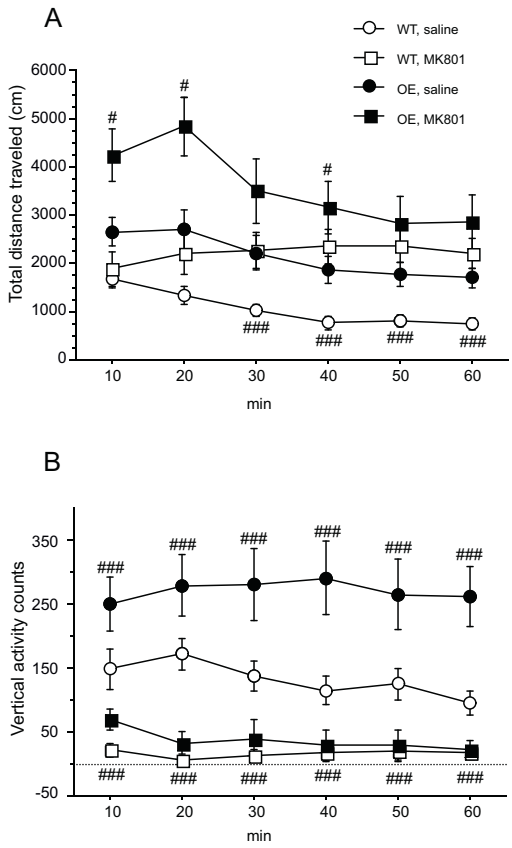

Fig. S2. Effects of MK801, an NMDA antagonist, on locomotor activity. Horizontal (*A*) and vertical (*B*) activities in the OF paradigm were recorded for 60 min immediately after MK801 administration (0.2 mg/kg). Graphs show the mean values ± SEM (10 min bins). ANOVA Fisher's PLSD test, n = 10 each genotype per treatment; $^{\#}$, $P < 0.05$, and $^{\#\#\#}$, $P < 0.0005$ (treatment effect for a given genotype).

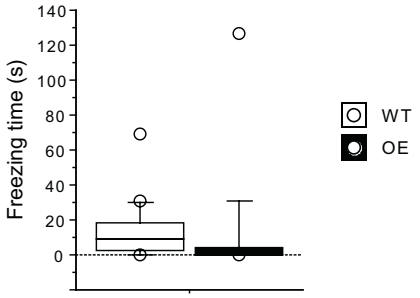

Fig. S3. Analysis of fear conditioning. Cue-induced freezing time was assessed during a test period of 5 min. Whiskers represent the 1.5 interquartile range. ANOVA Fisher's PLSD test (n = 15 each genotype).

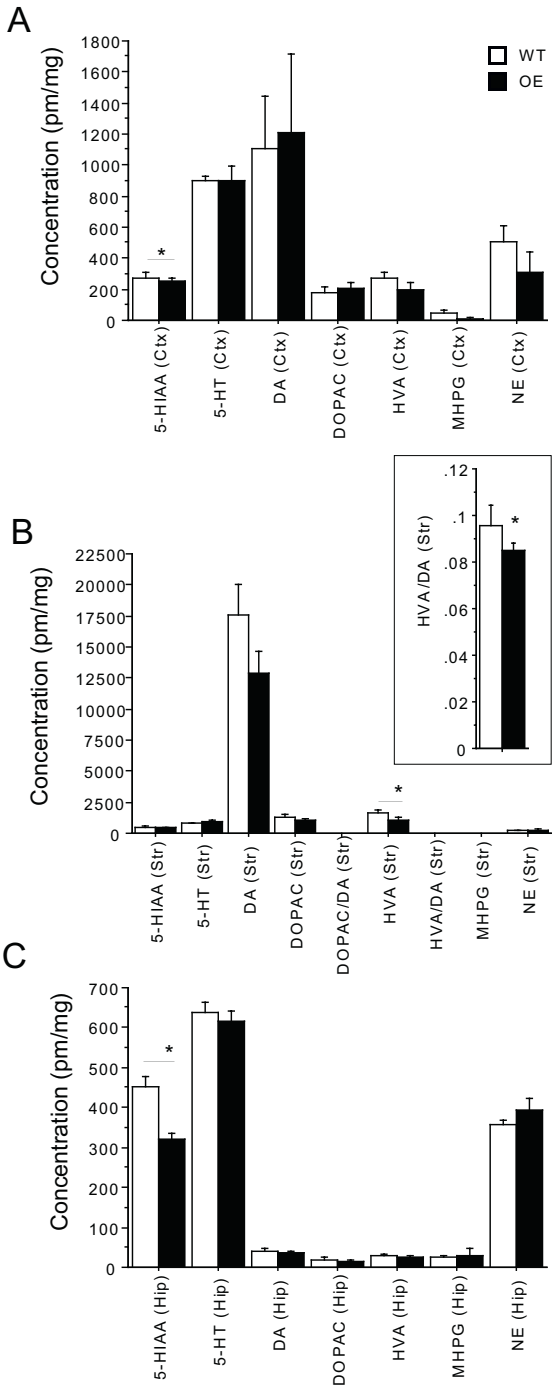

Fig. S4. The concentrations of serotonin (5-HT), dopamine (DA), norepinephrine (NE), and their corresponding metabolites, 5-HIAA, DOPAC, HVA, and MHPG, were measured by HPLC-coupled electro-chemical detection (pmol/mg). Samples were derived from (*A*) frontal cortex (Ctx, $n = 6$ each genotype), (*B*) striatum (Str, $n = 6$ each genotype), and (*C*) hippocampus (Hip, $n = 3$ each genotype). Concentrations are plotted as mean values ± SEM. ANOVA Fisher's PLSD test (*, $P < 0.05$). The bar graph inserted in *B* illustrates the ratio of the HVA to DA.