Supporting Information

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Materials and Methods

Subcellular Fractionation. Tissue was homogenized in a glass-glass potter in 0.4 mL of ice-cold 0.32 M sucrose containing 20 mM Hepes (pH = 7.4), 1 mM EDTA, 10 μg/mL aprotinin, 0.5 μg/mL antipain, 0.1 μg/mL pepstatin A, and 1 mM phenylmethanesulfonyl fluoride (Sigma Alrdich), as well as phosphatase inhibitors [mixtures A and B, 1% (vol/vol); Santa Cruz Biotechnology]. A 60-μL aliquot was reserved, sonicated, and stored at −80 °C. This aliquot served as the whole-cell lysate fraction. The resultant of homogenized tissue was centrifuged at 1,011 × *g* for 10 min at 4 °C to separate a pellet enriched in nuclear components and large cellular debris from the supernatant (S1). Next, 100 μL of sucrose buffer was added to the nuclear pellet. The pellet was sonicated and centrifuged at 16,181 × *g* for 5 min at 4 °C to remove insoluble debris. S1 was centrifuged at 16,181 × *g* for 10 min at 4 °C to obtain the cytosolic fraction (S2). The pellet (crude synaptosomal fraction) was resuspended and sonicated in tissue lysis buffer [60 mM Tris (pH 6.8), 2% SDS, and the same protease and phosphatase inhibitors as mentioned above]. Protein concentrations were measured using the Bradford protein assay (Bio-Rad).

Western Blot Analysis. Samples were lysed in the same buffer used for the BDNF ELISA. Samples (20 μg protein) were electrophoretically separated on an SDS polyacrylamide gel (7.5–12%). The phospho-protein was immunoblotted first, after which the membrane was stripped, washed, reblocked, and incubated with the total protein primary antibody. Semiquantitative assessment of protein bands was performed by measuring chemiluminescent signal using a ChemiDoc XRS Imager and Image Lab 4.0 Software (Bio-Rad).

Immunoprecipitation. A separate cohort of mice from the ELISA and Western blot analyses was used for immunoprecipitation. 2 μg of rabbit polyclonal anti-TrkB antibody (Abcam) was added to tissue lysate containing 10 mg of tissue and rotated at 4 °C for 3 h. Immunoprecipitation was carried out using the Dynabeads Protein G Immunoprecipitation kit (Invitrogen). The supernatant was removed, the tissue lysate-TrkB (tropomyosin receptor kinase B) antibody solution was added to 50 μL of Dynabeads, and the tubes were rotated at 4 °C overnight. The beads were washed according to the manufacturer's protocol. SDS/PAGE and immunoblotting was performed as described above. TrkB phosphorylation was measured by immunoblotting for phosphotyrosine, stripping the membrane, and then reprobing for TrkB. pTyr OD values (average of two bands) were divided by their corresponding TrkB OD values (average of two bands). The mutant values were normalized to WT values (percent control) collected in parallel from the same gel. The normalized values were then averaged and used for statistical analysis.

HPLC Analysis of D-Serine. Amino acids were derivatized using *o*-phthaldialdehyde (Alfa Aesar) and *N*-tert-butyloxycarbonyl-L-cysteine (Novabiochem). Amino acid peaks were resolved using a binary gradient of 25 mM sodium acetate (pH 6.5) and acetonitrile. The gradient progressed from 10 to 20% acetonitrile over the course of 25 min. Amino acid concentrations (μmol/g) were calculated by comparing an internal standard, L-homocysteic acid (L-HCA), to standard samples run at the beginning of each analysis using the following formula: (Peak height of L-HCA in standard sample/Peak height of D-serine in standard sample) × (Peak height of D-serine in tissue sample/Peak height of L-HCA in tissue sample) × [Amount of L-HCA in tissue sample (μmol)/Amount of tissue (g)]. Serum samples were treated identically to brain tissue samples.

Assessment of Kidney Tubular Integrity. Kidneys were removed from chronically treated WT and serine racemase-null mutant ($SR^{-/-}$) mice (n = 2/genotype) and placed in ice-cold 4% paraformaldehyde solution. Paraffin embedding, sectioning, hematoxylin and eosin staining was performed by the Rodent Histopathology Core Facility of the Dana-Farber/Harvard Cancer Center (P30 CA06516).

Trace Fear Conditioning. On day 1, each conditioning session consisted of a 3-min acclimation period followed by seven trials of the following structure: a 20-s tone at 75 dB followed by a 20-s trace period followed by a mild footshock (duration 2 s, amplitude 0.7 mA). Trials were roughly 4 min apart. On day 2, mice were placed in the same conditioning chambers as on day 1 and freezing behavior was measured for 8 min. On day 3, mice were placed in chambers with different contextual cues, including a smooth chamber floor, a peppermint odor (peppermint extract diluted 1:1 K), and a roof insert to distinguish the testing environment from the conditioning environment. The protocol from day 1 was repeated without the footshock. The house light in the chamber was illuminated during all sessions.

Hot-Plate Test. The mice were placed into a glass cylinder on a hot plate adjusted to 52 °C (Bioseb). Two trials separated with 5–10 min interval were carried out. For the first trial, the latency of the first reaction (licking, flinches, little leaps) was recorded, with a maximum of 30 s. For the second trial, in addition to the first reaction, the latency to jump was also recorded. A 3-min cutoff was used for the second trial.

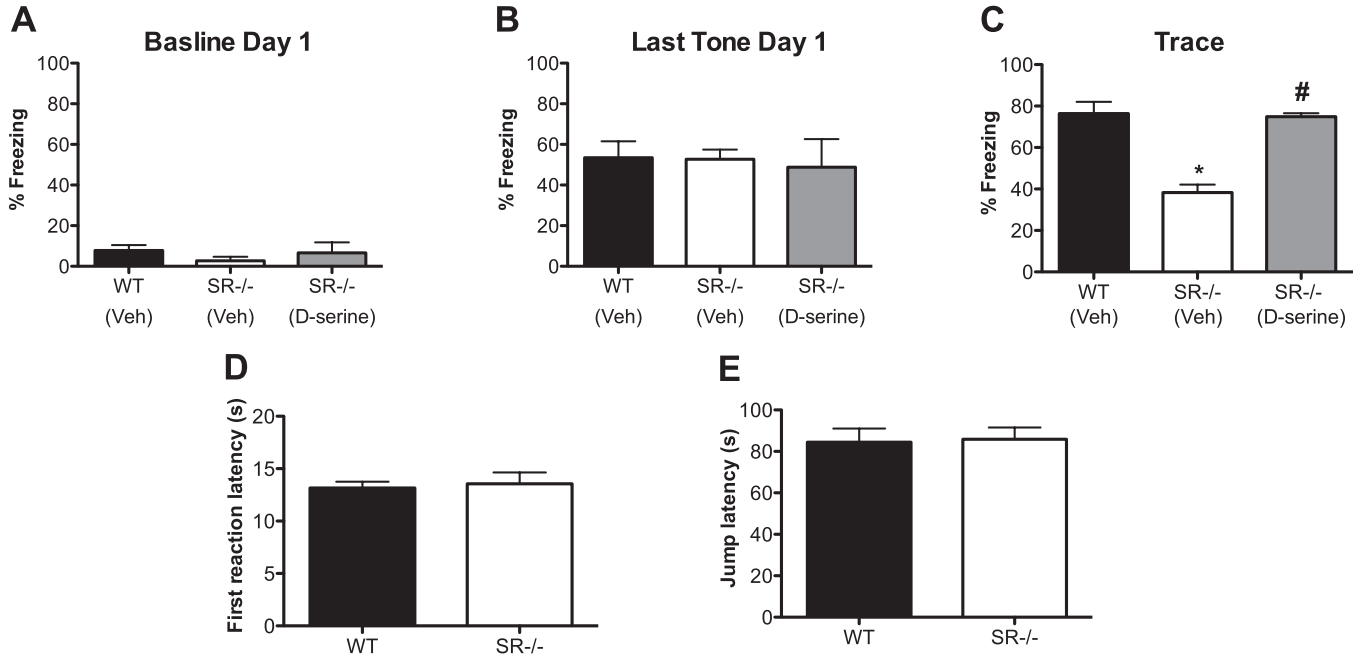

Fig. S1. Baseline freezing and pain sensitivity is unaltered in $SR^{-/-}$ mice. (*A*) WT ($n = 11$; black bars), $SR^{-/-}$ ($n = 5$; white bars), and $SR^{-/-}$ mice treated with D-serine ($n = 5$; gray bars) equally ($P > 0.05$) displayed very little nonassociative freezing during the 3-min acclimation period on day 1. (*B*) There was no significant difference in freezing behavior among groups across the seven tone presentations on day 1 [repeated-measures ANOVA; genotype: $F(2, 114) = 1.88$, $P = 0.2$] with all groups freezing equally by the last tone. (*C*) $SR^{-/-}$ mice froze less during the 20-s trace intervals (average freezing during each interval) on day 3, a deficit that was reversed by chronic D-serine treatment [$F(2,9) = 13.17$, $P < 0.005$; *, different from WT; #, different from SR−/− vehicle]. There was no difference between WT ($n = 12$) and $SR^{-/-}$ ($n = 12$) mice in thermal pain sensitivity in the hot plate assay, as measured by the latency of the first reaction (*D*; licking, flinches, little leaps) and (*E*) the latency to jump. All values represent the mean ± SEM.

Table S1. $SR^{-/-}$ mice exhibit many of the structural, neurochemical, and cognitive abnormalities that are observed in schizophrenia

Structural, molecular, and functional brain changes	Schizophrenia	$SR^{-/-}$
Reduced dendritic complexity	✓	✓
Reduced spine density	✓	✓
Cortical/hippocampal atrophy	✓	✓
Reduced BDNF/TrkB/Akt signaling	✓	✓
Reduced microRNA-132	✓	✓
Subtle cognitive impairments	✓	✓

Table S2. Primers used for quantitative PCR analyses

Gene	Source	Primer
GAPDH	Applied Biosystems	Mm99999915_g1
BDNF	Applied Biosystems	Mm04230607_s1
pri-miR-132	Applied Biosystems	Mm03306275_pri
premiR-132	N/A	5′ CGA CCA TGG CTG TAG ACT GTT 3
miR-16	N/A	5′ CCT GTC ACA CTA AAG CAG CA 3′
miR-132	Applied Biosystems	000457
snoRNA202	Applied Biosystems	001232
premiR-132	N/A	Forward: 5′AAC CGT GGC TTT CGA TTG TTA 3′
	N/A	Reverse: 5′CGA CCA TGG CTG TAG ACT GTT 3′
miR-16	N/A	Forward: 5′CCG CTC TAG CAG CAC GTA A 3′
	N/A	Reverse: 5′CCT GTC ACA CTA AAG CAG CA 3′

Table S3. Antibodies used for Western blot analyses

Antibody	Dilution	Supplier	Catalog No.
Primary			
Akt	1:1,000	Cell Signaling	9272
Akt1	1:1,000	Cell Signaling	2938
-actin	1:8,000	Abcam	8229
CREB	1:1,000	Cell Signaling	9197
eEF2	1:1,000	Cell Signaling	2332
Erk1/2	1:1,000	Cell Signaling	9102
GSK3a/b	1:1,000	Cell Signaling	5676
MeCP2	1:1,000	Millipore	07–013
MEK-1/2	1:1,000	Santa Cruz	sc-81504
mTOR	1:2,000	Millipore	07–231
p70 S6 kinase	1:1,000	Cell Signaling	2708
phospho-4E-BP-1 (Thr37/46)	1:1,000	Cell Signaling	2855
phospho-Akt (Ser473)	1:1,000	Cell Signaling	4058
phospho-Akt (Thr308)	1:1,000	Cell Signaling	4056
phospho-Akt1 (Ser473)	1:1,000	Cell Signaling	9018
phospho-CREB (Ser133)	1:1,000	Cell Signaling	9196
phospho-GSK3a/b (Ser21/9)	1:1,000	Cell Signaling	8566
phospho-eEF2 (Thr56)	1:1,000	Cell Signaling	2331
phospho-Erk1/2 (Thr202/Tyr204)	1:1,000	Cell Signaling	9101
phospho-MEK-1/2 (Ser218/Ser222)	1:1,000	Santa Cruz	sc-7995
phospho-p70 S6 kinase (Thr389)	1:1,000	Cell Signaling	9205
phosphotyrosine (pY99)	1:4,000	Santa Cruz	sc-7020
TrkB	1:1,000	BD Transduction	610101
TrkB (used for IP)	2 g	Abcam	ab33655
Secondary			
Goat anti-rabbit	1:5,000	Abcam	ab6721
Rabbit anti-mouse	1:3,000	Abcam	ab6728

CREB, cyclic AMP-responsive element binding; eEF2, eukaryotic elongation factor 2; GSK3, glycogen synthase kinase 3; MeCP2, Methyl CpG binding protein 2; mTOR, mammalian target of rapamycin; phospho-4E-BP-1, eukaryotic translation initiation factor 4E-binding protein 1; TrkB, tropomyosin receptor kinase B.