The Role of Dendritic Brain-Derived Neurotrophic Factor Transcripts on Altered Inhibitory Circuitry in Depression

Supplementary Information

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SUPPLEMENTARY METHODS AND MATERIALS

Human postmortem subjects

Postmortem brains were collected during autopsies conducted at the Allegheny County Medical Examiner's Office (Pittsburgh, PA) after consent from next of kin. The samples available in this brain collection have been previously characterized in microarray and micro-anatomical studies (1-4). In short, for all cases, a committee of experienced clinicians makes consensus DSM-IV diagnoses using information obtained from clinical records, toxicology exam and standardized psychological autopsy (5). The latter incorporates a structured interview, conducted by a licensed clinical psychologist with family members of the index subject, to assess diagnosis, psychopathology, medical, social and family histories, as well as history of substance abuse. Individuals were also screened for the absence of neurodegenerative disorders by neuropathological examination (6-8). All procedures were approved by the University of Pittsburgh's Committee for the Oversight of Research and Clinical Trials Involving the Dead and Institutional Review Board for Biomedical Research. Upon brain collection, coronal blocks are cut in ~2 cm blocks through the rostro-caudal extent of the brain and stored at –80°C. The RNA integrity of each brain is assessed by chromatography using the Agilent Bioanalyzer instrument (Agilent Bioanalyzer; Santa Clara, CA, USA). After careful examination of clinical and technical parameters, 19 pairs of MDD and unaffected control subjects were selected (detailed characteristics of the cohort in Supplementary Table S1). Each MDD subject was matched for sex and as closely as possible for age with one control subject. Subject groups did not differ in mean age, PMI, RNA integrity number (RIN), RNA ratio (ratio between 18S and 28S ribosomal RNA), or brain pH as determined by one way ANOVA ($p>0.05$).

Animals and unpredictable chronic mild stress

9-10 week old adult C57BL/6J male mice were divided into two groups and submitted to control housing condition or UCMS consisting of an 7-week regimen of pseudo-random unpredictable mild stressors: forced bath (~2 cm water in cage for 15 minutes), wet bedding, predator odor (1 hour exposure to fox or bobcat urine), light cycle changes, social stress (rotate mice into previously occupied cage), tilted cage (45°), mild restraint (50 mL Falcon tube with air hole for 15 minutes) and bedding changes. The progression of the UCMS syndrome was monitored weekly by assessing the degree of coat state degradation and weight changes for each mouse. All procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Behavioral testing

After UCMS, common tests for depression and anxiety-like phenotype were conducted. Behavior evaluated using the Elevated Plus Maze (EPM) used a cross maze with 2 open and 2 closed 30×5 cm arms under red light. Time spent in the open arms and percent entries (entries into open arms divided by entries into open or closed arm×100) in the open arms was recorded for 10 min to measure anxiety-like behavior. The total number of entries into any arm was used as an index of locomotor behavior. The Open Field test (OF) was performed with the aid of the ANY-maze video tracking system for the measurement of anxiety-like behavior. The center of the OF was defined as the centermost 51×51 cm arena. The time spent and percent distance traveled (distance in center divided by total distance×100) in the center of the arena was recorded for 10 min. The total distance travelled was recorded as an index of locomotor activity. The latency for food-deprived mice to feed in an aversive, novel environment was be used as an index of emotionality in the Novelty Suppressed Feeding test (NSF). Mice were food-deprived for 16 h prior to exposure to NSF. Testing was performed in a brightly lit 51×51 cm arena covered in bedding. Latency to eat a food pellet placed in the aversive center of the arena was recorded during a 12-minute session. Food consumption in the home cage (food eaten divided by body weight) during 5 min following NSF testing and percent weight lost during food deprivation was measured as controls for appetite differences. The Cookie Test (CT) is based on the conflict between the drive for the stimulus and the neophobic behavior of the mouse and utilizes a device containing three aligned compartments with the same dimension (20 × 20 × 20 cm). Mice were first familiarized with a chocolate cookie (Keebler® Fudge Stripes, US) 5 days before the first testing. At the time of testing, a small amount (2±1 g) of chocolate cookie was placed at the corner of the black compartment. The mouse was initially placed in the white compartment of the apparatus. The time of first cookie consumption (bites) was recorded within the 12 min test period. Next day, another session of testing was performed. An elongated time prior to cookie consumption was interpreted as anhedonia-like behavior, a habituation deficit or a combination of both effects.

Emotionality z-score

To assess consistency of behavior across tests, we integrated emotionality-related measures across tests as described earlier (9). Z-scoring is a mathematical tool used to normalize results within studies. These values indicate how many standard deviations (σ) an observation (X) is above or below the mean of the control group (μ). The equation is as follows: $z=\frac{X-\mu}{\sigma}$

After adjusting directionality of the scores so that increased score values reflected increased depressive-/anxiety-like behavior, z-scores of individual tests were averaged for group emotionality z-score.

RNA extraction and real-time quantitative polymerase chain reaction

For human studies, gray matter of dlPFC were collected in TRIzol and further purified with Qiagen RNeasy spin columns (QIAGEN, Valencia, CA, USA). For mice, mPFC punches were taken using 19G corer and processed for RNA extraction with Qiagen RNeasy micro kit. To generate cDNA, 100 ng of total RNA was mixed with qScript cDNA supermix (Quanta BioSciences, Gaithersburg, MD, USA) according to manufacturer's protocol. PCR products were amplified in triplets on a Mastercycler real-time PCR machine (Eppendorf, Hamburg, Germany) using universal PCR conditions. Results were calculated as the geometric mean of threshold cycles normalized to three validated internal controls (*ACTB, GAPDH*, and *PPIA*). Genes for qPCR verification were (i) *BDNF* transcript variants: *BDNF*-EXON 1, 2, 4, 6, CDS, L-3' UTR; (ii) *NTRK2* isoforms (full length (FL), truncated form (T1)); (iii) excitatory neuronal genes: *SLC17A7, GRIA1, GRIN 2A & 2B, DLG4*; (iv) inhibitory synapse-related genes: presynaptic genes (*SLC32A1, SST, NPY, CORT, PVALB, GAD 1 & 2*), postsynaptic GABA receptor genes (*GABRA 4 & 5, GABRB3*).

Small hairpin RNA, DNA constructs and adeno-associated virus (AAV)

Small hairpin RNA (shRNA) sequence targeting ggaaggctcggaagcaccct of L-3' UTR of mouse *Bdnf* was adapted from literature (10). Lentiviral vectors containing shRNA against *Bdnf* L-3' UTR or scrambled shRNA, and eGFP as a reporter gene were purchased from GeneCopeia (Rockville, MD, USA). AAV9-*Bdnf* L-3' UTR shRNA-GFP and control virus with scrambled shRNA were commercially prepared with the U6 promoter (Virovek, Hayward, CA, USA). Briefly, Bac-to-bac system (Invitrogen) was used to generate recombinant baculoviruses. First, shRNA was sub-cloned into pFastBac shuttle vector and transformed into DH10Bac competent bacteria. After antibiotic selection, recombinant Bacmid containing E. coli were cultured and miniprep DNAs were prepared. Recombinant baculovirus particles were generated by transfecting Sf9 cells with miniprep DNAs and amplified once. For AAV vector production, Sf9 cells were double-infected with recombinant baculovirus containing the shRNA and a helper recombinant baculovirus containing the AAV Rep and Cap genes. Three days post infection, the Sf9 cells were harvested and lysed in lysis buffer (50mM Tris-HCl, pH7.8, 50mM NaCl, 2mM $MgCl_2$, 1% Sarkosyl, 1% Triton X-100, and 140 units/mL Benzonase). After genomic DNA digestion, cell

debris was removed by centrifugation at 8,000 rpm for 30 min. To purify AAV vectors, the cleared lysates were loaded onto CsCl step-gradient and subjected to ultracentrifugation at 55,000 rpm for 20 hours. The viral band was drawn through a syringe with an 18-gauge needle and loaded onto a second CsCl and subjected to linear-ultracentrifugation at 65,000rpm for 20 hours. Then the viral band was drawn and passed through two PD-10 desalting columns (GE HealthCare) to remove the CsCl and detergents and at the same time exchanged to PBS buffer containing 0.001% pluronic F-68. qPCR was performed to determine the virus titer with primers corresponding to the target gene.

Primary culture and transfection of mouse cortical neurons

Primary cortex neurons obtained from E18 embryos of C57BL/6J mouse were purchased from Thermo Fisher Scientific (Waltham, MA, USA; A15585). After rapid-thaw, 3 $X10^5$ viable neurons were cultured on poly-D-lysine coated 12 mm coverslips in Neurobasal media (Thermo Fisher Scientific; 21103) supplemented with 2% B27 (Thermo Fisher Scientific; 17504), 0.5 mM GlutaMAX™-I Supplement (Thermo Fisher Scientific; 35050), and 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA; P4333). Half of the medium was replaced with fresh medium every third day to feed cells. At 7 days in vitro (DIV7), transfection was performed using 1 µL of Lipofectamine 3000 (Thermo Fisher Scientific; L3000015) and plasmid DNA (0.5 µg) in 50 µL of OPTI-MEM (Thermo Fisher Scientific; 31985062). Half of the culture media was replaced with fresh media after 3 days.

Fluorescent-activated cell sorting and Knock-down yield test

5 days after transfection, media was removed and rinsed with 500 µL of DPBS twice. 300 µL of 0.5% trypsin-EDTA was added to detach cells from the plate. After 10 min at 37°C, trypsin reaction was stopped by adding 300 µL of saline with 1% BSA, 4 mM EDTA, 20 U/mL of DNase1. Cells were collected by centrifugation at 300G, 5 min at 4°C and resuspended in 0.3 mL of FACS buffer (PBS, 0.5% BSA, 2 mM EDTA, 10 U/mL of DNase1). GFP-expressing cells were directly collected to Qiagen RNeasy lysis buffer containing microtube by FACS AriaII in McGowan Institute for Regenerative Medicine Flow Cytometry Facility (http://www.mirm.pitt.edu/flowcytometer/default.asp). Total RNA was extracted using Qiagen RNeasy micro kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocol. cDNA synthesis and qPCR were performed as described above.

Immunocytochemistry and image analysis

After 5 days of transfection, cells were rinsed twice with PBS and fixed with 4% paraformaldehyde (PFA) in PBS for 25 minutes. After subsequent rinses, cells were stained with rabbit anti-GFP (2 µg/mL, Life Technologies; A21311) and mouse anti-MAP2 (2 µg/mL, Sigma-Aldrich; M9942) to visualize dendrites of transfected cells. Images were obtained using Nikon 90i microscope and the morphology of dendrites were automatically traced and reconstructed by Imaris software (Bitplane AG, Concord, MA, USA). Quantification of the total length of dendrites, number of dendritic segments, Sholl analysis with concentric circles drawn every 10 µm from the soma were performed by the program.

Animals and stereotaxic surgery

Young adult C57BL/6J male mice were bilaterally injected with 400 nL of AAV9-*Bdnf* L-3' UTR shRNA-GFP virus or with AAV9-scrambled shRNA-GFP (control) with a titer of ~ 2 X 10^{12} genome copies/mL into the mPFC (A/P +2.0 mm, M/L ±0.4 mm, D/V -2.0 mm). After 2-3 weeks of recovery, mice were exposed to UCMS. For gene expression analysis, mice received *Bdnf* L-3' UTR shRNA in one hemisphere and scrambled shRNA in the other for intra-subject comparison.

Fluorescent in situ hybridization and laser microdissection

At post-surgery week 6, AAV-infused mice were transcardially perfused with 1 mL of PBS and lightly fixed with 1 mL of 4% PFA to preserve GFP signal and RNA integrity. Mouse brains were sectioned at 12 µm using a cryostat and thaw-mounted onto a polyethylene naphthalate-membrane slides (Leica Microsystems, Richmond Hill, ON, Canada, 11600289) that were pretreated with RNase Away (Thermo Fisher Scientific; 7005-11), UV at 254 nm for 30 min and coated with 0.01% Poly-L-Lysine (Sigma-Aldrich; P8920). To visualize *Sst*- and *Pvalb*-expressing GABA neurons, fluorescent in situ hybridization technique was used with modification for RNAscope (Advanced Cell Diagnostics, Hayward, CA, USA). Briefly, brain sections were examined under 5X objective in the fluorescence mode of Leica LMD7 system to identify shRNA expressing brain region which can be distinguished by GFP signal. After marking GFP (+) region with laser, brain sections were fixed with cold 4% PFA in PBS and dehydrated with ethanol. After protease treatment, antisense probe targeting *Sst* and *Pvalb*, preamplifier, signal amplifiers, and Atto 550 conjugated-label probes were serially hybridized in a HybEz hybridization oven at 40°C following manufacturer's protocol (Advanced Cell Diagnostics, Newark, CA). After final wash, slides were rinsed with PBS and dehydrated with

ethanol. ~200 *Sst* (+) or *Pvalb* (+) cells were collected from GFP (+) region and processed for RNA extraction using Picopure RNA isolation kit (Thermo Fisher Scientific; KIT0204). cDNA was synthesized using SuperScript VILO cDNA synthesis kit (Thermo Fisher Scientific; 11754050). GABA-related gene expression levels were measured with qPCR as described above.

Immunohistochemistry and image analysis

Mice were anesthetized and perfused through the heart with PBS, followed by fixative solution (11) containing 4% PFA in PBS. Fixed brains were removed from the skull, post-fixed overnight, and then dehydrated for 48 hours in 30% sucrose in PBS. Coronal sections with a thickness of 35 µm were collected in six serially adjacent sets and stored at 4°C in PBS with 0.1% sodium azide. After blocking with 10% normal goat serum in PBS with 0.3% Triton X-100, sections were incubated with Alexa Fluor® 488 conjugated rabbit anti-GFP (2 µg/mL, Life Technologies; A21311) diluted in PBS containing 1% normal goat serum. After wash, sections were mounted onto charged glass microscope slides (SuperFrost Plus; Fisher Scientific), air-dried and coverslipped with Vectashield hardset mounting media (Vector Laboratories Inc, Burlingame, CA, USA; H-1400). For BDNF immunostaining, 12 µm sections thaw-mounted onto polyethylene naphthalate-membrane slides were used. Antigen was retrieved by incubating the sections in sodium citrate buffer (Sigma-Aldrich, C9999) at 95°C for 10 min. Sections were cooled to room temperature and blocked with 20% normal donkey serum in PBS with 0.3% Triton X-100 and incubated with rabbit anti-BDNF antibody (1 µg/mL, Abcam; ab108319) overnight at 4°C. After wash, sections were incubated with donkey anti-rabbit antibody (4 µg/mL, Abcam; ab175470) and coverslipped with Slowfade diamond antifade mountant (Thermo Fisher; S36972). To identify shRNA expressing brain region, a consecutive section was used for GFP immunostaining with Alexa Fluor® 488 conjugated mouse anti-GFP (Santa Cruz Biotechnology, Dallas, TX, USA; sc-9996). BDNF signal intensity within the outlined area was measured using Slidebook 6.0 (Intelligent Imaging Innovations, Inc., Denver, CO, USA).

Statistical analysis

Gene expression differences between MDD and control subjects were determined by analysis of covariance (ANCOVA) using SPSS (SPSS, Inc., Chicago, IL, USA). To determine covariates to include in the gene-specific models, nominal factors (sex, suicide, antidepressants) were each tested as the main factor by ANOVA, scale covariates (age, pH, PMI, RNA ratio, RIN) were tested by Pearson correlation, and repeated measures were corrected by modified Holm-Bonferroni test. ANCOVA models including significant co-factors were then applied. Overall

correlation between *Bdnf* L-3' UTR and MDD-affected or unaffected genes were calculated with one sample t-test to 0. For cell culture and animal studies, Student's t-test was used to determine statistical significance between two groups. Repeated measures ANOVA was performed to determine interaction between shRNA and stress, effect of stress or shRNA on coat state over time.

Supplementary Figures and Tables

Table S1. Characteristics of human postmortem brain samples

	Control	MDD
n	19	19
Age (years)	48.1 ± 10.6	45.2 ± 10.1
Sex	M 10, F 9	M 10, F 9
Race	W 18, B 1	W 18, B 1
Postmortem interval (hours)	19.5 ± 5.1	20.1 ± 6.0
pH	6.6 ± 0.2	6.6 ± 0.2
RNA integrity number	8.0 ± 0.6	8.0 ± 0.5

Table S2. Cofactor analysis in postmortem subjects

Cofactor analysis		Age	PMI	pH	RNA ratio	RIN	Sex	Suicide	AD ATOD
BDNF L-3' UTR	All (n=38)	0.296	0.403	0.006	0.015	0.275	0.361		
	Control (n=19)	0.007	0.368	0.797	0.030	0.333	0.138		
	MDD (n=19)	0.950	0.505	0.001	0.392	0.347	0.944	0.302	0.327
SST	All (n=38)	0.023	0.473	0.127	0.030	0.794	0.215		
	Control (n=19)	0.010	0.596	0.706	0.675	0.920	0.860		
	MDD (n=19)	0.068	0.726	0.031	0.005	0.565	0.022	0.110	0.320
NPY	All (n=38)	0.541	0.586	0.073	0.293	0.747	0.077		
	Control (n=19)	0.642	0.720	0.808	0.912	0.924	0.190		
	MDD (n=19)	0.194	0.424	0.002	0.137	0.263	0.132	0.024	0.190
CORT	All (n=38)	0.158	0.368	0.175	0.011	0.588	0.139		
	Control (n=19)	0.005	0.536	0.700	0.044	0.820	0.305		
	MDD (n=19)	0.414	0.562	0.090	0.217	0.273	0.122	0.059	0.796
SLC32A1	All (n=38)	0.383	0.980	0.989	0.191	0.066	0.103		
	Control (n=19)	0.533	0.788	0.465	0.902	0.072	0.551		
	MDD (n=19)	0.103	0.885	0.731	0.007	0.707	0.010	0.046	0.596
GAD1	All (n=38)	0.811	0.848	0.517	0.239	0.737	0.146		
	Control (n=19)	0.813	0.934	0.761	0.626	0.510	0.558		
	MDD (n=19)	0.803	0.967	0.171	0.400	0.205	0.005	0.305	0.832
GAD2	All (n=38)	0.463	0.766	0.783	0.284	0.886	0.218		
	Control (n=19)	0.822	0.844	0.541	0.583	0.902	0.302		
	MDD (n=19)	0.500	0.977	0.589	0.758	0.684	0.239	0.906	0.187
GABRA5	All (n=38)	0.395	0.954	0.490	0.040	0.714	0.119		
	Control (n=19)	0.371	0.700	0.647	0.297	0.421	0.041		
	MDD (n=19)	0.152	0.903	0.155	0.088	0.177	1.000	0.072	0.316

Abbreviations: PMI, postmortem interval; RIN, RNA integrity number; AD ATOD, Antidepressant at time of death

Table S3. Sholl analysis results

	scrambled		shRNA		
Radius (µm)	mean	s.e.	mean	s.e.	p-value
10	7.5	0.8	5.8	0.9	0.180
20	10.6	1.3	8.1	1.2	0.195
30	12.3	1.2	10.0	1.4	0.234
40	12.9	1.3	8.8	1.2	0.036
50	14.6	1.5	8.9	1.2	0.010
60	16.9	2.1	8.3	1.2	0.004
70	17.7	2.0	8.9	1.5	0.004
80	20.0	2.2	8.5	1.2	0.001
90	19.9	2.1	9.6	1.4	0.001
100	18.9	2.0	9.4	1.3	0.002
110	20.4	1.7	9.7	1.3	8.4E-05
120	19.8	1.6	10.0	1.2	1.9E-04
130	19.6	1.8	9.5	1.5	4.7E-04
140	18.0	1.6	9.7	1.4	0.001
150	18.4	1.5	9.5	1.4	3.0E-04
160	17.7	1.5	9.0	1.3	4.1E-04
170	17.1	1.3	10.0	1.5	0.002
180	16.6	1.4	9.1	1.4	0.002
190	16.4	1.3	9.8	1.6	0.004
200	15.6	1.3	10.2	1.5	0.013
210	15.2	1.1	9.1	1.4	0.002
220	14.2	1.0	9.6	1.6	0.016
230	14.1	0.8	9.7	1.4	0.009
240	13.9	0.8	9.6	1.4	0.011
250	13.4	0.8	9.6	1.3	0.016

Table S4. Similarity between MDD- and *Bdnf* L-3'UTR KD-induced gene expression changes

	Cohort	MDD		BDNF L-3' UTR KD	
	Gene symbol	ALR	p-value	ALR	p-value
BDNF	BDNF-CDS	-0.02	0.45	-1.01	2.1E-03
	BDNF-EXON1	-0.87	0.06	-0.49	0.15
	BDNF-EXON4	-0.16	0.23	-0.69	0.03
	BDNF-EXON2	-0.88	0.23	-0.29	0.12
	BDNF-EXON6	-0.25	0.40	-0.41	0.23
	BDNF L-3' UTR	-0.37	3.7E-03	-0.69	0.01
NTRK2	NTRK2-FL	0.27	0.14	0.16	0.09
	NTRK2-T1	0.14	0.18	-0.16	0.30
Inhibitory presynaptic-related genes	SST	-0.75	1.5E-03	-0.54	8.0E-04
	NPY	-0.60	3.4E-04	-0.07	0.40
	CORT	-0.56	5.0E-06	-0.34	0.16
	PVALB	0.12	0.07	0.34	0.18
	SLC32A1	-0.45	0.01	-0.09	0.40
	GAD1	-0.30	0.01	0.03	0.44
	GAD2	-0.27	0.02	-0.28	0.08
Inhibitory postsynaptic-related genes	GABRA4	0.18	0.12	-0.19	0.37
	GABRA5	-0.30	3.2E-04	-0.24	0.25
	GABRB3	0.29	0.09	-0.16	0.26
Excitatory synapse-related genes	GRIA1	0.31	0.10	0.14	0.31
	GRIN2A	0.38	0.08	-0.03	0.45
	GRIN2B	0.39	0.09	0.19	0.30
	SLC17A7	0.22	0.22	0.03	0.47
	DLG4	0.18	0.25	0.08	0.18

Abbreviation: ALR, average log ratio (MDD/control, BDNF L-3' UTR KD/control).

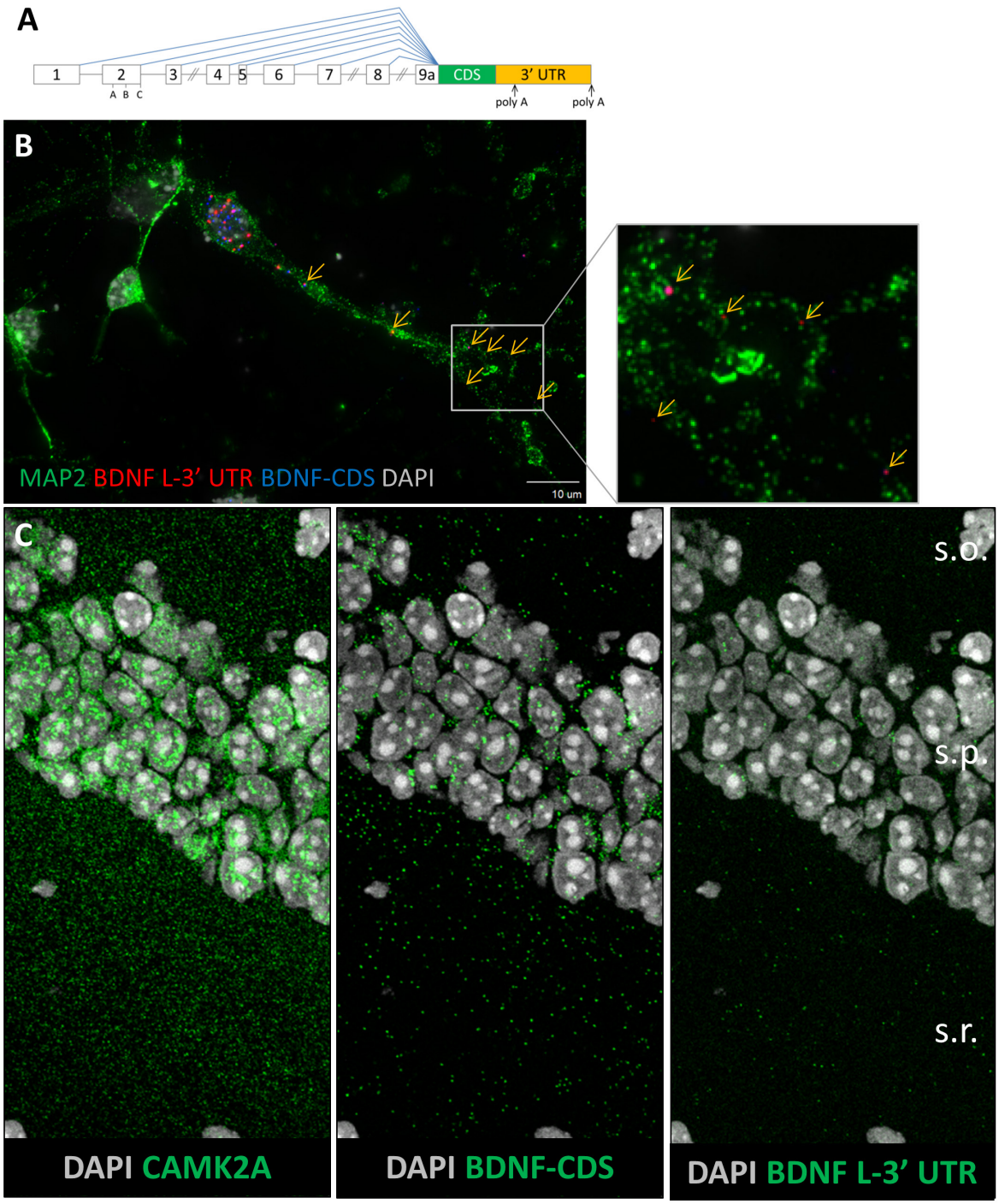

Figure S1. Dendritic localization of L-3' UTR (+) *Bdnf* transcripts in pyramidal cells. (A) Rodent BDNF gene structure. Human BDNF gene has two additional exons (5h, 8h). *Bdnf* L-3' UTR and CDS was labeled via fluorescent in situ hybridization in (B) primary cortical neuronal culture (C) hippocampus. Immunolabeling for *Map2* and in situ hybridization for *Camk2a* was utilized to trace dendrites of pyramidal neurons.

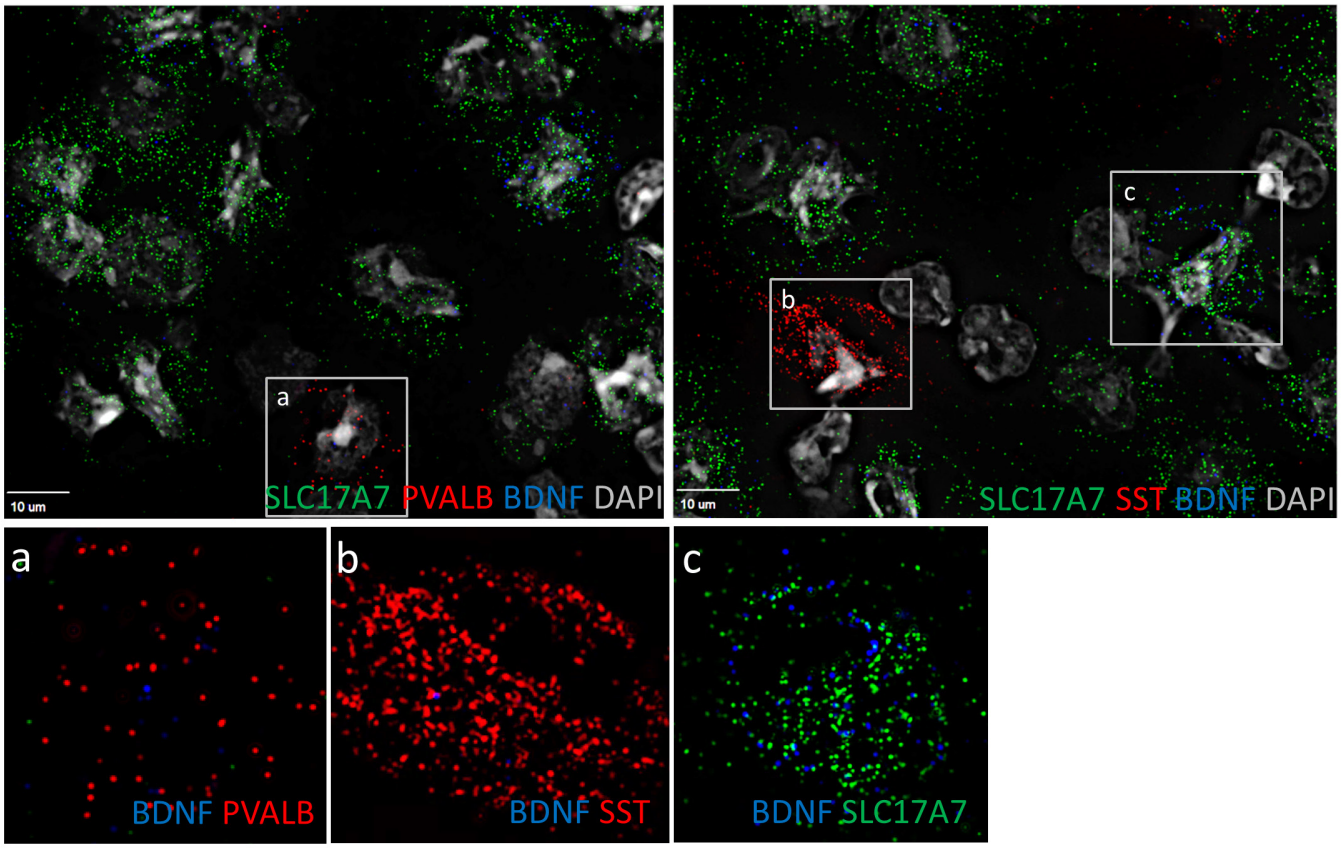

Figure S2. Glutamatergic-neuron specific *Bdnf* expression in mouse frontal cortex. Fluorescent in situ hybridization for *Bdnf*-CDS and three different cell markers reveals that BDNF is absent in GABA neurons expressing (a) *Pvalb* or (b) *Sst* and highly expressed in (c) glutamatergic neurons expressing *Slc17a7*.

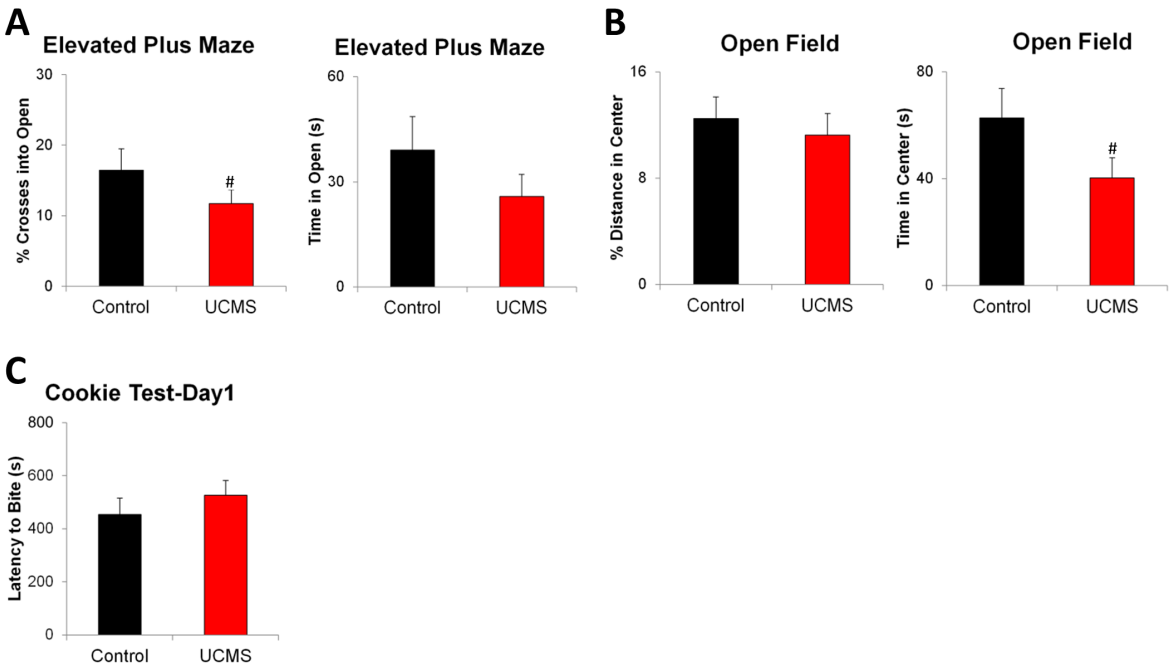

Figure S3. UCMS-induced behavioral changes in mice. (A) Chronic stress-associated behavioral changes of mouse in elevated plus maze (left: crosses into open arms compared to closed arms, right: time spent in open arms), (B) open field (left: distance traveled in center compared to total distance, right: time spent in center area), (C) latency to bite cookies in day 1 of cookie test (n=12/group; *p<0.05, **p<0.01). Data are represented as mean ± SEM.

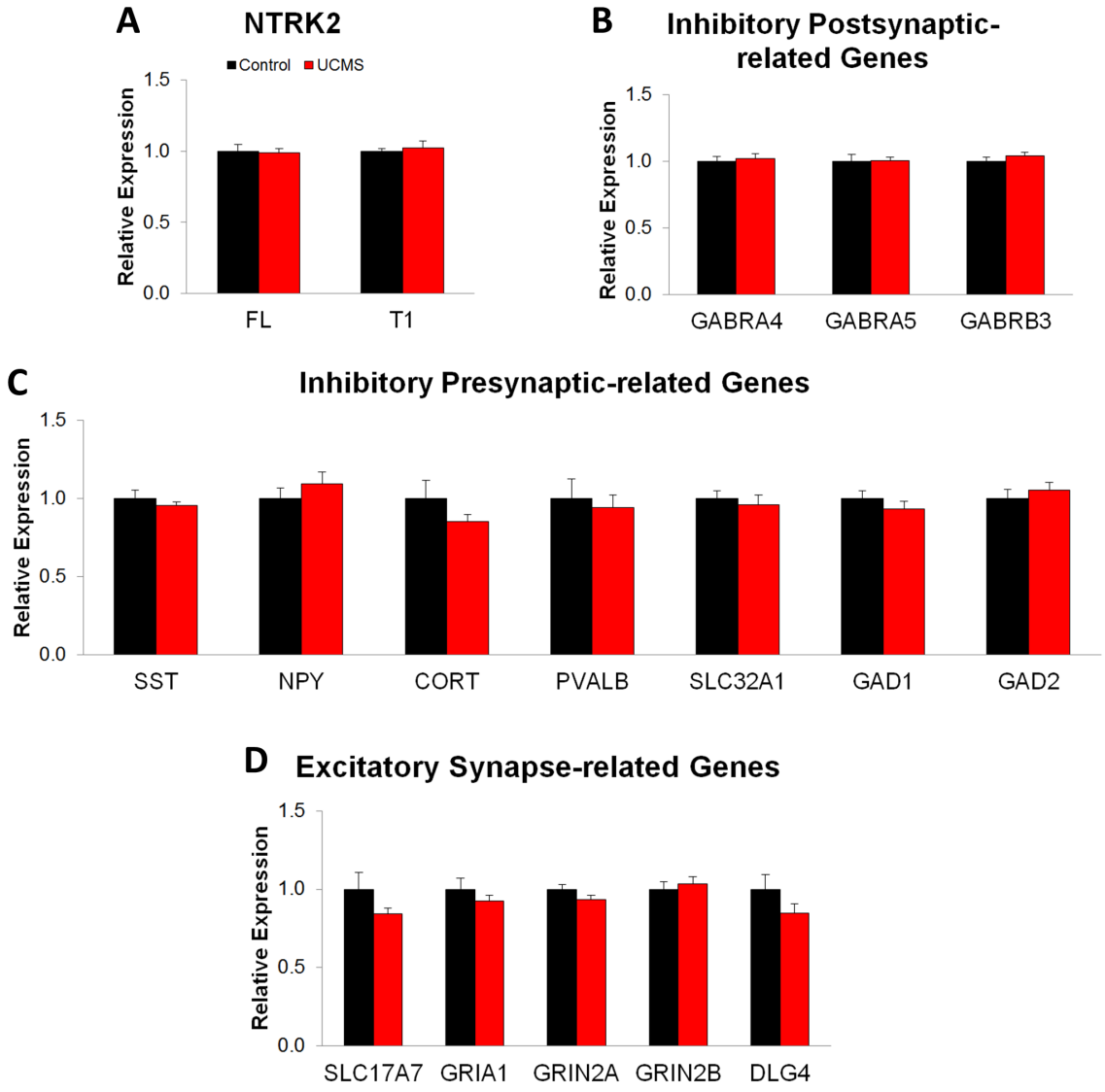

Figure S4. UCMS-induced gene expression changes in mouse mPFC. Relative expression level of (A) *Ntrk2* isoforms, (B-D) selected sets of synapse related genes (n=12/group; *p<0.05, **p<0.01). Data are represented as mean ± SEM.

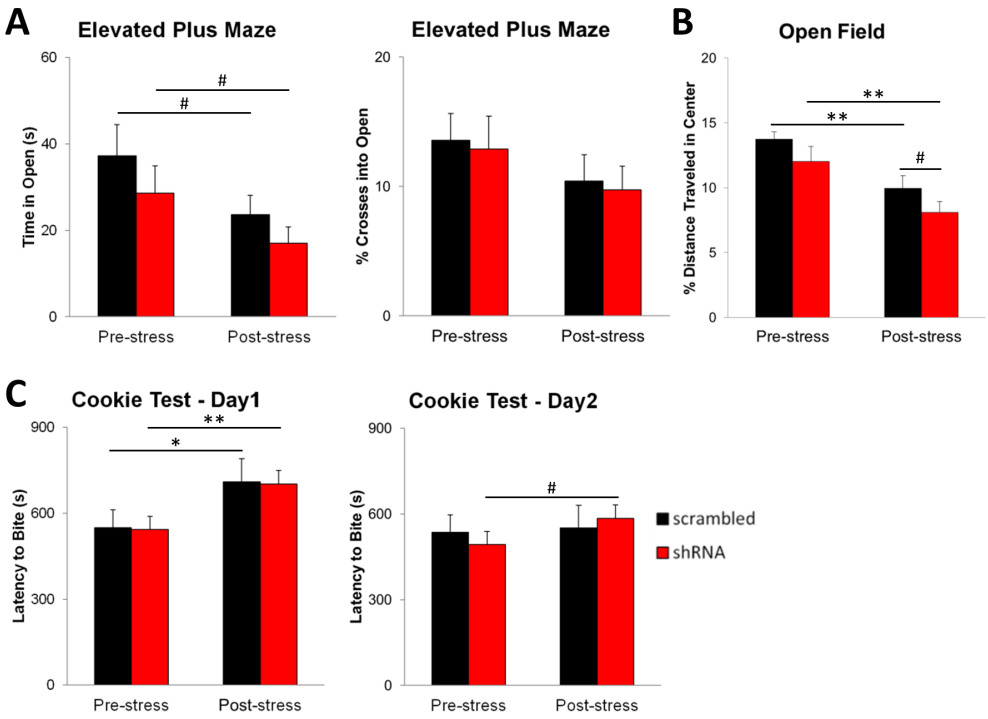

Figure S5. *Bdnf* L-3' UTR KD and stress-induced behavioral changes. (A) Stress-associated behavioral changes of shRNA treated mouse in elevated plus maze (left: time spent in open arms, right: crosses into open arms compared to closed arms), (B) open field (distance traveled in center area). (C) Latency to bite cookies in day 1 and day 2 of cookie test (scrambled: n=9, shRNA: n=13; $^{\#}p<0.1$, *p<0.05, **p<0.01, ***p<0.001). Data are represented as mean ± SEM.

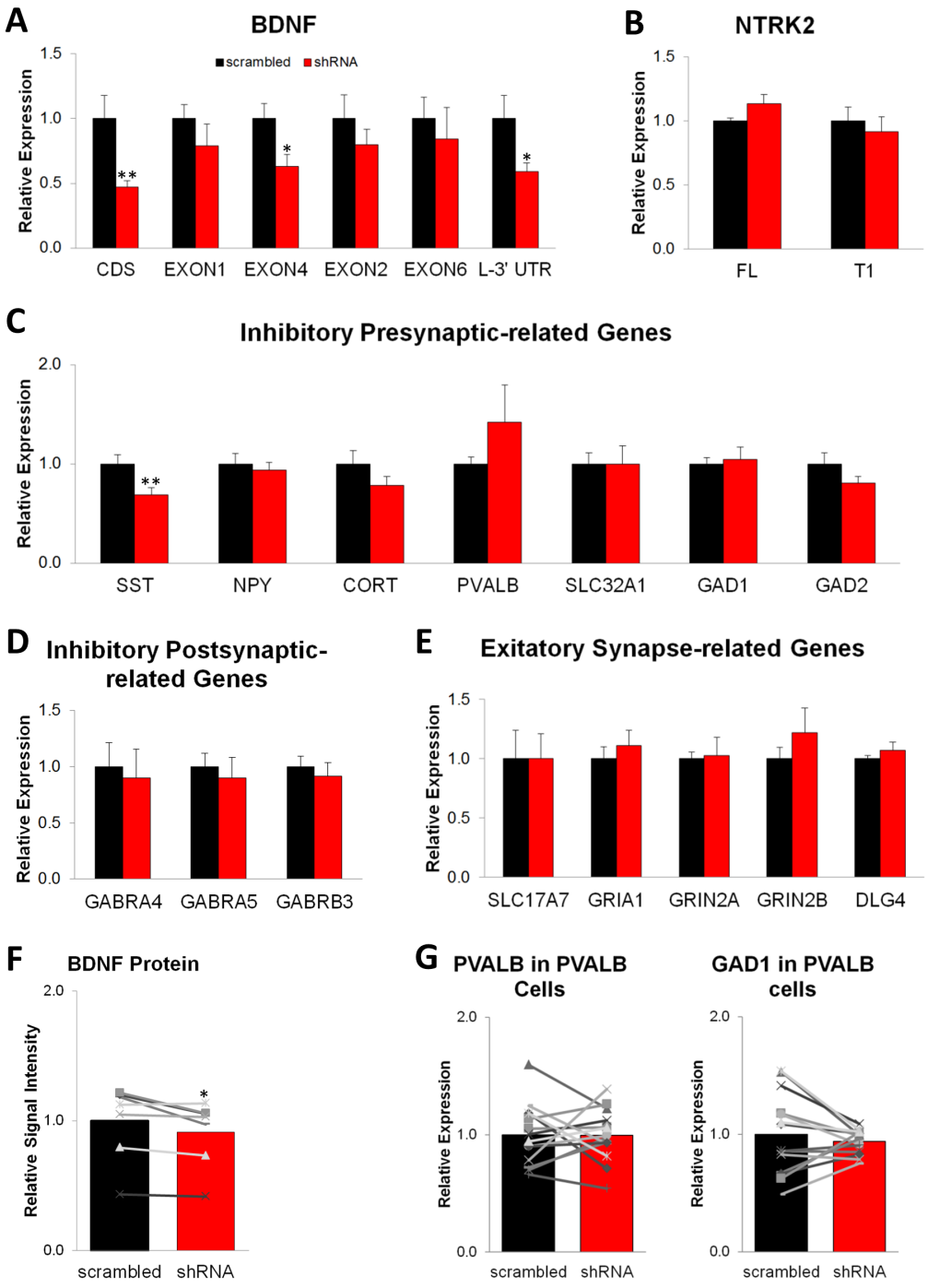

Figure S6. Gene expression changes by shRNA treatment at post-operation week 6. Relative expression level (*Bdnf* L-3' UTR shRNA/scrambled shRNA) of (A) *Bdnf* transcripts, (B) *Ntrk2* isoforms, (C-E) selected sets of synapse related genes (n=6/group). (F) BDNF immunostaining intensity in shRNA infused brain region (n=7/group). (G) Relative expression level of *Pvalb* and *Gad1* in *Pvalb*+ cells after shRNA treatment (n=14/group; *p<0.05, **p<0.01, ***p<0.001). Data are represented as mean ± SEM.

Supplementary References

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