

Supporting Information

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SI Materials and Methods

Two-Dimensional Gel Electrophoresis and In-Gel Analysis. Protein samples were prepared as described in *Materials and Methods* for synaptic plasma membrane preparation, except that striatal tissues from five mice were pooled for each sample, and all steps were performed in the presence of phosphatase inhibitors (Phosphatase Inhibitor Mixture-1; Sigma). Prepared membranes were resuspended in 0.32 M sucrose, 4 mM Hepes, pH 7.4, and pelleted by benchtop ultracentrifugation at $200,000 \times g$ (Ultima 100X; Beckman). Protein pellets were frozen in liquid nitrogen and submitted to Applied Biomix for 2D DIGE analysis. Protein samples were processed as described (1). Equal amounts of NR1-KD and WT protein samples were pooled and labeled with Cy2 as an internal standard. WT and NR1-KD samples were labeled with Cy3 or Cy5, and three gels were run for comparative analysis of three separate preparations. The three gels were matched by biological variance analysis module of DeCyder 6.5. Spot volume was normalized within the gel, and abundance of each spot was normalized against the internal Cy2 standard so that spots could be compared across gels. The ratio of NR1-KD over WT was calculated for each spot, and average ratio and *P* values (Student *t* test and one-way ANOVA) from three replicate samples were calculated using DeCyder 6.5 software. Spots with *P* values less than 0.05 were selected for protein identification. Protein spots of interest on preparative 2D gels were picked by Ettan Spot Picker (Amersham BioSciences) and subjected to in-gel trypsin digestion, peptide extraction, and desalting before MALDI-TOF/MS-MS (ABI 4700; Applied Biosystems). A GPS Explorer workstation was used to search the database (National Center for Biotechnology Information nonredundant) to match MS and MS/MS data to proteins in the database.

Immunogold EM. Mice were anesthetized with a lethal dose of chloral hydrate and perfused transcardially with 4% paraformaldehyde in PBS solution, pH 7.4. Striatum was dissected and 0.5-mm³ pieces were postfixed for 1 h in 1% glutaraldehyde, 0.1 M sodium cacodylate, and embedded in Lowicryl HM20 resin. Ultrathin sections (50–60 nm) were cut, supported on a grid, and incubated in 0.1 M ammonium chloride followed by blocking in Aurion blocking solution (Electron Microscopy Sciences). Grids were incubated with 25 ng/mL rabbit affinity-purified anti-DISC1-Mid (Invitrogen) or 25 ng/mL rabbit affinity-purified anti-HA in 5% FCS, PBS solution, for 1 h at room temperature, rinsed with PBS solution, and incubated with Aurion 10-nm gold-conjugated, goat anti-rabbit IgG at 1:25 dilution (Electron Microscopy Sciences). After PBS solution washes, sections were postfixed in 2% glutaraldehyde, 0.1 M sodium cacodylate, rinsed with water, and sections were stained with uranyl acetate and lead citrate. Sections were examined in a CM 12 electron microscope at a magnification of 15,000 \times , and images of synaptic fields were collected. Image analysis was performed using Nikon Elements software, using an embedded scale bar to calibrate length and area measurements of asymmetric synapses and mitochondria, respectively. For each animal, 10 images of synapses were acquired and analyzed. Synapse length measurements were made for all visible synapses in a field, and area measurements were made for all mitochondria visible within the same field. Gold particles were counted when overlapping with visible asymmetric synapses, or within the measured boundaries of the mitochondria. The majority of synapse-associated DISC1 was postsynaptic, and pre- and postsynaptic DISC1 was included in the analysis. Statistical significance was determined using Excel software by two-tailed Student *t* test with three animals per genotype.

DISC1 Immunohistochemistry. Mice aged 12 wk were anesthetized with a lethal dose of chloral hydrate and perfused transcardially with 4% paraformaldehyde in PBS solution, pH 7.4. Dissected brains were cryoprotected in 30% sucrose, embedded in optimal cutting temperature media, and 10- μ m thin sections were cut with a cryostat. Staining for DISC1 was performed as previously described (2) with minor modifications. Sections were permeabilized (0.1% Triton X-100 in PBS solution) and blocked [2% normal goat serum (NGS)] before incubation overnight at 4 °C with primary antibody mExon3 (1:400). Sections were incubated with secondary goat anti-rabbit Alexa 568 (1:400; Invitrogen), washed, and stained with DAPI before mounting with ProLong Gold antifade reagent (Invitrogen).

GFAP Immunohistochemistry. Mice subchronically treated with MK801 (0.2 mg/kg/h) for 7 d were anesthetized with isoflurane (5%) and perfused transcardially with 4% paraformaldehyde in PBS solution, pH 7.4. After overnight fixation, 100- μ m coronal sections were cut with a vibratome. Floating sections were blocked with 10% NGS in Tris-buffered saline solution, pH 7.4, 0.1% Tween 20 (i.e., TBST). Sections were incubated overnight at 4 °C with mouse anti-GFAP primary antibody (1:1,000) in 2% NGS TBST (clone N206A8; University of California, Davis/National Institutes of Health NeuroMab Facility). Subsequently, sections were washed with TBST and incubated with secondary anti-mouse Alexa 568 antibody (Invitrogen) in 2% NGS TBST. Sections were mounted with Vectashield (Vector Laboratories) and imaged with an Olympus confocal microscope and Fluoview software.

Locomotor Behavior Analysis. Locomotor activity was measured in 12-wk-old mice during their light cycle (1000 hours to 1400 hours) by using a digital activity monitor (VersaMax; Accuscan Instruments) as described previously (3). Mice were placed in a novel open field, 20 cm \times 20 cm \times 45 cm, and their activity monitored by beam breaks over a 2-h period. Total distance measurements were collected in 5-min bins. Statistical significance was determined with GraphPad Prism software by two-way ANOVA or, for cumulative total distance, by two-tailed Student *t* test with eight animals per genotype.

Social Behavior Analysis. Sociability was measured as described by Moy et al. (4), with some modifications. Test mice aged 12 wk were placed in a 60 cm \times 40.5 cm \times 22 cm arena made of opaque white Plexiglas, and containing two inverted wire cups (Galaxy Cup; Spectrum Diversified Designs). One cup was empty, and served as a nonsocial novel stimulus, whereas the other cup housed a novel, sex-matched C3H/HeJ mouse. Circular zones of 5 cm surrounding each wire cup were defined as the “social zone” and “nonsocial zone.” The test mouse was allowed to explore the arena for 10 min; during this period, the position and movements of the test mouse were recorded by overhead videocamera and analyzed by Viewer2 software (Bioobserve). The amount of time spent in each zone was recorded, as well as the number of independent visits to each zone. Sociability was expressed as the amount of time during the 10-min period that the test mouse was located in the social zone, with the center of body mass in close proximity to the novel C3H mouse. A second parameter, visit duration, described the average length of visits to the social zone; this was calculated by dividing the total length of time in the social zone by the number of independent visits to the zone. Statistical significance was determined using Excel software by two-tailed Student *t* test with eight animals per genotype.

1. Paczkowski M, Krisher R (2010) Aberrant protein expression is associated with decreased developmental potential in porcine cumulus-oocyte complexes. *Mol Reprod Dev* 77:51–58.
2. Seshadri S, et al. (2010) Disrupted-in-Schizophrenia-1 expression is regulated by beta-site amyloid precursor protein cleaving enzyme-1-neuregulin cascade. *Proc Natl Acad Sci USA* 107:5622–5627.
3. Gainetdinov RR, et al. (1999) Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science* 283:397–401.
4. Moy SS, et al. (2004) Sociability and preference for social novelty in five inbred strains: An approach to assess autistic-like behavior in mice. *Genes Brain Behav* 3:287–302.

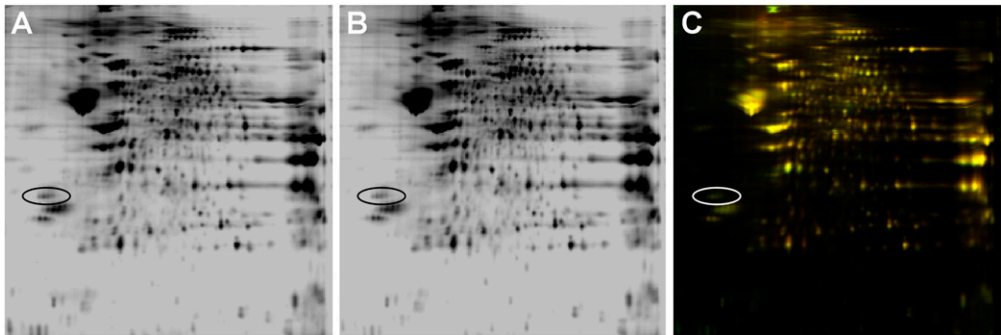


Fig. S1. Two-dimensional DIGE identifies 14-3-3ε reduction in NR1-KD synaptic protein fraction. Representative images of a 2D-DIGE gel from striatal synaptic plasma membrane samples from WT (A) and NR1-KD (B) mice in grayscale. WT protein samples were labeled with Cy3 (green), and NR1-KD protein samples were labeled with Cy5 (red). (C) Merged color image of the two fluorophores. The three circled proteins were identified as 14-3-3ε by MS. These three spots likely represent phosphorylated forms of 14-3-3ε.

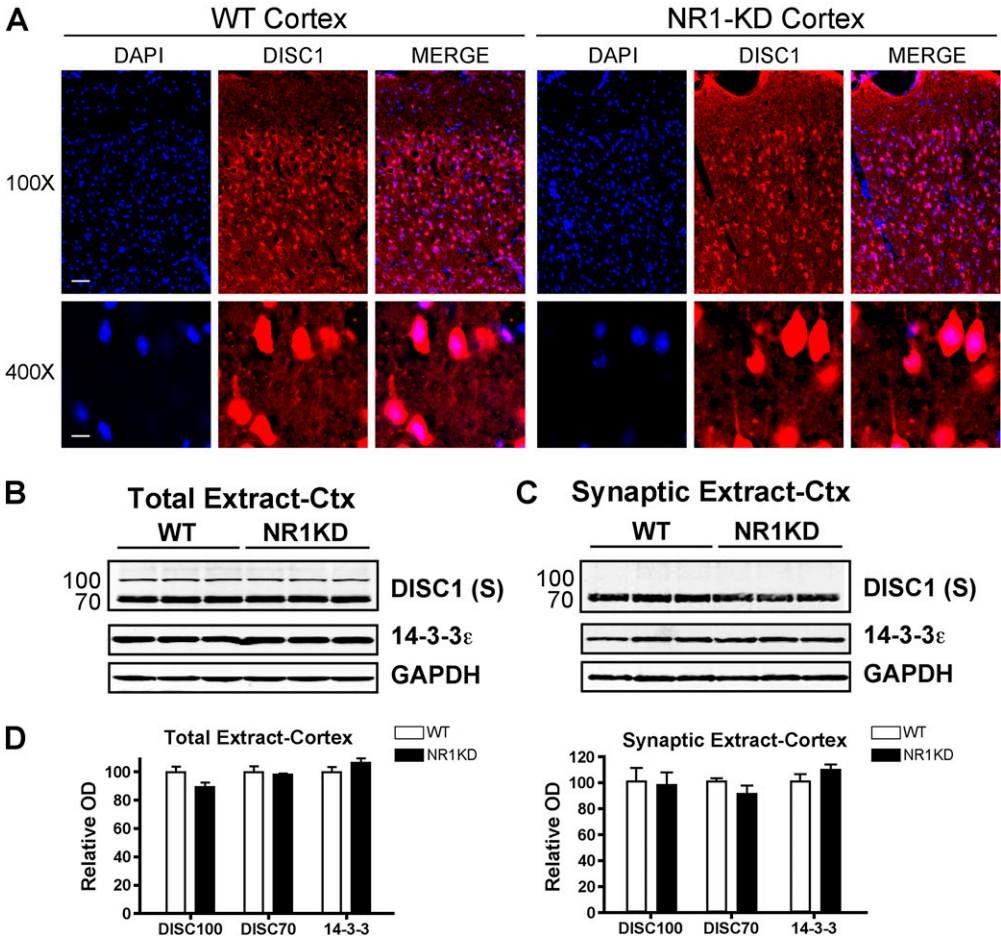


Fig. S2. DISC1 and 14-3-3ε protein levels are unchanged in the cortex of NR1-KD mice compared with WT animals. (A) Immunofluorescence images of DISC1 levels from cortical sections of WT and NR1-KD. Nuclei were imaged using DAPI labeling (blue), and DISC1 was imaged using the mExon3 primary antibody and a secondary anti-mouse Alexa 568 antibody (red). (Scale bars: 100× magnification, 50 μm; 400× magnification, 10 μm.) Western blot analysis of DISC1 and 14-3-3ε levels from total (30 μg) (B) and synaptic plasma membrane fraction (25 μg) (C) of WT and NR1-KD mice. (D) Relative levels of DISC1 and 14-3-3ε normalized to GAPDH levels. (n = 3 for each treatment group; *P < 0.05, two-tailed t test).

Fig. S3. DISC1 immunoreactivity is reduced in the striatum but not the hippocampus of NR1-KD mice compared with WT animals. (A–C) Immunofluorescence photomicrographs of DISC1 immunoreactivity from striatal sections of WT and NR1-KD mice. (Magnification, 200 \times ; scale bar, 20 μ m.) (D and E) Immunofluorescence photomicrographs of DISC1 immunoreactivity from hippocampal sections of WT and NR1-KD mice at low resolution (Magnification, 25 \times ; scale bar, 100 μ m.) (F) High-resolution immunofluorescence images of DISC1 levels in the CA3 region of the hippocampus of WT and NR1-KD mice (magnification, 200 \times ; scale bar, 20 μ m.) Nuclei were imaged using DAPI labeling (blue), and DISC1 was measured using the mXon3 primary antibody and a secondary anti-mouse Alexa 568 antibody (red).

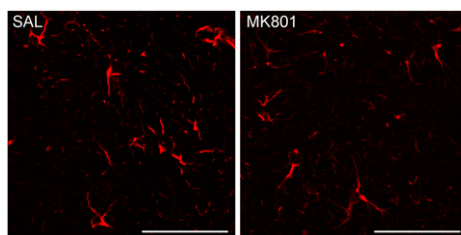


Fig. S4. Subchronic administration of MK801 does not induce neurotoxicity. GFAP immunoreactivity, used as an indicator of glial cell activation and neurotoxicity, is detected in saline-treated (SAL) and MK801-treated sections. However, GFAP immunoreactivity is not increased in the striatum of MK801-treated mice. (Scale bar: 40 μ m.)

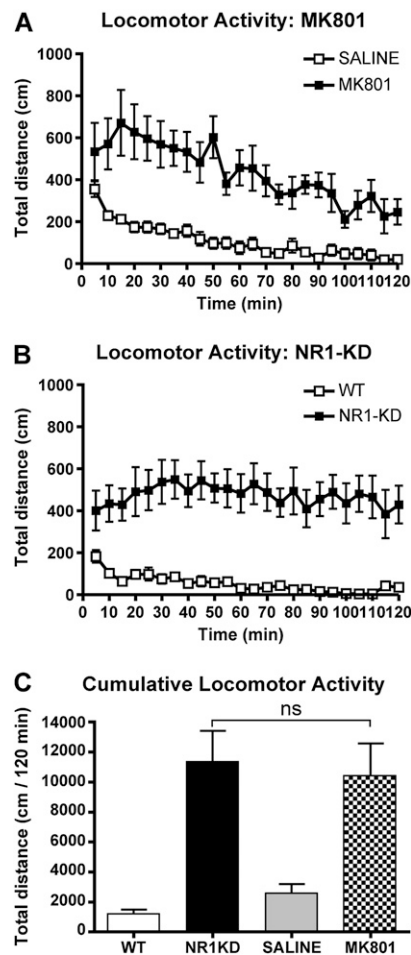


Fig. S5. Subchronic MK-801 treatment in WT mice increases locomotor activity similar to genetic reduction of NMDA receptors in NR1-KD mice. (A) Locomotor activity of WT mice in a novel environment, following 14 d of osmotic minipump infusion. Groups of mice ($n = 9$, age 12 wk) were implanted with Alzet pumps delivering 0.2 mg/kg/h of MK-801 (closed square) or saline solution (open square). (B) Locomotor activity of WT mice (open square) and NR1-KD mice (closed square; $n = 8$ for each genotype, age 12 wk). (C) Cumulative locomotor activity over a period of 2 h. Comparison of total distance traveled showed no difference between NR1-KD mice and WT mice treated with MK-801 (ns, nonsignificant; $P > 0.05$, Student t test).

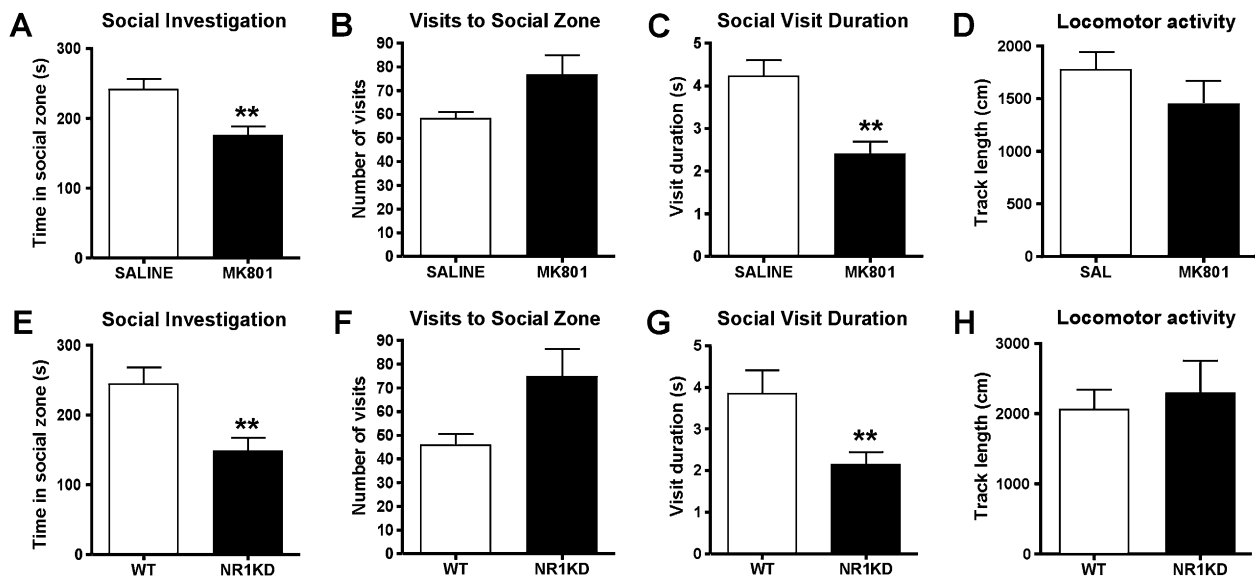


Fig. S6. Subchronic MK-801 treatment in WT mice decreases sociability similar to genetic reduction of NMDA receptors in NR1-KD mice. (A) Sociability of WT mice implanted with saline solution (SAL) or MK-801 (MK801) was determined by measuring the amount of time, over a 10-min period, test animals spent in a 5-cm zone (social zone) surrounding a novel mouse. MK-801 treatment reduced the amount of time spent in social investigation ($n = 8$ for each group). (B) The number of discrete visits to the social zone during the testing period. The number of visits was slightly increased in MK-801-treated mice, but the trend was nonsignificant. (C) The average duration of each social contact, calculated as the total time spent in social investigation divided by the number of separate contacts with the novel mouse. MK-801 treatment reduced the average length of each social contact, but not the number of contacts. (D) Locomotor activity during the 10-min trial, expressed as track length (i.e., total distance traveled). (E) Sociability of WT and NR1-KD mice (NR1KD) was determined by measuring the amount of time, over a 10-min period, test animals spent in a 5-cm zone (social zone) surrounding a novel mouse. NR1KD mice spent less time than WT controls in social investigation ($n = 8$ for each group). (F) The number of discrete visits to the social zone during the testing period. The number of visits was slightly increased in NR1-KD mice, and the trend was nonsignificant. (G) The average duration of each social contact, calculated as the total time spent in social investigation divided by the number of separate contacts with the novel mouse. NR1KD made more separate contacts with the novel mouse, but the duration of each contact was significantly shorter than WT controls (** $P < 0.005$, Student t test).

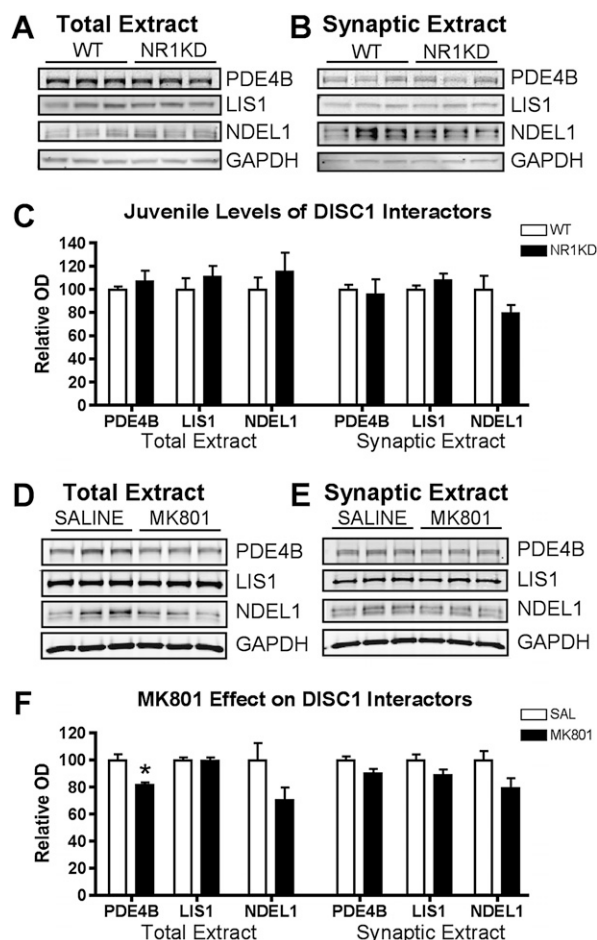


Fig. S7. Levels of DISC1-interacting proteins in juvenile NR1-KD mice or adult mice treated with MK801. (A) Western blot of total striatal extracts (25 μ g) from WT and NR1-KD mice at 2 wk of age, blotted for DISC1-interacting proteins PDE4B, LIS1, and NDEL1. (B) Western blot of striatal synaptic plasma membrane extracts (15 μ g) from the same experimental groups. (C) Relative levels of PDE4B, LIS1, and NDEL1 normalized to GAPDH levels ($n = 6$ for each genotype; $*P < 0.05$, two-tailed t test). (D) Western blot of total striatal extracts (25 μ g) from saline solution-treated (SAL) or MK801-treated (MK801) adult WT mice at 12 wk of age, blotted for DISC1-interacting proteins PDE4B, LIS1, and NDEL1. (E) Western blot of striatal synaptic plasma membrane extracts (15 μ g) from the same experimental groups. (F) Relative levels of PDE4B, LIS1, and NDEL1 normalized to GAPDH levels ($n = 6$ for each genotype; $*P < 0.05$, two-tailed t test). MK801 treatment at 0.2 mg/kg/h by osmotic minipump caused a down-regulation of total levels of PDE4B.

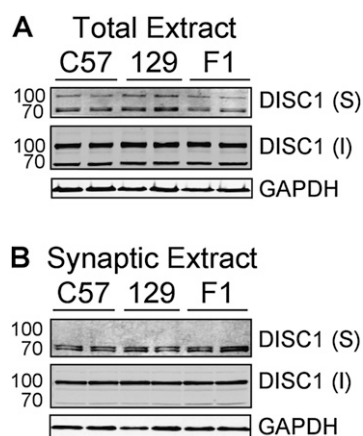


Fig. S8. Determination of DISC1 immunoreactivity in three genetic backgrounds. The genetic background used in all of the studies is an F1 resulting from the cross of an inbred or congenic C57BL/6J mouse with an inbred or congenic 129X1SvJ mouse. Striatal protein extracts from each of these genetic backgrounds were submitted to Western blotting to determine whether the antibodies used in these studies equally detect the DISC1 isoforms in the three strains. Western blot analysis of DISC1 levels from total (30 µg) (A) and synaptic plasma membrane fraction (25 µg) (B) of C57BL/6J, 129X1SvJ, and F1 mice using Santa Cruz (S) and Invitrogen (I) antibodies. GAPDH immunoreactivity is used as a loading control.