Supporting Information

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SI Materials and Methods

Preparation of the KO Construct. Mouse genomic fragments used to generate *Lynx2* mutant mice were derived from C57B6 genomic BAC RP23–401F24. The DNA fragments serving as the 5′ arm of homology (4.4 kilobase [kb]), the loxP-flanked region containing exons 1 and 2 (2.8 kb) and the 3′ arm of homology (1.1 kb) were amplified by long-distance PCR (Expand Long Template PCR System, Roche), cloned into pCR4-TOPO (Invitrogen) and sequenced. These fragments were then inserted into vector pEasy-Flox (1). The linearized targeting construct was electroporated into Bruce4 ES cells (2), and homologous recombinants were identified by Southern Blot analysis. Recombinant clones were injected into C57B6-Tyr(c)-2J blastocysts to generate chimeric founders that transmitted the mutant allele. The loxP-flanked region and the neomycin selection marker were deleted *in vivo* using the EIIa::Cre deleter transgene (3).

***In Situ* Hybridization.** Adult brain sections were prepared as described (4). Digoxigenin- (Dig-) labeled riboprobe was transcribed using 2 μmol/l Dig-NTP (Boehringer-Mannheim) in the transcription reaction. Sections were incubated at 60 °C overnight in hybridization buffer (50% formamide, 5xSSC, 5x Denhardt's reagent, 500 μg/ml herring sperm DNA, and 250 μg/ml yeast tRNA [Sigma R6750]) and washed extensively with 50% formamide and 2x SSC at 60 °C. Riboprobe was detected with anti-Dig Fab fragments conjugated to Alkaline Phosphatase and NBT/BCIP substrate mixture.

Social Interaction Test. The apparatus for social interaction test was built based on the design described previously (5). The test box consisted of three compartments divided by partitions with removable doors. Before the test, the Stranger mouse was placed under a wire cage in the first compartment, an empty wire cage was placed in the third compartment, and the test mouse was placed in the middle compartment for 10 minutes for habituation, before the doors were removed. Time spent in each compartment and the time spent sniffing the empty cage and the cage with the Stranger mouse were recorded for another 10 minutes and analyzed by Ethovision equipment and software.

Passive Avoidance Test. On Day 1, each mouse was placed in the light compartment facing away from the dark compartment while the guillotine door was closed. The door was elevated after a 30-second exploration and it was closed again when the mouse entered the dark compartment with all four paws. The latency of the mice to go into the dark compartment was recorded and used as a measure of baseline anxiety. On Day 2, the mice were placed in the light compartment, facing away from the dark compartment and were allowed to explore for 30 s. Then the guillotine door was elevated and when the mouse entered the dark compartment with all four paws, the door was closed and the mice received a 2-second electric shock with 0.5 mA amplitude. After 30 seconds the mice were removed from the dark compartment and latency of the mice going in to the dark compartment was recorded. On Day 3, the mice were again placed in the light compartment, facing away from the dark compartment. After 5 seconds, the guillotine door was elevated and the latency of the mice to enter the dark compartment was recorded.

Fear Conditioning Test. After 24 hours, the mice were placed in the conditioning environment and freezing behavior was recorded for 5 minutes for contextual conditioning. For cued conditioning, mice were placed in a novel environment with a modified ceiling, walls and a novel smell (orange extract) for 3 minutes, and then presented with an 80-db tone for another 3 minutes, and freezing behavior was scored.

Hot-Plate Test. Mice were placed on a 55 °C hot-plate (Columbus Instruments, OH) with a cut-off time of 60 seconds. A stopwatch was used to measure the time between the placement of the mice and the following nociceptive endpoints: hind pawlicking, jumping or rapid thumping of the hind paws.

Finding Buried Food Test. *Lynx2*$^{-/-}$ and WT mice were first habituated to eat OREO cookies. Mice were fasted without food and water for 18 hours before the test. Each test mouse was placed in the center of a clean mouse cage and a quarter of a cookie was buried under ≈1 cm of bedding on the corner of the cage. A stopwatch was used to measure the time spent by each mouse to find the cookie and start eating it.

Whole Cell Recordings in Medial Prefrontal Cortex Slices. Brains were rapidly removed and submerged in an ice-cold sucrose replacement solution containing (in mmol/l) 200 sucrose, 2.5 KCl, 1.2 NaH_2PO_4, 1.3 $MgCl_2$, 8 $MgSO_4$, 20 glucose, and 46 $NaHCO_3$, equilibrated with 95% O_2/5% CO_2. Coronal prefrontal cortex slices (350 μm thick) were made using a microtome (Leica Microsystems, Nussloch GmbH). The slices were placed in a holding chamber containing artificial cerebrospinal fluid (ACSF) comprised of (in mmol/l) 124 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 2 $CaCl_2$, 20 glucose, and 26 $NaHCO_3$, equilibrated with 95% O_2/5% CO_2. The slices were maintained at 32 °C temperature for 30 min; then they were kept at room temperature for use. To increase slice viability, 5 μmol/l glutathione, 500 μmol/l pyruvate, and 250 μmol/l kynurenic acid were added to the sucrose replacement buffer and ACSF buffer in all experiments.

After a recovery period of at least 1 hour, a slice was transferred to a submerged brain slice recording chamber, where it was perfused continuously with oxygenated ACSF at 2–3 ml/min at room temperature. Whole cell recordings were performed in layer V pyramidal neurons in the medial prefrontal cortex. The patch pipettes were pulled from PP-830 puller (Narishige, Japan) and filled with the following internal solution (in mmol/l): 125 potassium gluconate, 10 HEPES, 1 $MgSO_4$, 1 $CaCl_2$, 0.5 EGTA, 2 MgATP, 0.3 mmol/l NaGTP at a pH of 7.3 and osmolarity of ≈295 mOsm. Filled patch pipettes had resistances ranging from 3–5 MΩ. Spontaneous excitatory synaptic transmission currents (sEPSCs) were recorded by an Axon MultiClamp 700B amplifier (Molecular Devices, Sunnyvale, CA) in voltage clamp mode. Data were filtered at 2 kHz, digitized at 10 kHz with DigiData 1440A (Molecular Devices) and acquired by the Clampex 10.0 program (Molecular Devices). sEPSCs were recorded at a holding potential of -70 mV; which is near the reversal potential for the $GABA_A$ receptor-mediated chloride channels. With 10-minute stable recordings after forming whole cell configuration, 5 consecutive recordings (5 × 60 seconds) were performed as control. After 1 minutes of 10 μmol/l nicotine for 1 minutes or 300 nmol/l for 2 minutes, another 5 consecutive recordings (5 × 60 seconds) were made for examining the effects of nicotine. The sEPSCs were detected and analyzed using Mini Analysis Program (Synaptosoft, Decatur, GA). Amplitude and area thresholds were set to 5 pA and 20 fC, respectively. The peak amplitude and intervent interval of

sEPSCs from 60-second episodes were used to generate cumulative probability plots, and the statistical significance was determined by Kolmogorov-Smirnov test for each neuron. The mean values of sEPSC amplitude and frequency (mean ± SEM.) from the 5 × 60 second episode before nicotine were grouped as control, The mean values of first 2 × 60 second episode after nicotine application were grouped and divided by the control value to calculate the fold increase of amplitude and frequency. Student's *t* test was used to compare the effects of nicotine; P value <0.05 was considered statistically significant. The mean values of sEPSC amplitude and frequency (mean ± SEM.) in WT and *Lynx2*$^{-/-}$ from the 5 × 60 second episode before nicotine application were also grouped and used to compare WT and *Lynx2*$^{-/-}$ mice. The Student *t* test was used for statistical analysis.

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4. Gong S, *et al.* (2003) A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 425:917–925.
5. Nadler JJ, *et al.* (2004) Automated apparatus for quantitation of social approach behaviors in mice. *Genes Brain Behav* 3:303–314.

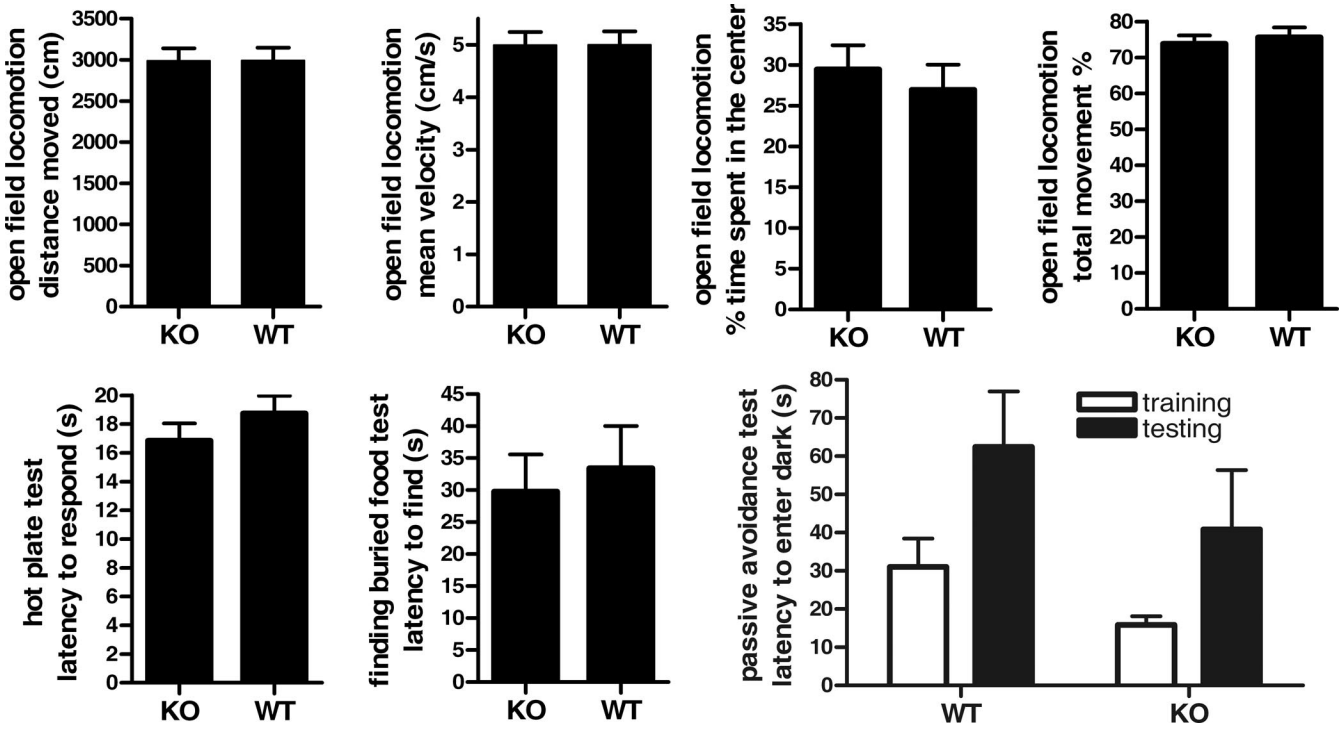

Fig. S1. There were no gross abnormalities in motor behavior of *Lynx2*$^{-/-}$. There were no significant differences between gross motor behavior of *Lynx2*$^{-/-}$ and WT mice in open field exploration test. Also, no significant differences were observed in pain sensitivity in hot plate test, in olfactory senses in finding buried food test and in learning in passive avoidance test