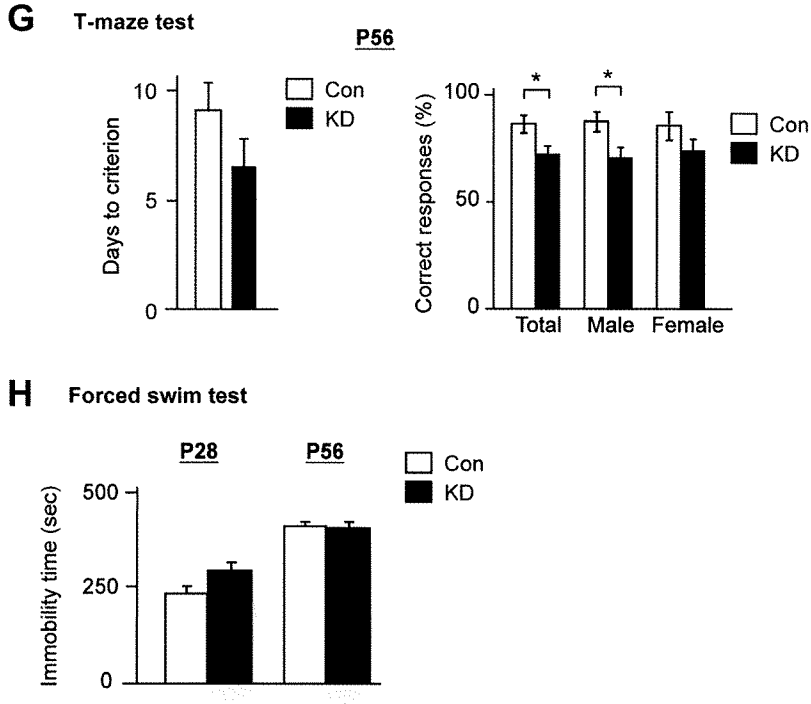

Figure S5. Performance of DISC1 KD mice in prepulse inhibition test (PPI) and novel object recognition test (NORT) as well as T-maze and forced swim tests

(A) *In utero* gene transfer with another well-established shRNA to DISC1 (milder effect on DISC1 knockdown by this shRNA was published (Kamiya et al., 2005)) also results in deterioration of PPI at P56. Consistent with the milder level of suppression of DISC1, this DISC1 KD mouse displays a trend toward subtle PPI deficits. N=11-21 per condition. (B) Effect of clozapine (CLZ) (3 mg/kg, *i.p.*) on the startle response at P56. Neither knockdown of DISC1 nor the treatment with CLZ show effects on startle amplitude. N=16 per condition. Con, control mice. (C) Sex differences in the effect of CLZ on the impairment of PPI in KD mice at P56. Treatment with CLZ (3 mg/kg, *i.p.*) significantly ameliorates the impairment of PPI in male DISC1 KD mice (*p<0.05, **p<0.01). Treatment of CLZ also improves PPI deficits in female DISC1 KD mice, although the effect was milder. N=8 per condition. VEH, vehicle; Con, control. (D) Performance in NORT at P28 and P56. Retention session was carried out 24 h after training session. DISC1 KD mice significantly impaired performance in the test runs at P28 and P56 (*p<0.05, **p<0.01). N=8-12 per condition. (E) Performance in NORT at P56, when retention session was carried out 1 h after training session. No difference is observed in the exploratory preference of NORT between DISC1 KD and Con mice. N=5 per condition. (F) Effect of CLZ on the impairment of NORT in DISC1 KD mice at P56. Retention session was carried out 24 h after training session. Treatment with CLZ (3 mg/kg, *i.p.*) 30 min before training sessions improved the impairment of NORT in DISC1 KD mice (*p<0.05, **p<0.01). N=7 per condition. VEH, vehicle. (G) Performance in T-maze test at P56. Left, there is no difference in days to reach the criterion during training between DISC1 KD and Con mice. Right, working memory performance for DISC1 KD and Con mice with delay intervals of 15 sec is shown. The percentage of correct responses is significantly decreased in DISC1 KD mice compared with Con mice with delay intervals of 15 sec (*p<0.05). N=8 per condition. (H) Performance in forced swim test. DISC1 KD mice do not differ from Con mice in immobility time at P28 and P56. N=9-12 per condition. Error bars show mean + SEM.

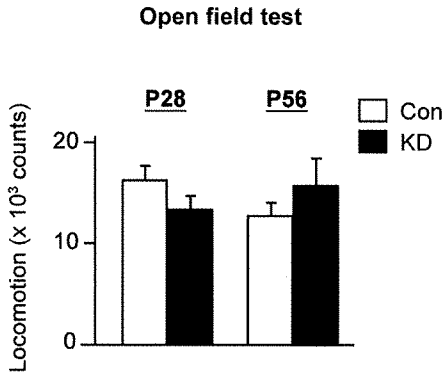

Figure S6. No change in locomotor activity of DISC1 KD mice in open field test
Performance in locomotor activity test. DISC1 KD mice do not differ from Con mice in spontaneous locomotion at P28 and P56. N=12-13 per condition. Error bars show mean + SEM.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Semi-quantitative population analysis of GFP-positive cells in cerebral cortex

In order to evaluate the feasibility of *in utero* gene transfer technique for modulating gene expression in PFC, a GFP expression construct was incorporated in the bilateral ventricular zone at E14 by our protocol (see **EXPERIMENTAL PROCEDURES** in main text). The localization of GFP-positive cells was analyzed semi-quantitatively in serial coronal sections (+2.34 mm, +1.94 mm, +1.18 mm, +0.98 mm, and -1.34 mm from Bregma) from six randomly selected brains at P56. Images were acquired with a confocal microscope (LSM510; Carl Zeiss, Grottingem, Germany). GFP signal intensity in medial prefrontal cortex (mPFC), dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC), moter cortex (mCx), sensory cortex (sCx), cingulate cortex (cgCx), retrosplenial cortex (rsCx), and other areas were quantified by using MetaMorph software version 7.1 (MDS Analytical Technologies, Sunnyvale, CA). These areas were defined by landmarks and neuroanatomical nomenclature in the atlas of Franklin and Paxinos (Franklin and Paxinos, 2007). The percentage of GFP signal intensity of each area in relation to total intensity of all areas was calculated.

Constructs

The expression construct of RNAi-resistant full length DISC1 ($DISC1^{R}$), which has three silent mutations in the target sequence of RNAi, was used for rescue experiments to test functional complementation in neuronal migration assay. Another short hairpin RNA (shRNA) to DISC1 (5'-CGGCTGAGCCAAGAGTTGG-3'), which was well characterized in our previous publication (Kamiya et al., 2005), was used in the PPI test.

Morphological analysis of dendrites

Brains were fixed with 4% paraformaldehyde and coronal sections were obtained with a cryostat at 20 μm (CM 1850, Leica, Bannockburn, IL). GFP-positive pyramidal neurons in layer II/III in mPFC at P14 were subjected to morphological analysis of dendrites. A z-series of six images were collected at 2-μm depth intervals with a confocal microscope (FV300, Olympus Optical, Tokyo, Japan). Acquisition parameters were kept the same for all scans. Reconstructed images were generated by compressing the Z stacks. For the quantification of dendritic complexity, a region of interest was randomly selected (300 μm wide x 200 μm high) in layer II/III of mPFC where GFP-positive cells were located, in an unbiased manner. We analyzed 30 GFP-positive cells from 3 individual animals per group (DISC1 KD or Con mice). The ratio of GFP signal intensity in processes (dendrites and axons) and in total areas (processes and cell body) was measured. In order to quantify GFP signal intensity in a single GFP-positive neuron, the GFP-positive dendrites originating from other adjacent GFP-positive neurons, which were distinguished based on observation of uncompressed z-scan images, were manually erased by using Adobe Photoshop 6.0 (Adobe Systems Inc, San Jose, CA). In each GFP-positive neuron, the ratio of GFP signal intensity between in the main apical shaft of dendrites and in the area in a circle with 50-μm diameter centered on the cell body was then measured by using MetaMorph software version 7.1 (MDS Analytical Technologies, Sunnyvale, CA).

Monitoring of body weight

Body weights of DISC1 KD mice and Con mice were measured at P14, P28, and P56. The data were analyzed in total or in each gender group. All the animals used for measurement of body weight were those also used for

other characterizations.

Nissl staining and GFAP staining

Histological procedures were performed as previously described (Kamiya et al., 2005) with minor modifications (see **EXPERIMENTAL PROCEDURES** in main text). For immunohistochemistry of GFAP, an antibody from Millipore was used (1:300 dilution). Nissl staining with cresyl violet was carried out as published (Yu et al., 2003). The areas of GFAP-positive cells/mm^2 and numbers of Nissl-stained cells were measured in a region of interest by imaging software WinRoof (Mitani Co. Ltd., Fukui, Japan) after Images were acquired with a confocal microscope (LSM510) or a light microscope (Axioskop2 plus; Carl Zeiss, München, Germany). Three sections per brain were analyzed from each of 6 DISC1 KD mice and 6 Con mice (total 18 sections from each group).

Protein extraction and immunoblotting

Frontal cortex dissected from whole brains were homogenized at 4 °C in a lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM sodium orthovanadate, 2 mM EDTA, 50 mM, NaF, 0.1 % SDS, 1% NP-40, 1 % sodium deoxycholate, 20 μg/ml aprotinin, 20 μg/ml leupeptin, and 20 μg/ml pepstatin, pH 7.4). Extracted protein samples were mixed with SDS-PAGE loading buffer after protein concentration was measured. Each protein sample was analyzed with SDS-PAGE followed by Western blotting. The following primary antibodies were used; anti-GFAP (1:1000, Millipore Corporation), anti-TH (1:1000, Millipore Corporation), anti-D1R (1:250, Sigma-Aldrich), anti-D2R (1:500, Millipore Corporation), and anti-β-actin (1:500, Santa Cruz Biotechnology, Santa Cruz, CA). Quantitative densitometric measurement of Western blotting was performed using a densitometer (Atto

Instruments, Tokyo, Japan).

Quantitative real-time PCR

Total RNA was isolated from frontal cortex by using an RNeasy Kit (Qiagen, Valencia, CA) and converted into cDNA with a SuperScript^{TM} First-Strand System for RT-PCR Kit (Invitrogen, Carlsbad, CA). The mRNA levels of D1R and D2R were determined by real-time RT-PCR with a TaqMan probe. PCR-cycling parameters are 2 min at 50°C and 2 min at 95°C as initial steps, followed by 40 cycles of 15 sec at 95°C and 1 min at 57.7°C for D1R as well as 57.5°C for D2R in an iCycle iQ Detection System (Bio-Rad Laboratories, Inc., CA, USA). The expression levels were calculated as described previously (Wada et al., 2000). The following primers and probes against mouse D1R, D2R, and β-actin (an internal control) were used.

D1R:

Forward: 5'-AGATGCCGAGGATGACAACTG-3'

Reverse: 5'-TGTAAAAGCTTATGAGGGAGGATG-3'

Probe: 5'-TGGCGTATGTCCTGCTCAACCTCGTGT-3'

D2R:

Forward: 5'-CCTTCATCGTCACCCTGCTG-3'

Reverse: 5'-CTGAAAGCTCGGCTGCTACG-3'

Probe: 5'-TGTTGACCCGCTTCCGACGCTTGC-3'

β-actin:

Forward: 5'-GGGCTATGCTCTCCCTCACG-3'

Reverse: 5'-GTCACGCACGATTTCCCTCTC-3'

Probe: 5'-CCTGCGTCTGGACCTGGCTGGC-3'

Neurochemical assessment (HPLC and microdialysis)

Experiments were performed as described in **EXPERIMENTAL PROCEDURES** in main text.

Behavioral analysis

Prepulse inhibition: See **EXPERIMENTAL PROCEDURES** in main text.

Novel object recognition task: The novel object recognition test was carried out as described previously (Mouri et al., 2007) with minor modifications. The experimental apparatus consisted of a Plexiglas open field box (30 x 30 x 35 cm), with the floor covered in sawdust. The test procedure consisted of three sessions: habituation, training, and retention. At P28 or P56, each mouse was individually habituated to the box, with 10 min of exploration in the absence of objects each day for 3 consecutive days (habituation session). On day 4, two novel objects were symmetrically fixed to the floor of the box, 8 cm from the walls, and each animal was allowed to explore the box for 10 min (training session). The objects were different in shape and color, but similar in size. Animals were considered to be exploring the object when the animal's head was facing the object or the animal was touching or sniffing the object. After training sessions, mice were immediately returned to their home cages. The animals were then placed back into the same box with one of the familiar objects in the training session and one novel object 1 h or 24 h after training sessions. The animals were allowed to explore freely for 10 min and the time spent exploring each object was recorded (retention session). Clozapine (3 mg/kg, *i.p.*) (Sigma-Aldrich) was

administered 30 min before training sessions.

T-maze test: The T-maze test was carried out as described previously (Clapcote et al., 2007; Kellendonk et al., 2006) with minor modifications. The maze consisted of a stem, 80 x 5.5 cm; arms, 45 x 5.5 cm; walls, 10 cm high. Mice were habituated to the maze with three trials in which they had to collect food pellets (20-mg dustless precision pellets, BIO-SERV, Frenchtown, NJ). Mice were tested in 4 trials per day. At the beginning of the trial, both arms were baited and during each trial, the randomly chosen right or left goal arm was blocked and the mouse had to run and eat the bait in the open arm. After consuming the bait in the open arm, the mouse was removed from the maze. After a 5-sec delay, the mouse was placed again at the end of the stem of the T-maze, the block was removed and a correct choice was scored when the mouse visited the other arm. The inter-trial delay was at least 20 min. When a mouse met the correct choice criterion (11 out of 12 choices for 3 days), testing 15-sec delay intervals was started on the following day. Mice were tested on 4 trials of delay for 3 days.

Forced swim test: A mouse was placed in a transparent glass cylinder (8 cm in diameter x 20 cm high), containing water at 22-23 $^{\circ}$C at a depth of 15 cm, and was forced to swim for 9 min. The duration of immobility was measured by using digital counters with infrared sensors (Scanet MV-10 AQ; Merquest, Toyama, Japan) (Noda et al., 2000). The immobility time was calculated as follows: 540 (sec) – swimming time (sec) = immobility time (sec).

Statistical Analysis

The Student's t-test was used in comparing two sets of data. Statistical differences among more than three groups were determined using one-way analysis of variance (ANOVA), two-way ANOVA, or ANOVA with

repeated measures followed by the Bonferroni multiple comparison tests. A value of $p<0.05$ was considered statistically significant. All data are expressed as the mean ± SEM.

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