Archival Report

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

Hyunjung Oh, Sean C. Piantadosi, Brad R. Rocco, David A. Lewis, Simon C. Watkins, and Etienne Sibille

ABSTRACT

BACKGROUND: A parallel downregulation of brain-derived neurotrophic factor (BDNF) and somatostatin (SST), a marker of inhibitory gamma-aminobutyric acid interneurons that target pyramidal cell dendrites, has been reported in several brain areas of subjects with major depressive disorder (MDD). Rodent genetic studies suggest that they are linked and that both contribute to the illness. However, the mechanism by which they contribute to the pathophysiology of the illness has remained elusive.

METHODS: With quantitative polymerase chain reaction, we determined the expression level of *BDNF* transcript variants and synaptic markers in the prefrontal cortex of patients with MDD and matched control subjects (n = 19/group) and of C57BL/6J mice exposed to chronic stress or control conditions (n = 12/group). We next suppressed *Bdnf* transcripts with long 3′ untranslated region (L-3′-UTR) using short hairpin RNA and investigated changes in cell morphology, gene expression, and behavior.

RESULTS: L-3′-UTRs containing *BDNF* messenger RNAs, which migrate to distal dendrites of pyramidal neurons, are selectively reduced, and their expression was highly correlated with *SST* expression in the prefrontal cortex of subjects with MDD. A similar downregulation occurs in mice submitted to chronic stress. We next show that *Bdnf* L-3′-UTR knockdown is sufficient to induce 1) dendritic shrinkage in cortical neurons, 2) cell-specific MDD-like gene changes (including *Sst* downregulation), and 3) depressive- and anxiety-like behaviors. The translational validity of the *Bdnf* L-3′-UTR short hairpin RNA–treated mice was confirmed by significant cross-species correlation of changes in MDD-associated gene expression.

CONCLUSIONS: These findings provide evidence for a novel MDD-related pathological mechanism linking local neurotrophic support, pyramidal cell structure, dendritic inhibition, and mood regulation.

Keywords: BDNF, Dendrite, GABA, Major depressive disorder, SST, Stress

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Major depressive disorder (MDD) is characterized by persistent low mood and/or anhedonia and cognitive impairment. These symptoms are associated with dysfunction of brain networks involved in cognition, emotion, and reward (1). The prefrontal cortex (PFC) plays a role in cognition and emotion regulation and is affected by stress (2). Consistent with functional impairment and atrophy (3–5), postmortem studies reported decreased neuronal body size, spine loss, and reduced cellular density in the PFC of subjects with MDD (6,7).

Decreased gamma-aminobutyric acidergic (GABAergic) neurotransmission in MDD has been revealed by transcranial magnetic stimulation (8) and proton magnetic resonance spectroscopy (9,10). Decreased expression of GABA-related genes has been observed in corticolimbic areas of patients with MDD (11–14). Downregulation of somatostatin (SST), a molecular marker of dendritic-targeting interneurons, was consistently found, whereas parvalbumin (PVALB), the marker of perisomatic-targeting interneurons, remained relatively unaffected by disease [although see Tripp *et al.* (14)]. Downregulation of SST is also observed in other neurological diseases (15), suggesting a vulnerability of GABA interneurons coexpressing this marker. The underlying mechanism is unknown; however, chronic stress and corticosterone exposure induced endoplasmic reticulum stress in these neurons (16). Another mechanism could be the loss of neurotrophic support. MDD and prolonged stress reduce *BDNF* expression (17,18), which might compromise structural integrity of various cells (19,20). GABAergic cells do not produce BDNF and rely on supply from other cell populations, mostly pyramidal neurons (21–23). In MDD, *SST* reduction is accompanied by *BDNF* and/or neurotrophic receptor tyrosine kinase 2 (*NTRK2*, also known as *TRKB*) downregulation (11,14) and genetic studies in mice revealed that *Sst* expression is highly dependent on *Bdnf* expression (11,14,24), together suggesting a pathogenic mechanism linking *BDNF* and GABA.

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Local action of *BDNF* is closely associated with neural plasticity. In addition to its autocrine action regulating dendritic and synaptic plasticity of pyramidal cells (25,26), *Bdnf* can alter the presynaptic GABAergic system in a paracrine manner (23,27). In a previous transcriptome analysis of human PFC, we found that the expression of *GABRA5*, a subunit of $GABA_A$ receptor enriched adjacent to dendritic GABAergic synapses that mediates the function of *SST* neurons (28), showed the highest correlation with *BDNF* levels among all investigated genes (29). This suggests that local *BDNF* supply may play a role in maintaining the function of *SST* neurons on pyramidal cell dendrites. Interestingly, *Bdnf* transcripts are present in dendrites (30–32), and cellular localization of *Bdnf* transcripts corresponds with phospho-NTRK2 immunostaining (30), suggesting that *BDNF* may be locally translated and may act on dendrites. Studies showed that dendritic *BDNF* is implicated in stress-related mood disorders: dendritic *Bdnf* transcripts are decreased by stress (33–35) and increased by antidepressant treatment (36). Knockdown of dendritic *Bdnf* impairs structural integrity of primary hippocampal neurons (30,37), replicating the effects of chronic stress.

Here, we investigated changes in dendritic *BDNF* transcripts in the dorsolateral PFC (dlPFC) of subjects with MDD and medial PFC (mPFC) of mice exposed to chronic stress, and we tested whether such changes were linked to dendritic-targeting interneuron markers. Using short hairpin RNA (shRNA), we then knocked-down dendritic *Bdnf* mRNA in primary neuronal culture and in mouse mPFC to assess whether reduced dendritic *Bdnf* expression was sufficient to induce MDD- and stress-related phenotypes. We predicted that decreased local neurotrophic support contributes to selective disturbance in dendritic structure and in dendritic-targeting GABAergic neurons, as a putative mechanism leading to mood dysregulation.

METHODS AND MATERIALS

A complete description can be found in the Supplement.

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. For all cases, a committee of experienced clinicians makes consensus DSM-IV diagnoses using information obtained from clinical records, toxicology exams, and standardized psychological autopsies (38). Individuals were screened for the absence of neurodegenerative disorders by neuropathological examinations (39–41). 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. After careful examination of clinical and technical parameters, 19 pairs of subjects with MDD and control subjects were selected (Supplemental Table S1).

Animals and Unpredictable Chronic Mild Stress

Nine- to 10-week-old C57BL/6J male mice were divided into two groups and submitted to control housing condition or unpredictable chronic mild stress (UCMS) consisting of a 7-week regimen of pseudorandom unpredictable mild stressors. The progression of the UCMS syndrome was monitored weekly by assessing coat state and weight changes for each mouse. All procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Behavioral Testing and Emotionality *Z* Score

Tests for depressive- and anxiety-like phenotypes included the elevated plus maze, open field, novelty suppressed feeding, and cookie tests. To assess behavioral consistency across tests, we integrated emotionality-related measures across tests as described earlier (42).

RNA Extraction and Real-Time Quantitative Polymerase Chain Reaction

For human studies, gray matter of dlPFC were collected in Trizol and further purified with RNeasy Micro Kit (QIAGEN, Valencia, CA). For mice, mPFC punches were taken and processed with RNeasy Micro Kit. To generate complementary DNA, total RNA was mixed with qScript cDNA Supermix (Quanta BioSciences, Gaithersburg, MD). Polymerase chain reaction products were amplified in triplets on a Mastercycler real-time polymerase chain reaction machine (Eppendorf, Hamburg, Germany).

shRNA, DNA Constructs, and Adeno-associated Viral Vector

shRNA sequence targeting long 3′ untranslated region (L-3′-UTR) of mouse *Bdnf* was adapted from (37). Lentiviral vectors containing shRNA against *Bdnf* L-3′-UTR or scrambled shRNA and enhanced green fluorescent protein (GFP) reporter gene were purchased from GeneCopeia (Rockville, MD). Adeno-associated viral 9 (AAV9) vectors containing *Bdnf* L-3′-UTR shRNA-GFP or scrambled shRNA were commercially prepared (Virovek, Hayward, CA).

Primary Culture, Transfection, and Fluorescent-Activated Cell Sorting of Mouse Cortical Neurons

Primary mouse neurons (A15585; Thermo Fisher Scientific, Waltham, MA) were cultured in Neurobasal media (21103; Thermo Fisher Scientific) supplemented with B27 (17504; Thermo Fisher Scientific), GlutaMAX-I (35050; Thermo Fisher Scientific). At 7 days in vitro, transfection was performed using Lipofectamine 3000 (L3000015; Thermo Fisher Scientific). GFP-expressing cells were collected by fluorescent-activated cell sorting into RNeasy lysis buffer.

Immunostaining and Image Analysis

After 5 days of transfection, cells were fixed with 4% paraformaldehyde and stained with rabbit anti-GFP (A21311; Life Technologies, Carlsbad, CA) and mouse anti-MAP2 (M9942; Sigma-Aldrich, St. Louis, MO). The morphology of dendrites was automatically traced and analyzed by Imaris software (Bitplane AG, Concord, MA). To quantify BDNF protein in AAV-infused region, immunostaining was performed with rabbit anti-BDNF (ab108319; Abcam, Cambridge, UK) and mouse anti-GFP (sc-9996; Santa Cruz Biotechnology, Dallas, TX).

Animals and Stereotaxic Surgery

Nine- to 10-week-old C57BL/6J male mice were bilaterally injected with AAV9-*Bdnf* L-3′-UTR shRNA-GFP or with AAV9-scrambled shRNA-GFP into the mPFC (anteroposterior +2.0 mm, mediolateral ± 0.4 mm, dorsoventral −2.0 mm). Behavioral tests were performed before and after UCMS exposure. For gene expression analysis, mice received *Bdnf* L-3′-UTR shRNA in one hemisphere and scrambled shRNA in the other for intrasubject comparison.

Fluorescent In Situ Hybridization and Laser Capture Microdissection

AAV-infused mice were perfused and lightly fixed with 1 mL of 4% paraformaldehyde. Mouse brains were sectioned and thaw-mounted onto a polyethylene naphthalate-membrane slides (Leica Microsystems, Concord, ON, Canada). *Sst*- and *Pvalb*-expressing neurons were visualized by fluorescent in situ hybridization (RNAscope; Advanced Cell Diagnostics, Newark, CA). Briefly, brain sections were permeabilized with protease and antisense probe targeting *Sst* and *Pvalb,* pre-amplifiers, amplifiers, and Atto 550 conjugated-probes were serially hybridized at 40°C. After dehydration, ∼200 *Sst*+ or *Pvalb*+ cells were collected using a LMD7 system (Leica Microsystems) and processed for RNA extraction.

Statistical Analysis

Gene expression differences between subjects with MDD and control subjects were determined by analysis of covariance using SPSS (IBM Corp., Armonk, NY). To determine covariates to include in gene-specific models, nominal factors were tested as main factors by analysis of variance, scale covariates were tested by Pearson correlation, and repeated measures were corrected by modified Holm-Bonferroni test. Analysis of covariance models including significant cofactors were then applied. For cell culture and animal studies, statistical significance between two groups was determined with Student's *t* test. Repeated-measures analysis of variance was performed to determine interaction among shRNA and stress, effect of stress, or shRNA on coat state over time.

RESULTS

Parallel Downregulation of Dendritic *BDNF* Transcripts and Dendritic Targeting Interneuron Markers in the PFC of Human Subjects With MDD

The *BDNF* gene is composed of at least nine exons and makes diverse transcript variants using a combination of alternative splicing and polyadenylation sites (Supplemental Figure S1A). The spatial segregation of *BDNF* mRNA is encoded by different untranslated regions; transcript variants with exons 2 and 6 and the L-3′-UTR can migrate to distal dendrites, whereas mRNAs containing exons 1 and 4 and the short 3′-UTR are restricted to the soma and proximal dendrites (30,43,44). We used quantitative polymerase chain reaction to quantify *BDNF* expression, focusing on transcript variants with known cellular localization, its receptor *NTRK2,* and synaptic function-related genes whose expressions are closely linked to *BDNF* levels in human PFC (29). Primers targeting *BDNF* protein coding sequence were included to measure pan-*BDNF* level. Several *BDNF* variants displayed nominal changes, but only L-3′-UTR was significantly reduced in MDD samples ($F = 6.98$, $p = .012$) (Figure 1A). The dendritic localization of *Bdnf* L-3′-UTR was confirmed in mouse primary cortical neurons and hippocampal tissue (Supplemental Figure S1B, C). Expression changes were not observed for *NTRK2* isoforms and excitatory synaptic-related genes; however, inhibitory synaptic genes displayed disease-related downregulation (Figure 1B–E). Interestingly, genes for all three dendritic-targeting interneuron markers (*SST, NPY, CORT*) and for dendritically localized *GABRA5* were decreased in subjects with MDD (*SST:* $F = 15.03$, $p = 4.6 \times 10^{-4}$; *NPY:* $F = 10.56$, $p = .003$; *CORT*: $F = 19.76$, $p = 8.5 \times 10^{-5}$; *GABRA5:* $F = 10.18$, $p = .003$), whereas expression of *PVALB,* the molecular marker of perisomatic-targeting interneurons, was not affected ($F = 1.94$, $p = .173$).

Despite the absence of *BDNF* messenger in interneurons (Supplemental Figure S2), *BDNF* L-3′-UTR level showed positive correlations to MDD-affected genes such as *SST* ($r = .53$, $p = .001$), but not to genes such as *PVALB* that are unaffected by disease ($r = -.25$, $p = .131$) (Figure 1F). The average correlation values among *BDNF* L-3′-UTR and MDD-affected or unaffected genes were 0.43 ± 0.11 ($p = 5.7 \times 10^{-5}$) and 0.08 ± 0.15 ($p = .178$) (Figure 1G), respectively. No other *BDNF* transcript variants including total *BDNF* showed significant correlation to MDD-affected genes (coding sequence: $r = -.14 \pm .07$, $p = .104$; EXON1: $r = -.09 \pm .04$, $p = .082$; EXON4; $r = -.13 \pm .09$, $p = .222$; EXON2: $r = .11 \pm .10$, $p = .310$; EXON6: $r = -.09 \pm .09$, $p = .320$).

Consistent with previous finding (13), female subjects with MDD showed greater *SST* reduction than did male subjects (Supplemental Table S2). However, correlation between *BDNF* L-3′-UTR and *SST* was comparable in both sexes (male: $r = .51$, female: $r = .48$). We observed age-related reduction of *BDNF* L-3′-UTR, *SST,* and *CORT* transcripts in control subjects; however, such age effects were less robust and not significant in subjects with MDD, which is consistent with prior findings in other cohorts (13,14). Other parameters including antidepressants or suicide were not consistently associated with the expression of all tested genes.

Together, selective downregulation in the expression of dendritic *BDNF* and dendritic-targeting interneuron markers was observed in the dlPFC of subjects with MDD. The high correlation between expressions of *BDNF* L-3′-UTR and specific GABA-related genes suggests that reduced dendritic *BDNF* may be associated with functional alterations of dendritic-targeting GABAergic neurons.

Chronic Stress Downregulates Dendritic *Bdnf* Transcript in the Rodent mPFC

Next, we tested whether chronic stress, a major risk factor for MDD, was sufficient to induce MDD-like gene expression changes in mice. UCMS increased the latency to bite on day 2 of the cookie test ($p = .03$) (Figure 2A) and the food pellet in the novelty suppressed feeding test ($p = .03$) (Figure 2B). The effect of stress was variable in other tests (Supplemental Figure S3). To assess behavioral consistency across tests, we derived behavioral *Z* scores (16,42) and confirmed that UCMS-exposed mice exhibited higher depression-/anxiety-related behaviors, denoted as behavioral emotionality, than did control mice ($p = .003$) (Figure 2C).

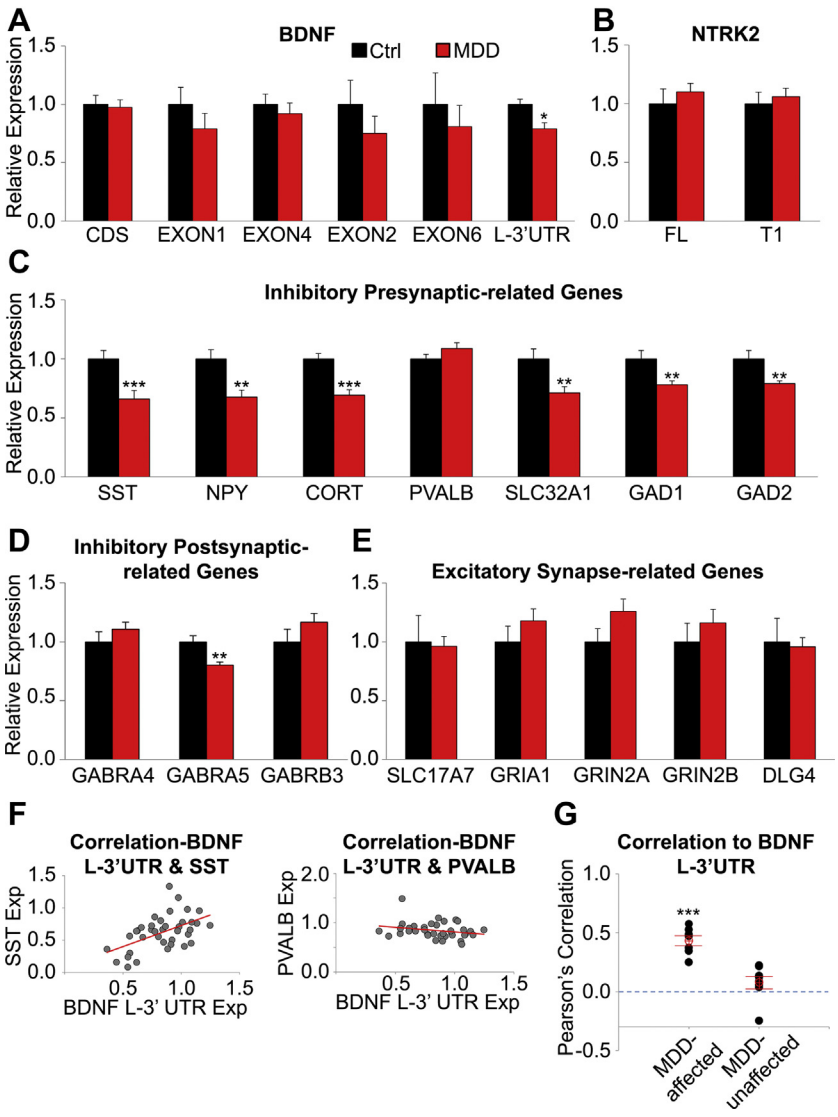

Figure 1. Major depressive disorder (MDD)-related changes in *BDNF* transcript variants and synaptic markers in human dorsolateral prefrontal cortex. **(A)** Relative expression (Exp) level (MDD/control [Ctrl]) of *BDNF* transcripts, **(B)** NTRK2 isoforms, and **(C–E)** selected synapse-related genes (n = 19 subjects/group). *BDNF* EXON1 and EXON4 are associated with localization in soma and proximal dendrite. *BDNF* EXON2, EXON6, and long 3′ untranslated region (L-3′-UTR) are associated with localization in distal dendrites. **(F)** Pearson's correlation among *BDNF* L-3′-UTR and *SST* and *PVALB*. *BDNF* L-3′-UTR is positively correlated with *SST,* a dendritic targeting interneuron marker, but not with *PVALB,* a marker of perisomatic targeting interneurons. **(G)** Overall correlation between *BDNF* L-3′-UTR and MDD-affected or unaffected synaptic markers. Significant positive correlations between *BDNF* L-3′-UTR level and MDD-associated gene expression changes suggest a contribution of dendritic *BDNF* to transcriptome alterations. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. Data are represented as mean ± SEM. CDS, coding sequence.

We found a significant reduction of *Bdnf* L-3′-UTR in the mPFC of UCMS-exposed mice (p = .029) (Figure 2D). This expression change was not observed for other genes (Supplemental Figure S4). Despite an absence of difference at the group level, there was a positive correlation between *Bdnf* L-3′-UTR and *Sst* (r = .43, p = .036) (Figure 2E) and negative correlations between behavioral emotionality and both *Bdnf* L-3′-UTR (r = −.43, p = .036) and *Sst* (r = −.41, p = .047) (Figure 2F). In comparison, *Pvalb* expression was not correlated with either *Bdnf* L-3′-UTR level (r = −.23, p = .281) or emotionality (r = .17, p = .420).

In summary, UCMS induced a selective downregulation in L-3′-UTR-containing *Bdnf* mRNAs in the mouse mPFC, which was significantly correlated with behavioral emotionality and *Sst* levels. These results suggest a role for dendritic *Bdnf* in anxiety- and depressive-like measures via regulation of dendritic-targeting interneurons.

Knockdown of Dendritic *Bdnf* Transcript Is Sufficient to Induce MDD- and Stress-Associated Phenotypes

We next tested whether altered dendritic *Bdnf* expression was causal to MDD- and stress-related dendritic shrinkage of cortical neurons. Using GFP as a marker (Figure 3A), we collected shRNA-expressing primary neurons with fluorescent-activated cell sorting and confirmed that shRNA treatment knocked down *Bdnf* L-3′-UTR expression (Figure 3B), without affecting total *Bdnf* levels. shRNA expression reduced the number of intersections in distal dendrites without changes in proximal dendrites (0 to ∼30 μm from soma) (Figure 3C, Supplemental Table S3). The total length of all dendrites of shRNA-treated neurons was shorter than that of control neurons (p = .009) (Figure 3D) while the number of dendritic segments remained unchanged (p = .91) (Figure 3E).

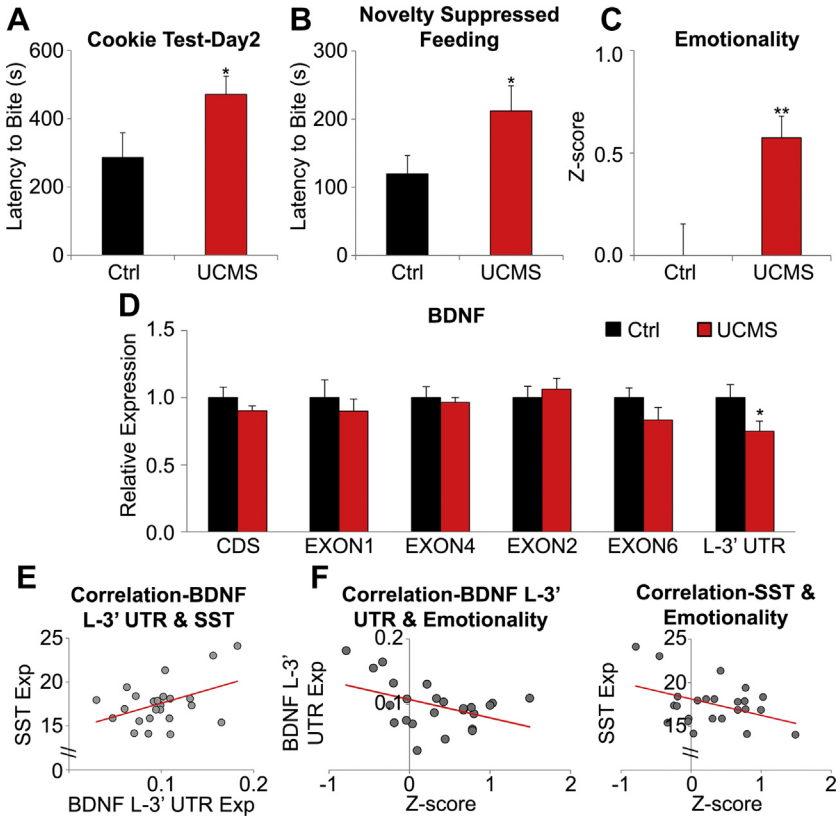

Figure 2. Chronic stress-related changes of anxiety-/depressive-like behaviors and expression of *Bdnf* transcript variants and *Sst* in mouse medial prefrontal cortex. **(A)** Stress-associated behavioral change in the cookie test (increased latency to bite cookie) and **(B)** novelty suppressed feeding (increased latency to bite food pellet). **(C)** Increased depressive-/anxiety-like phenotype in stressed mice compared with control (Ctrl) animals. **(D)** Relative expression (Exp) level (stress/control) of *Bdnf* transcript variants. **(E)** Positive correlation between *Bdnf* long 3′ untranslated region (L-3′-UTR) and *Sst* suggests a functional link between the two genes in mouse brain. **(F)** Behavioral emotionality was negatively correlated to *Bdnf* L-3′-UTR (left) and *Sst* (right). n = 12/group; $^{*}p < .05$; $^{**}p < .01$. Data are represented as mean ± SEM. CDS, coding sequence; UCMS, unpredictable chronic mild stress.

Next, we injected shRNA-expressing AAV in mouse mPFC (Figure 4A) to investigate whether low dendritic *Bdnf* expression is associated with behavioral changes. Behavioral tests were performed after recovery and then after UCMS (Figure 4B). Stress exposure did not affect body weight (Figure 4C) but did induce a progressive worsening of the coat state (Figure 4D), indicating that both groups responded to UCMS ($p = 3.6 \times 10^{-28}$). Notably, over time, shRNA-treated mice displayed worse coat state than the control group did ($p = .002$). shRNA-treated mice showed trend-level differences in the open field ($p = .052$) (Figure 4E) and in emotionality Z scores before UCMS ($p = .093$) (Figure 4F).

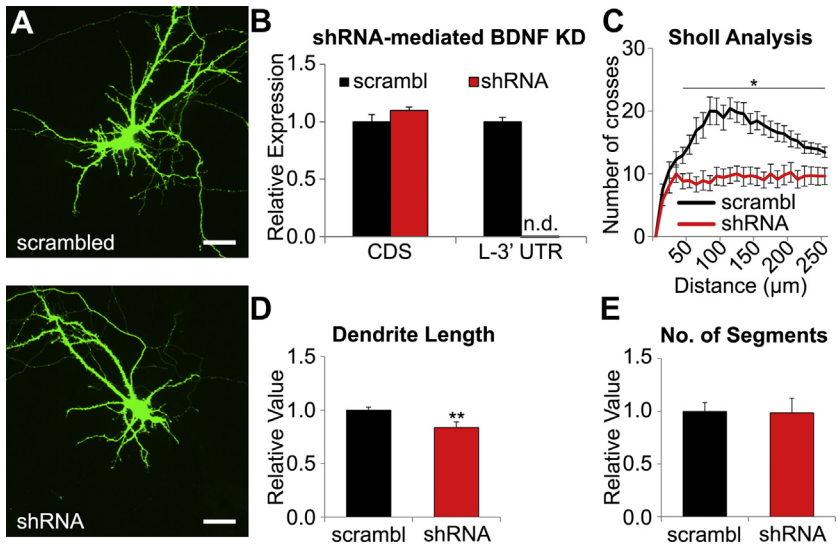

Figure 3. *Bdnf* long 3′ untranslated region (L-3′-UTR) knockdown-induced structural changes of primary mouse cortical neurons. **(A)** Representative image of primary mouse cortical neuron expressing scrambled (scrambl) short hairpin RNA (shRNA) and *Bdnf* L-3′-UTR-targeting shRNA. Scale bar = 50 μm. **(B)** Relative expression (shRNA/scrambled) of *Bdnf*–coding sequence (CDS) and *Bdnf* L-3′-UTR in shRNA-expressing cells compared with control cells. Expression of L-3′-UTR was not detectable (n.d.) after shRNA treatment. **(C)** Sholl analysis for shRNA-treated and control neurons showed that shRNA treatment disrupted integrity of distal dendrite. **(D)** Relative value of total length of dendrites of shRNA-treated and control neurons. **(E)** Relative value of number of dendritic segments of shRNA-expressing neurons compared with control neurons. Total dendritic length was significantly decreased without changes in branch number (control: n = 14, shRNA: n = 10; $^{*}p < .05$, $^{**}p < .01$). Data are represented as mean ± SEM. KD, knockdown.

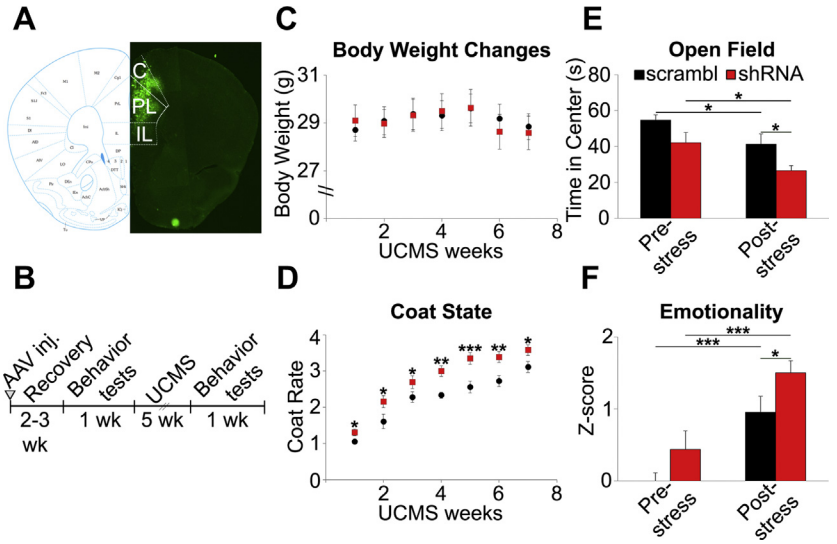

Figure 4. Effect of *Bdnf* long 3′ untranslated region (L-3′-UTR) knockdown and chronic stress on behavior. **(A)** Representative image of green fluorescent protein expression following adeno-associated viral (AAV) injection (inj) in medial prefrontal cortex. The mouse medial prefrontal cortex map is from Franklin and Paxinos (66). **(B)** Schematic of the experimental design. **(C)** No body weight changes by chronic stress. **(D)** Progressive coat state changes with stress exposure. **(E)** Short hairpin RNA (shRNA)-treated mice spent less time in the center of the open field apparatus after unpredictable chronic mild stress (UCMS). **(F)** shRNA-treated mice group showed significantly higher emotionality *Z* scores than control mice did after stress exposure (scrambled [scrambl]: $n = 9$, shRNA: $n = 13$; $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$). Data are represented as mean ± SEM. C, cingulate cortex; IL, infralimbic cortex; PL, prelimbic cortex.

After UCMS, shRNA-treated mice spent less time in the open field center ($p = .009$) (Figure 4E). Although other behavioral measures did not show significant group differences (Supplemental Figure S5), shRNA-treated mice displayed higher emotionality than did control vector-treated mice after UCMS ($p = .029$) (Figure 4F). There was no interaction between shRNA treatment and chronic stress on emotionality ($p = .763$) or coat state ($p = .270$).

To examine whether knockdown of dendritic *Bdnf* was sufficient to induce MDD-associated molecular changes, we infused AAV-expressing *Bdnf* L-3′-UTR shRNA into one hemisphere and scrambled shRNA into the other (Figure 5A) and sacrificed mice at 2, 4, and 6 weeks after surgery. Gene expression changes were not found at postsurgery week 2. *Bdnf* L-3′-UTR was significantly downregulated at week 4 ($p = .013$) and week 6 ($p = .030$), whereas *Sst* level was reduced at week 6 only ($p = .002$) (Figure 5B). *Sst* and *Bdnf* L-3′-UTR expression showed positive correlation at week 6 ($r = .67$, $p = .016$), whereas *Pvalb* expression did not show such relationship ($r = -.05$, $p = .866$) (Figure 5C), confirming the selective effect of *Bdnf* L-3′-UTR on *Sst*. In contrast to the knockdown yields observed in primary culture, total and EXON4+ *Bdnf* mRNA levels were reduced by shRNA ($p = .009$ and $p = .035$, respectively) (Supplemental Figure S6A), suggesting differences between in vitro and in vivo *Bdnf* transcripts regulation, and/or in vivo functional adaptations. Notably, total and EXON4+ *Bdnf* levels were not correlated with *Sst* level (total: $r = .18$, $p = .566$; EXON4: $r = .39$, $p = .205$). The expression of other genes was not changed by shRNA treatment (Supplemental Figure S6B–E). BDNF protein levels were also significantly reduced by shRNA treatment (91.4 ± 9.6%, $p = .037$) (Supplemental Figure S6F).

To validate the translational value of our findings, we compared shRNA-induced changes of *Bdnf*-, synaptic function-related genes in mice to expression changes observed in subjects with MDD compared with changes in control subjects. We found a significant correlation ($r = .45$, $p = .009$) (Figure 5D, Supplemental Table S4), demonstrating that dendritic *Bdnf* knockdown in mice replicated gene expression changes observed in human MDD.

Finally, we collected *Sst*- or *Pvalb*-expressing cells from AAV-infused mPFC using laser microdissection to investigate whether *Bdnf* L-3′-UTR knockdown induced gene expression changes in a cell type–specific manner. Expression of *Sst* and *Gad1* mRNAs was significantly reduced in *Sst*-positive cells by shRNA treatment ($p = .001$ and $p = .014$, respectively) (Figure 5E), but *Pvalb* and *Gad1* remained unchanged in *Pvalb*-positive interneurons ($p = .478$ and $p = .217$, respectively) (Supplemental Figure S6G).

In summary, *Bdnf* L-3′-UTR knockdown recapitulated MDD- and chronic stress-associated molecular, structural, behavioral phenotypes. Our observations that *Sst* reduction followed dendritic *Bdnf* knockdown, and that shRNA-treated mice showed transcriptome profiles similar to those for MDD, suggest that MDD-related dendritic *Bdnf* downregulation is causally implicated in MDD-related GABAergic gene changes and associated symptoms (Figure 6).

DISCUSSION

MDD is a devastating disease characterized by low mood, anhedonia, and cognitive deficits. Studies suggest that impaired neural plasticity and GABAergic function are associated with MDD; however, the mechanism underlying these two phenomena has not been clearly defined. In the current study, we report a selective downregulation of dendritic *BDNF* mRNA in the dlPFC of subjects with MDD and a parallel downregulation of dendritic-targeting interneuron markers without affecting a perisomatic-targeting interneuron marker. Strong and selective correlations between expressions of *BDNF* L-3′-UTR and inhibitory synaptic genes were observed, suggesting that an MDD-related decrease in dendritic *BDNF* transcripts contributed to impairment of dendritic-targeting interneurons. However, human postmortem brains do not allow examining dynamic molecular changes induced by the disease; therefore, our findings could be the results of the disease, not the cause.

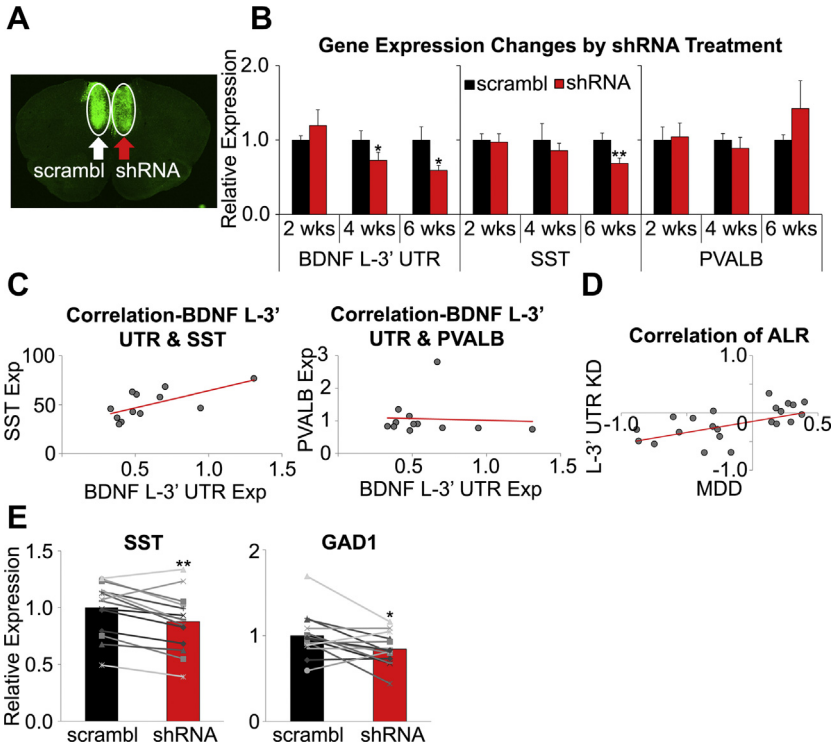

Figure 5. *Bdnf* long 3′ untranslated region (L-3′-UTR) knockdown-related gene expression (Exp) changes. **(A)** Representative image of green fluorescent protein expression following adeno-associated viral injection in medial prefrontal cortex. **(B)** Expression changes in core genes at 2, 4, and 6 weeks after surgery. Changes in *Bdnf* L-3′-UTR expression were observed from week 4, and *Sst* expression change was observed only in week 6. **(C)** Correlation between expression of *Bdnf* L-3′-UTR and *Sst* (left) or *Pvalb* (right) level at postsurgery week 6. Significant correlation to *Bdnf* L-3′-UTR level was observed in *Sst*, but not in *Pvalb* (n = 6/group at each time point). **(D)** Similarity between major depressive disorder (MDD)–induced and *Bdnf* L-3′-UTR knockdown-induced gene expression changes (average log ratio [ALR]: [MDD/control, BDNF L-3′-UTR knockdown/control]). **(E)** *Sst* and *Gad1* in *Sst*+ cells were significantly downregulated by short hairpin RNA (shRNA) treatment (n = 14/group; $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$). Data are represented as mean ± SEM. scrambl, scrambled.

To test a putative causal link, we used animal models. The UCMS paradigm is known to reproduce phenotypes resembling human MDD (45), although it is a model of chronic stress-induced pathologies, rather than MDD itself. We observed a selective decrease in *Bdnf* L-3′-UTR in the mPFC of mice exposed to UCMS. Although *Sst* expression did not show significant group differences, it was correlated positively with *Bdnf* L-3′-UTR level and negatively with behavioral emotionality. The fact that *Sst* downregulation did not reach statistical significance may relate to differences in timescale; subjects had experienced MDD for months to years, whereas mice were exposed to mild stress for only 7 weeks. Moreover, we performed animal experiments in male mice, and it is not known whether greater effects would be observed in female mice.

Knockdown of *Bdnf* L-3′-UTR replicated MDD-like structural, behavioral, and molecular phenotypes. We observed a trend for elevated anxiety- and depression-like behaviors in shRNA-treated mice at baseline and a significant elevation after UCMS in shRNA-treated animals compared with control animals. Following shRNA-induced *Bdnf* knockdown, *Sst* expression was reduced and showed positive correlation to *Bdnf* L-3′-UTR level. These progressive molecular changes were consistent with nonsignificant group differences in baseline emotionality at postsurgery weeks 2 and 3 and with the earlier UCMS-induced fur degradation in shRNA-treated mice. Moreover, cell-specific analyses suggest that *Sst*+ cells are more vulnerable than *Pvalb*+ interneurons to low level of dendritic *Bdnf* as revealed by reduced *Gad1* expression.

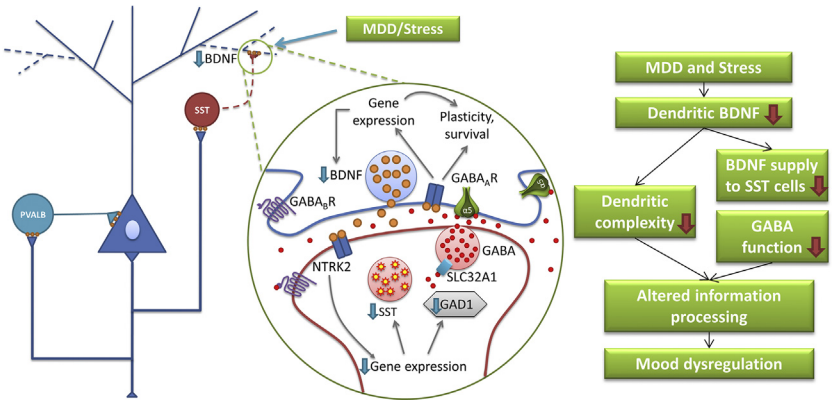

Figure 6. Model of brain-derived neurotrophic factor (BDNF) role in major depressive disorder (MDD)- and stress-related molecular and behavioral phenotypes. Results from the current study suggest a sequence of events associated with MDD-related brain changes. First, prolonged stress induces selective deficit of dendritic BDNF indicated by low *BDNF* long 3′ untranslated region expression in the prefrontal cortex. Second, reduced *BDNF* supply to dendritic compartments and gamma-aminobutyric acid (GABA) interneurons targeting distal dendrites of pyramidal cells leads to impaired function of dendritic-targeting interneurons (e.g., downregulation of somatostatin [SST] and GABA synthesizing enzyme), disrupted dendritic integrity, altered filtering and processing of excitatory input onto pyramidal cell dendrites, and finally, mood dysregulation. GABA$_A$R, GABA type A receptor.

Notably, although the mouse studies were inspired by findings in human postmortem brains, species differences may limit their interpretability. While not all MDD-associated gene expression changes are recapitulated, we observed a positive correlation between *Bdnf* L-3′-UTR shRNA-induced gene expression changes in mice and those observed in human MDD brain samples. Hence, the collective results suggest a novel mechanism underlying the pathophysiology of MDD, linking local neurotrophic support, pyramidal cell structure, and dendritic inhibition (Figure 6).

Contribution of Low Dendritic *BDNF* to Impaired Structural Integrity and Function in the dlPFC of Subjects With MDD

Previous studies showed that about 50% of *Bdnf* transcripts had L-3′-UTR (31,46). Knockdown of *Bdnf* L-3′-UTR in mice was sufficient to induce stress- and MDD-associated morphological changes in cortical neurons. In agreement with a human postmortem study reporting shortened dendritic processes without changes in the number of processes in basilar dendrites of pyramidal neurons in dlPFC of subjects with psychiatric illnesses including MDD (47) and with animal studies showing stress-induced structural changes mainly occurring on the distal portion of apical dendrites (4,19,20,48,49), shRNA targeting *Bdnf* L-3′-UTR induced retraction of distal dendrites of primary cortical neurons.

Animal studies show that dendritic *Bdnf* transcripts are closely linked to stress-related mental illness (33–35). Human genetic studies also support the idea that the dendritic action of *BDNF* is essential to mood regulation. The most common single nucleotide polymorphism in human *BDNF,* resulting in Valine to Methionine substitution at codon 66 (Val66Met), has been implicated in defective fear extinction (50) and increased susceptibility to psychiatric diseases (51). Mice harboring the Met allele exhibit impaired dendritic transport of *Bdnf* mRNA (52,53), reduced activity-dependent BDNF secretion (54), impaired synaptic plasticity in mPFC (55), and elevated anxiety-like behavior that is not normalized by fluoxetine (56). Our finding that emotionality is significantly correlated to *Bdnf* L-3′-UTR expression provides evidence that dendritic *Bdnf* is important for the function of mood regulatory systems.

How, then, can the loss of dendritic *BDNF* contribute to functional abnormalities? First, defects in activity-dependent BDNF supply might play a role. A recent study found that over 2000 transcripts are localized in dendrites and/or axons, maybe for faster on-demand protein supply by local synaptic translation (32). Not only does L-3′-UTR+ *Bdnf* mRNA preferentially exist in dendrites (31), but it stays dormant in resting state, yet binds to a polyribosome responding to neuronal activity (46). Given that MDD may be conceived as a disease of maladaptive processes, impaired activity-dependent neuroplasticity may underlie the inability to cope with stress. A second possible mechanism could be through reduced GABAergic transmission. Knockdown of dendritic *Bdnf* mRNA and/or blockade of dendritic transport downregulate GABAergic receptors and GABA-synthesizing enzymes (57,58). In addition, SST acts as inhibitory neuropeptide, and *Sst* knockout is sufficient to induce depressive-/anxiety-related behavior in mice (16). These data imply that the depressive-like behavioral outcome may derive at least in part from low inhibitory control.

Close Link Between Pyramidal Cell Dendritic *BDNF* Transcripts and Dendritic-Targeting Interneurons and Its Implication for the Pathology of MDD

SST and *PVALB* are expressed in distinct classes of cortical interneurons. Multiple lines of evidence imply that *BDNF-NTRK2* signaling plays a crucial role in interneuron development and maintenance of GABAergic function. Our studies have consistently found that low *BDNF* function is closely linked to reduced expression of GABA-related genes observed in the brains of patients with MDD (11,14,24). As *Ntrk2* is predominantly expressed in *Pvalb*+ interneurons compared with other interneuron populations (21,22), higher *BDNF* dependency of *SST* than *PVALB* raised questions about underlying mechanisms. Our current study suggests that selectivity of MDD- and stress-induced effects may be achieved through reduced dendritic *BDNF* function, leading to altered neuronal plasticity, behavior, and impaired function of dendritic-targeting interneurons.

To date, it is not clearly defined how dysfunction of *SST*+ interneurons contributes to the pathophysiology of mood disorders (59). In a previous study, we reported that acute inactivation of *Sst*+ neurons in mPFC can increase anxiety- and depressive-like behavior (60). The mPFC, the target of our study, has excitatory projections to amygdala to produce fear/stress response (61). Hence, low *Bdnf* L-3′-UTR-mediated dysfunction of *Sst*+ neurons may result in increased mPFC pyramidal neuron baseline activity leading to amygdala hyperactivity. In addition, distal dendrites mainly receive inputs from other cortical areas, and regulation of dendritic excitability is crucial for functional computations [reviewed in Larkum (62)]. Considering that dendritic excitation mainly occurs in actively behaving animals, one can assume that proper control of dendritic activity is crucial for regulation of active behavior. For instance, *Sst*+ interneurons in barrel cortex are inactivated during active whisking, which leads to increased excitability for the sensorimotor stimuli (63). During contextual fear learning, *Sst*+ interneurons in the basolateral amygdala are inactivated by multisensory environmental context, whereas an aversive event inactivates both *Pvalb*+ and *Sst*+ interneurons (64,65). Hence, loss of dendritic inhibition may result in increased signal-to-noise ratio and failure to discriminate different stimuli (59).

Combining current knowledge and our experimental results, we propose a biological model linking low level of BDNF, structural changes, and GABAergic dysfunction, three phenomena commonly observed in the PFC of MDD subjects and stressed animals (Figure 6). MDD and stress may disturb dendritic *BDNF,* which leads to low neurotrophic supply to dendrites and dendritic targeting interneurons. Reduced BDNF signaling may contribute to decreased dendritic complexity of pyramidal neurons and low inhibitory function via reduced *GAD* (i.e., low GABA production) and *SST* expression in *SST*+ cells, finally culminating in altered information processing by cortical microcircuits, leading to behavioral symptoms of MDD.

In conclusion, our results contribute to our understanding of *BDNF* function under control and disease states and provide

evidence that MDD-related downregulation of dendritic *BDNF* contributes to selective impairment of dendritic-targeting interneurons in subjects with MDD and that maintaining the integrity of the *BDNF-NTRK2* system is critical for mood regulation.

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ARTICLE INFORMATION

From the Campbell Family Mental Health Research Institute of CAMH (HO, ES) and Departments of Psychiatry (ES) and Pharmacology and Toxicology (ES), University of Toronto, Toronto, Ontario, Canada; and the Department of Psychiatry (SCP, BRR, DAL, ES) and the Center for Biologic Imaging (SCW), Department of Cell Biology and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Address correspondence to Etienne Sibille, Ph.D., Campbell Family Mental Health Research Institute of CAMH, 250 College Street, Room 134, Toronto, Ontario M5T 1R8, Canada; E-mail: etienne.sibille@camh.ca.

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